ISSN 2313–5891 (Online)
ISSN 2304–974X (Print)

Ukrainian Food Journal

Volume 10, Issue 1
2021

Kyiv **Київ**

2021

Ukrainian Food Journal is an international scientific journal that publishes innovative papers of the experts in the fields of food science, engineering and technology, chemistry, economics and management.

Ukrainian Food Journal – міжнародне наукове періодичне видання для публікації результатів досліджень фахівців у галузі харчової науки, техніки та технології, хімії, економіки і управління.

Ukrainian Food Journal is abstracted and indexed by scientometric databases:

Ukrainian Food Journal індексується наукометричними базами:

Index Copernicus (2012)
EBSCO (2013)
Google Scholar (2013)
UlrichsWeb (2013)
CABI full text (2014)
Online Library of University of Southern Denmark (2014)
Directory of Research Journals Indexing (DRJI) (2014)
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European Reference Index for the Humanities and the Social Sciences (ERIH PLUS) (2014)
Directory of Open Access Journals (DOAJ) (2015)
InfoBase Index (2015)
Chemical Abstracts Service Source Index (CASSI) (2016)
FSTA (Food Science and Technology Abstracts) (2018)
Emerging Sourses Citaton Index (2018)

Ukrainian Food Journal включено у перелік наукових фахових видань України з технічних наук, категорія А (Наказ Міністерства освіти і науки України № 358 від 15.03.2019)

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Scientific Council of the National University of Food Technologies approved this issue for publication. Protocol № 8, 25.03.2021

Рекомендовано вченою радою Національного університету харчових технологій. Протокол № 8, 25.03.2021

Ukrainian Food Journal publishes original research papers, short communications, scientific news in the related fields and also literature reviews.

Topics coverage:

Food engineering	Food nanotechnologies
Food chemistry	Food processing
Food microbiology	Economics and management in food industry
Physical property of food	Automation of food processing
Food quality and safety	Food packaging

Periodicity. Journal is issued four times per year.

Reviewing a Manuscript for Publication. All scientific articles submitted for publication in "Ukrainian Food Journal" are double-blind peer-reviewed by at least two reviewers appointed by the Editorial Board: one from the Editorial Board and one reviewer that is not affiliated to the Board and/or the Publisher.

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Trends and expected benefits of the breaking edge food technologies in 2021–2030

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Keywords:

Nutrition
Food safety
Processing
Biotechnology
Personalization

Article history:

Received 23.09.2020
Received in revised form 01.12.2020
Accepted 25.03.2021

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DOI: 10.24263/2304-974X-2021-10-1-3

Abstract

Introduction. The review considered the major trends in the world development of new food processing technologies in 2021–2030 that are as follows:

Material and method. Morphological analysis of clusters of scientific knowledge about food science.

Result and discussion. Major trends in the world development of new food processing technologies:

1. More strict regulations of food safety including QPCR and DNA-sequencing detection of emerging food-borne pathogens, comprehensive control of minor chemical pollutants of food;

2. Production of functional food including food for babies, children, pregnant women, elderly, for sport activities, military food, meals ready-to-eat, microelements-enriched food, nutraceuticals, and clinical food;

3. Emerging environmentally-friendly and energy-saving food processing including non-thermal physical methods such as cold plasma processing, high pressure homogenization, pulsed electric fields, ultrasound-assisted extraction, novel food-drying technologies, novel ethanol production technologies, novel extraction technologies, disinfection and sterilization of food and equipment, novel food packaging technologies, food processing intensification using biotechnological methods, involvement of new food staff and materials for food production;

4. Biotechnological food processing using enzymes proteinases, glutamine transferases, galactosidases, enzymes of extremophilic and psychrophilic microorganisms, alive microorganisms as probiotics or starter cultures, microbial metabolites, and new sources of food such as insects and artificial meat;

5. Personalization of food processing and distribution including adaptation of the food processing to the nutritional needs of the different medical, racial, religious, and regional customer groups, computerization of the personal food production and consumption, and a problem of consumer acceptance of a new food, 3D printing of personal food. Commercial food became so diverse that the specific nutritional computer programs with the comprehensive information on this food as well as personal diet requirements will be used for the optimization of the production and delivery of the personal-specified food.

Conclusions. Review information can be valuable for researchers and managers to prioritize the research and innovation directions.

Trend 1. Enhanced food safety

Food safety regulations

Development of new food technologies increases the risk of the foodborne diseases and food contamination. For example, about 48 million people in U.S. get sick and 3,000 die each year from foodborne diseases (CDC, 2018). In Europe, there are 23 million cases of foodborne diseases causing 5,000 deaths every year by WHO estimation (Flynn et al., 2019).

Food is an essential source of human exposure to harmful for health chemicals. Food contamination is also increasing, for example in U.S. more than half of the foods tested had pesticide residues. Therefore, new and more strict regulations for the food manufacturers and distributors were established there by the shifting of the focus from responding to prevention of foodborne illness (Food Safety Modernization Act, 2020). The main idea of this legislation is that prevention of foodborne illness and food contamination is both a significant public health problem and a threat to the economic well-being of the food system. So, food industry executives have to make sure their enterprises are complying with the latest regulations to ensure food quality from farm to fork.

Current rules and related programs of Food Safety Modernization Act (FSMA) are as follows:

1. Accredited third-party certification;
2. Current good manufacturing practice, hazard analysis, and risk-based preventive controls for human food;
3. Current good manufacturing practice, hazard analysis, and risk-based preventive controls for animal feed;
4. Foreign supplier verification programs;
5. Laboratory accreditation;
6. Food traceability;
7. Mitigation strategies to protect food against intentional adulteration;
8. Sanitary transportation of human and animal food;
9. Standards for the growing, harvesting, packing, and holding of produce for human consumption;
10. Voluntary qualified importer program (food safety modernization act, 2020).

Food industry needs more scrutiny and transparency because of the inherent risk involved with allowing untested ingredients to enter the market.

Similar activities are performing currently in Europe to enhance transparency and sustainability of the EU risk assessment in the food chain (EU risk assessment in the food chain, 2019). The main points of this regulation are as follows:

1. Ensuring more transparency so that citizens will have automatic access to all studies and information submitted by industry in the risk assessment process;
2. Increasing the independence of studies;
3. Strengthening the governance and the scientific cooperation in eu;
4. Developing comprehensive risk communication throughout the risk analysis process, combined with open dialogue amongst all interested parties.

The National Center for Food Safety Risk Assessment was created in China. It is a key contributor to the food safety standards using international best practices and CODEX ALIMENTARIUS of FAO-WHO (Zhang et al., 2018; The Codex Alimentarius, 2020). The center comprises four networks of food safety in China including the foodborne disease surveillance network, the biological hazards (bacteria, viruses and parasites) monitoring in

foods network (Pei et al., 2015), chemical hazards monitoring in foods network, and the microbial DNA fingerprint profile network (Wu and Chen, 2018).

The Codex Alimentarius of FAO-WHO (The Codex Alimentarius, 2020) considers such important standards of food safety as:

1. Quality of animal feed that plays a vital role in the production of safe and quality products of animal origin;
2. Antimicrobial resistance, which is a global threat of increasing concern to human and animal health and the economic wellbeing of farming households;
3. Biotechnology of food, mainly of genetically modified organisms (gmos) and other potentially unsafe biotechnological methods and products;
4. Chemical contaminants of food and feed, especially pesticides, that may pose a risk to animal and human health;
5. Nutrition and labelling ensuring information for the choice of healthy and safe foods.

The types of food safety events were distributed in 2017 as follows: biological (microbiological) hazards – 64%, chemical hazards – 16%, physical hazards – 2%, allergens – 7%, hazards of unknown origin – 11% (WHO/FAO, 2018).

Detection of emerging food-borne pathogens

Data on *Salmonella* isolates in food for 2012 -2016 showed that in 61% cases there were contaminated chicken and chicken products, in 16% cases there were pork and pork products, and only small quantity of beef and eggs, 1.5 and 1%, respectively, were contaminated with *Salmonella* (Schlundt et al., 2020). Using novel methods there were discovered new 175 pathogenic microbiological species considered to be "emerging" (Schlundt et al., 2020) and a lot of them could be the agents of foodborne diseases. Innovative approach in the evaluation of bacteriological safety of food is detection of wide-spread antibiotic-resistant microorganisms (ARM) in food. Resistance of pathogens to antibiotics considered as the greatest and most urgent global risk (UN, 2016).

Analysis of ARM in food is developing in Nanyang Technological University, Food Technology Centre, Singapore by the team of Prof. Jørgen Schlundt (Schlundt et al., 2020). For detection of foodborne pathogen and ARM in food there are used not only conventional microbiological methods but mainly identification and subtyping of isolates of foodborne bacterial pathogens by matrix-assisted laser desorption ionization-time of flight mass spectrometry (Aung et al., 2020) and whole genome sequencing, which is delivering sufficiently high resolution and epidemiological concordance (Schlundt et al., 2020). Whole genome sequencing will be the most advanced method in the nearest future to study food biosafety and foodborne diseases.

Another modern approach to study microbiological safety of food is pulsed-field gel electrophoresis (PFGE), which is a DNA fingerprint for a bacterial isolate. PFGE could be used effectively to investigate bacterial isolates from sick people, the contaminated food, and the sites of food production (Pulsed-field Gel Electrophoresis, 2016).

Control of chemical pollutants of food

Major chemical pollutants of feed and food are as follows:

1. Mycotoxins such as aflatoxins, fumonisins, mycotoxins of *Fusarium spp*. Such as trichothecenes T-2 toxin, HT-2 toxin, deoxynivalenol (DON), nivalenol, zearalenone,

and some other toxins such as ochratoxins of *Aspergillus ochraceus*, *A. Carbonarius*, *A. Niger*, *Penicillium verrucosum*; some substances, for example patulin produced in the rotten apples and extracted to the apple juice, are rather carcinogenic than toxic substances;

2. Polycyclic aromatic hydrocarbons (pahs) such as naphthalene, benzopyrene, chrysene, benz(a)anthracene, and others;
3. Recalcitrant organic pollutants such as aldrin, dieldrin, heptachlor, endrin, DDT, hexachlorocyclohexanes, polychlorinated biphenyls, dioxins, and others. Major part of these pollutants entering human organism with food of animal origin, mainly from beef and dairy food;
4. Hormones in meat that was used as animal growth promoters: oestradiol, testosterone, progesterone, zeranol, trenbolone acetate and melengestrol acetate, and substances having a thyrostatic action and of beta-agonists (EUR-LEX EUROPA; Guide to cross compliance in England, 2020);
5. Chemical food preservatives and other chemical food additives to control ph, foam, oxidation, color, flavor, emulsification, thickening, food energy;
6. Some toxic or carcinogenic substances are producing during food processing, for example, acrylamide that is produced from potato or cereals at temperature above 120 ^{o}C;
7. Indirect chemical food additives that are used in food production and packaging from food contact materials (FCM): food containers, processing machinery, packaging materials, kitchenware and tableware (WHO, 2019; EU food chemical safety, 2018). For Ukraine, it is important a problem of high concentration of water-retention substances, phosphates and salt in the meat and meat products, which are increasing a risk of cardiovascular diseases and strokes of people with hypertension (http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3278747).

The general trend in USA and in Europe that will be developed in 2021–2030 is strict legislation on authorization, comprehensive testing of safety, and limited use of food chemical additives. For all food contaminants must be established maximum levels to protect public health (WHO, 2019; EU food chemical safety, 2018). The European Commission publishes a factsheet on food contaminants (Managing food contaminants – European Commission, 2007).

Ukrainian food manufacturers are introducing safety control procedures of the Hazard Analysis and Critical Control Point (HACCP) system for correct organization of food production, analysis of reasons for mistakes, and determining methods of fixing them (The system of hazard analysis and critical control points, 2019).

Currently, HACCP systems in Ukraine were implemented only at large, export-oriented enterprises (EU in Ukraine, 2019), so it is a need for wider comprehensive implementation during 2021 – 2030.

Trend 2. Functional food and nutraceuticals

Functional food

Every kind of food has physiological and health effects. For example, consumption of processed meat correlated with the risk of colorectal and stomach cancer, and it was calculated that diminishing of the processing meet consumption in USA using 10% excise

tax would prevent 77,000 colorectal and 12,400 stomach cancer cases and generate net savings of about US$41 billion from healthcare and societal perspectives (Kim et al., 2019). Modern trend in the food technology is production of the functional food that has scientifically defined and described physiological benefits to consumers (Granato et al., 2020). There are used functional food ingredients such as vitamins, proteins, microelements, polyunsaturated fatty acids, antioxidants, polyphenols, probiotics and prebiotics, fructose, and many others. Functional food is developing and marketed for different age groups, patients with specific diseases, and groups with different nutritional requirements or customs (Bogue et al., 2017).

Food for babies and children

Micro- and macronutrients in the food for babies and children is most important factor of their growth and the development of cognitive and psychomotor skills (Gutierrez, 2020). As shown in the cited book, food industry should develop different infant milk formulas, addition of probiotics and prebiotics, specific baby food from cereals, optimization of micro- and macronutrients for special diet regimes to prevent allergies of babies and children to gluten, casein, phenylalanine, intolerance to lactose, to avoid children obesity, and many other specific dietary requirements for babies and children.

So, differentiation and development of functional food for babies and children will remain one of the major trends in the food technologies in 2021–2030.

Food for pregnant women

Food for the vulnerable population of pregnant women is not differentiated too much but there are strict requirements for food safety (Flynn et al., 2019), food avoidances, food additions like protein, iron, folate, and fiber (Siega-Riz et al., 2002), 50 types of food taboos for pregnant women in some countries (Iradukunda, 2020).

So, there are a lot of opportunities for the development and commercialization of new kinds of food for the pregnant women.

Food for elderly people

Food for elderly people should be personalized because their nutrition is age-specific, their food choice is mediated by medicine and health-related pubic information (Herne, 1995), and there are numerous factors important for health of elderly people (Rusu et al., 2020). This food must be personalized by the content of macro- and micro-components that is adapted to personal age-related diseases. The texture of food should be adapted to the chewing and swallowing problems. The food taste should be personally adapted because it is changed with ageing (Laureati et al., 2006). Even in developed countries, significant part of elderly long-term-care home residents are malnourished (Nieuwenhuizen et al. 2010) due to physiological and psychological problems with not personalized food (Rusu et al., 2020).

So, development of food for elderly people should be done as a personalized food or at least as food for the groups of elderly people differentiated by the medical indications.

Food for sportsmen

It is well known that dietary supplements are very popular in sport diet and the doses of these supplements are higher than in the normal diet (Burke and Read, 1993). Sport food

provides enhanced consumption of special nutrients or just more convenient consumption of nutrients for athletes. Very popular is also a sport drink, which is usually balanced by carbohydrates and salts. For example, solution containing glucose, maltodextrins, and electrolytes are suitable for sport activity sources of water, energy, and optimal dosage of potassium and sodium ions to avoid dehydration and at the same time an intestinal absorption of water (Burke and Read, 1993). Another type of a sport drink is a solution containing up to 25% of carbohydrates to support high carbohydrate intake by the athletes. There are used also in sport diet the liquid meals with essential components, for example iron, vitamins, and easily digested components for consumption at conditions when it is not suitable consume the solid food. It is clear that the production and consumption of the food developed for specific sport activity will be increased in 2021–2030.

Military food and ready-to-eat meals

The major trend in military food is production of diverse, specialized or the type of military activity "Meals, Ready-to-Eat" (MREs) including not only food and drinks but also flameless heater and accessories. A MRE contains usually one-third of the Military Recommended Daily Allowance of proteins, carbohydrates, fat, vitamins, and minerals containing 1,250 calories, 13% protein, 36% fat, and 51% carbohydrates (Scott and Albert, 2006). In USA, soldiers can choose from up to 24 entrees, and more than an additional 150 items in the MRE chain and a minimum shelf life at 27 °C is 3.5 years (https://www.goarmy.com/soldier-life/fitness-and-nutrition/components-of-nutrition/meals-ready-to-eat.html). MREs should be developed and produced also for civilians: refugees from violence or military actions region, those who is suffering from poverty, population in the area of natural disaster or great industrial accident, the travelers in air and on land, or just those who prefer to it MRE. There is also known set "First Strike Ration" (FSR) that is used for the whole day and twice easier MRE (https://www.gaydamak.com.ua/suhpaj-armii-ssha-first-strike-ration-sus_24-ru).

Food for Ukrainian soldiers is cooked and supplied by catalogue. There is also used "Daily set of food" or "Suhpay" ("Dry meals" in English) that means set of dry food" (https://portion.com.ua/category/nabory; reibert.info/lots/sutochnyj-polevoj-nabor-produktov-dpnp-p-1-s.309448). However, increase of the production and using of the MREs in Ukraine could be useful not only for the military personnel, but also for civilians, for example travelers. Just for Ukraine, abbreviation of this type of food must be not "MRE" because it means in Ukrainian "Dying", but may be better use something like REM, "Ready-to-Eat Meals".

It could be also commercially viable if the food industry will start production of the diverse, tasty, low-calorie menu of MREs that will restrict intake to approximately 1,400 calories per day for men, and to 1,200 calories per day for women.

So, production of diverse MREs in Ukraine should be widely developed in food industry during 2021–2030.

Microelements-enriched food

Proper nutrition includes recommended daily allowance of essential and probably essential microelements (WHO, 1996) such as zinc, iron, manganese, copper, chromium, iodine, fluorine, selenium, molybdenum, boron, chlorine, nickel, silicon, sodium, cobalt, and strontium (Nieder et al., 2018; WHO, 1996; Mehri, 2020). For example, trivalent chromium is essential for diabetic patients receiving parenteral nutrition; copper and especially zinc are

included in the activity center of many enzymes; selenomethionine and selenocysteine from food participate in the immune responses; thyroid functions, and reproduction; four human enzymes requiring molybdenum as cofactor; iodine is an essential for the functioning of the thyroid hormones; iron and manganese are the essential elements being a component of many metalloenzymes; essential silicon is supplied mainly with the plant foods (Mehri, 2020).

Support of the microelements levels in an organism can be done using dietary diversification, food fortification, and increasing content or availability of microelements in food products. Current and developing trend in the production of the functional foods enriched with microelements is an addition of fruits and vegetables, edible mushrooms, biomass of seaweeds, cultivated microscopic algae, yeasts, or bacteria to conventional food to optimize the content of microelements, as well as vitamins. The food additions and food compositions must be specific for the regions and the customer groups. Usually, the dominant elements to fortify food are iron, calcium, zinc and iodine (Gharibzahedi and Jafari, 2017).

Trace essential elements added to the food can be toxic elements depending on the dosage. Therefore, the food fortification with microelements, vitamins, other food additives must be under strict and obligatory regulations on their content and forms in food (Konikowska and Mandecka, 2018; Poniedziałek et al., 2020).

So, production of microelements-enriched food and nutraceuticals with microelements and vitamins in Ukraine should be widely developed in food industry during 2021–2030.

Selenium-enriched food

Selenium is an essential microelement being cofactor of many enzymes, which are participating in antioxidant defense, immunomodulation, thyroid functioning, and sperm motility (Kora, 2020). Meanwhile, diet in some regions is deficient in selenium. For example, bread enriched with biomass of yeast or plant sprouts with increased content of organic forms of selenium is recommended for the population of North Ukraine, Belorussia, and Poland where acidic soil diminished the content of organic forms of selenium in cereals (Stabnikova et al., 2005; 2008; 2019). Therefore, supplementation of the food with organic form of selenium became a trend in food industry of many countries (Yang and Dong, 2017; Kieliszek, 2019). However, because of the narrow gap between recommended consumption dosage and toxic dosage of selenium and some other microelements it could be better to produce and consume such microelements as nutraceuticals, see below.

Good source of organic forms of selenium as well as other macro- and microelements, antioxidants, vitamins, dietary fibers, unsaturated fatty acids is the biomass of edible fungi (Kora, 2000), biomass of yeasts (Stabnikova et al., 2005; 2008). biomass or extracts of edible seaweeds, which are intensively studied as a functional food addition (Roohinejad et al., 2017; Shennon and Abu-Ghannam, 2019; Corsetto et al., 2020). Kappa carrageenan, agar, alginate and other gelling substances from seaweeds are also used in foods (Rhein-Knudsen et al. 2015; Zollman 2019). It is expected that applications of seaweeds and their components in food technologies will be increased especially in case if seaweed harvesting will be increased on the Pacific seashores of Chili, Canada, and Russia.

Nutraceuticals

Nutraceuticals is a food with some physiological benefits and manufactured usually as the pharmaceutical product, *i.e.* as the capsules, pills, or extracts with clearly defined dosage of pure or at least determined biologically active substances. They are used as dietary

supplements, dietary nutrition for chronic diseases (gastrointestinal, diabetes, cancer) and for clinical nutrition.

Important direction in nutraceutics is production of nutraceutical lipids (Akoh et al., 2017). Lipospheres are used in medicine for drug delivery (Bunjes, 2005). So, preparation of the colloidal lipid nanostructures, usually from phospholipids forming bilayer structures, and the preparation of the nanoparticles of encapsulated or solubilized hydrophobic bioactive compounds (Akhavan et al., 2018; Babazadeh and Ghanbarzadeh, 2017; Huang et al., 2017; Santos et al., 2019) could improve bioaccessibility of the lipids, protect them from oxidation, and fortify the food, for example with lipophilic vitamins or with omega-3 fatty acids (Awad et al., 2009; McClements, 2018). This is very popular direction in nutraceuticals. Lipid-containing nutraceuticals are prepared very often with lycopene, other carotenoids, or quercetin to increase shelf-life of the unsaturated fatty acids of lipids (Huang et al., 2017; Zardini et al., 2018). Lipid-based nutraceuticals can be considered as food that is preventing disease due to anti-inflammatory, wound healing, and other medicinal effects (Shin et al., 2015).

The biomass of seaweeds or their extracts are widely used for production of nutraceuticals. It is known that countries with regular consumption of seaweeds by population have significantly lower than average frequency of dietary-related disease such as type 2 diabetes and obesity as well as some types of cancer (Shannon and Abu-Ghannam, 2019). The seaweed extract increased the oxidative stability of fish oil-loaded capsules with dextran as the main biopolymer wall material (Hermund et al., 2019).

Generally, nutraceuticals are a type of the functional food and should be developed and manufactured on the modern food processing plants in 2021–2030.

Trend 3. Environmentally-friendly and energy-saving food processing

Emerging non-thermal and energy saving processing

There are intensively developing novel food processing technologies that are environmentally-friendly and energy-saving ones. For example, developing of modern food processing technologies limiting the thermal degradation of the biologically active compounds and saving taste and aroma of food are based on cold plasma, pressurized fluids, pulsed electric fields, ohmic heating, radiofrequency electric fields, ultrasonics and megasonics, high hydrostatic pressure, high pressure homogenization, hyperbaric storage, and negative pressure cavitation extraction (Misra et al., 2017). These processes can be not only fast, environmentally-friendly and energy-saving but also can ensure food safety and high nutritional value (Misra et al., 2017).

Cold plasma processing

Plasma is ionized gas containing electrons, ions, neutral molecules, and atoms. A high-temperature plasma is a fully ionized one but in non-thermal, partially ionized temperature plasma remains so low that can be used in biological applications (Sakudo et al., 2019; 2020). Cold plasma is a useful method for inactivation of microorganisms of meats and vegetables, microflora of milk and dairy products, browning enzymes polyphenoloxidase and peroxidases, thus improving food preservation (Thirumdas, 2015; Misra et al., 2016; Coutinho et al., 2018). Cold plasma inactivates the microbial contaminants on both animal and plant food for 3 – 120 s by 10^5 magnitude. It is due to UV light and chemical products

that are accompanying ionization (Niemira, 2012; Pankaj et al., 2018). It can be used also for decontamination of food packaging materials (Pankaj et al., 2014). However, there are still challenges in application of this technology in food industry such as regulatory approval, design of the plasma source, and process control (Keener and Misra, 2016).

High pressure homogenization

Batch or continuous high pressure (HPH, 100–300 MPa) or ultra-high pressure (UHPH, 300–450 MPa) homogenization is performing due to flow of a pressurized fluid through a system that produce strong turbulence, cavitation, and temperature increase. This treatment repeated 5-10 times enhances emulsion stability, diminishes particle size, increases availability of nutritional components, and inactivates microbes (Martínez-Monteagudo et al., 2017; Sevenich and Mathys, 2018; Levy et al., 2020). It can be used also for extraction of bioactive compounds from the foodstuff or food processing waste (Juric et al., 2019), modification of rheological properties of biopolymers (Xie et al., 2018), and food sterilization (Sevenich and Mathys, 2018). However, processing cost of HPH and UHPH is relatively high, from 0.5 to 1.5 €/kg and processing ate is relatively low, approximately 264 kg/h for a system with 55 L vessel (Sevenich and Mathys, 2018). Additionally, food processing regulations in EU (EC No.258/97) requires that each food treated with HPH or UHPH needs to be tested is there equivalent to an existing food in the EU or not (Sevenich and Mathys, 2018).

Pulsed electric fields

Pulsed electric fields (PEF) is a new non-thermal food processing and preservation technology that is acting on cells due to electroporation of cell membrane (Saulis, 2010). It is a non-thermal method that does not deteriorate food quality (Mohamed and Eissa, 2012; Barbosa-Cánovas and Zhang, 2019). Pulsed electric field treatment has positive effect not only for the food pasteurization but also for the extraction, for the drying through decreasing drying temperature or decreasing freezing time (Barba et al., 2015; Sitzmann et al., 2016). Useful food processing technology could be PEF-assisted cold pasteurization of liquid foods (Sitzmann et al., 2016).

Novel extraction technologies

Ultrasound-assisted extraction (UAE) acting by production of the cavitation bubbles in biomaterials is used for in food processing nutraceuticals, methane biogeneration and other biorefinery processes from food and agricultural wastes (Chemat et al., 2017; Wen et al., 2018; Martínez-Patiño et al., 2019). The benefits of UAE are fast extraction, low consumption of energy, and improvement in bioavailability of food components. It is most applicable in extraction of oil, proteins, polysaccharides, polyphenols, natural colorants such as anthocyanin (Pinela et al., 2019), antioxidant phenolic compounds from different plant materials (Chen et al., 2018; Görgüç et al., 2019; Sharayei et al., 2019; del Mar Contreras et al., 2020). UAE is used as a pre-treatment step in the processing of plant-based food, mainly of high-cost raw materials (Vilkhu et al., 2008) and for the extraction of thermo-labile compounds (Medina-Torres and Ayora-Talavera, 2017; Jalili et al., 2018).

There are known also other "green" extraction methods such as microwave-assisted extraction, high-pressure assisted extraction, high voltage electric discharges assisted extraction, pulsed electric fields assisted extraction, supercritical fluids extraction with low

expenditure of energy and solvents (Putnik et al., 2018). These methods are useful for lower cost, non-thermal extraction of biologically active compounds for example antioxidant phenols, vitamins, carotenoids, essential oils, phytosterols, antimicrobial compounds from fruits, berries, and vegetables in production of nutraceuticals and non-caloric sweetener from *Stevia rebaudiana* (Putnik et al., 2018). Natural antioxidants like polyphenols are often extracted from the berries wastes, grape pomaces, citrus and pomelo waste, and use of novel extraction technologies can increase the yield of nutraceutical product by 50%, however industrial innovative solutions for extraction of biologically active compounds are very specific and were not sufficiently tested in food industry yet (Putnik et al., 2018). So, industrialization of these novel extraction technologies is a current task of food sciences.

Novel food-drying technologies

Novel drying technologies such as infrared-assisted freeze drying (Hnin et al., 2019), microwave-assisted convective drying (Kumar and Karim, 2019), and ultrasound-assisted convective drying (Szadzińska et al., 2019) are more energy-saving than conventional freeze drying or convective and often improving food quality. Infrared-assisted freeze drying at 45 – 55ºC could save up to 14% of the drying time and up to 19% of the energy compared to conventional freeze drying (Hnin et al., 2019). The drying time was shorter by up to 64% and energy consumption is lower up to 23% for ultrasound-assisted convective drying as compared to convective drying of raspberries (Szadzińska et al., 2019). Energy saving for microwave-assisted convective drying were even higher, up to 54% as compared to convective drying. Biomimetic technologies such as electronic nose and computer vision altogether with artificial intelligence technologies can significantly improve different drying technologies (Sun et al., 2019).

Membrane distillation of ethanol

The conventional distillation and rectification of ethanol produced by yeast fermentation consume tremendous amounts of energy. Therefore, a lot of technologies have been tested to replace conventional distillation and rectification of ethanol by membrane distillation and rectification (Banat and Simandl, 1999; Curcio and Drioli, 2005). For example, with the feed concentration of ethanol 10 % (w/w), at temperature range of 40–70°C, ethanol selectivity was 2–3.5 for PVDF membrane used for water-ethanol separation (Banat and Simandl, 1999). Economic consideration showed that integrating distillation with membrane- based separation can really reduce the ethanol production cost (Gavahian et al., 2019; Khalid et al., 2019). Ohmic-assisted volume heating and distillation of ethanol has such benefits as saving time and energy (Gavahian et al., 2018; 2019). This technology can be used also for essential oil extraction from plants. The ethanol fermentation can be integrated with the membrane distillation of ethanol so that the productivity of fermentation in the membrane bioreactor was 5.5 g of ethanol/L/h instead of 2.6 g of ethanol/L/h in the reactor without membrane distillation (Gryta, 2001)

Disinfection of equipment

For the cleaning and disinfection of food processing equipment, it must be sequentially cleaned, washed, disinfected and rinsed. Novel physical cleaning methods of equipment are dry-ice cleaning, ice-pigging where ice-water mixture is used to remove and carry off particles from equipment, and ultrasonic vibration to clean the membranes that are used for

filtration (Otto et al., 2011). Hydrogen peroxide 5% solution (Moretro et al., 2019) or electrolyzed water (Tango et al., 2019) are both effective in cleaning and disinfection of food-processing equipment. The sterilization of equipment can be done also by heating, using different liquids mainly phenolic or quaternary ammonium compounds, and using such gaseous chemicals as ethylene oxide and hydrogen peroxide vapor (Chauhan and Jindal, 2020).

The cutting-edge advancements in sterilization of food industry equipment came from the space research because spacecrafts must be not contaminated to avoid investigation problems. Initial thermal treatment of the spacecraft equipment was replaced by carbon dioxide snow cleaning, vapor hydrogen peroxide sterilization, and gamma irradiation sterilization (Gradnini et al, 2019). Cold plasma technology can be used to inactivate pathogens on the surface of food processing equipment (Sakudo et al., 2019; 2020; Katsigiannis et al., 2021).

Food processing plants has to be also cleaned or even disinfected to prevent biocontamination of food with the fungal spores, bacterial cells or spores, and viruses. Equipment and technologies for air disinfection are common for all bioaerosols: aseptic filtering through the fibers, hydrophobic membrane filtration, chemical fogging, ozonation, and UV radiation (Masotti et al., 2019).

Decontamination of fresh vegetables, fruits, and berries

To diminish spoilage of the vegetables, fruits and berries and the risk of infectious diseases and helminthosis this production is washed by solution of chlorine that is giving toxic by-products. Now this practice is prohibited in EC and is replaced with decontamination using hydrogen peroxide, ozone, organic acids, as well as irradiation and ultrasound (Meireles et al., 2016; Deng et al., 2020). Biotechnological products such as polysaccharides, biosurfactants, and probiotics can be used to diminish microbial contamination of fresh vegetables and fruits and to increase significantly shelf-life of vegetables and fruits (Pirog et al., 2019; Gregirchak et al., 2020). However, this direction is not developed yet.

The main point of the decontamination studies is to find optimum between maximum of antimicrobial activity and minimum of produce deterioration (Deng et al., 2020).

Novel food packaging materials and technologies

Disinfection of the packed food and packaging materials, for example using cold plasma or the dielectric barrier discharge plasma (Peng et al., 2020), are also important for extended shelf life.

Nowadays the implementation of logistics packaging systems is an integral part of any production of finished products. Packaging turns product into the commodity. To ensure a synergistic connection of three systems – products, packaging materials, packaging machines is the condition for high-quality packaging operations. Each of these systems develops independently, but during packing features and stages of development of other systems are considered (Dudeja et al., 2016).

A lot of innovative food packaging materials are developed using such conditions as to be convenient for packing and distribution, with extended shelf life, maintained good quality of the products (Majid et al., 2018). Absorption of oxygen in the pack is most important for the long-term storage of the food products (Pasichnyi et al., 2018). Disinfection of the packed food and packaging materials, for example using cold plasma or the dielectric barrier discharge plasma (Peng et al., 2020), are also important for extended shelf life.

Food products as objects of packaging must meet all the requirements of consumers and have the properties necessary for the implementation of the certain technologies of packaging, storage, transportation and sale. Different packaging technologies also give different results in terms of product preservation and waste minimization. A packaged product is a single system of interaction between packaging and product. Packaging creates a separate medium which should be safe for storing products. Therefore, the processes of interaction and the formation of barriers take place between the packaging, the product and the medium (. Mannheim et al., 1990; Svensson, 2003). These processes include:

- Biochemical processes in the product;
- Interaction between internal and external media;
- Interaction between the product and the internal medium;
- Penetration of liquids, steam, gas, sunlight and more from the outside;
- Loss of products or its components;
- Interaction between the external medium and the packaging material;
- The influence of the external medium on the packaging material.

The study and research of these processes make it possible to minimize the waste of packaged products.

The efficiency of logistics involves the performance of a significant number of functions that rely on packaging (Aggarwal, 2020). Key features include:

- Operational feature provides protection of packaged products from mechanical and physico-chemical damages;
- Technological feature ensuresrational, with minimal losses production, storage and transportation of packaged products;
- Ecological feature provides the use of cheap, environmentally friendly, fast-renewable and affordable packaging materials;
- Special feature depends on the properties of the product, its physical condition, shelf life, consumption conditions;
- Sanitary and hygienic features provide neutrality and safety of packaging for the products.

Along with these functions, today it is important to digitalize packaging, i.e. to create intelligent packaging.

The implementation of the sustainable program involves the development of the packaging industry in the direction of a closed cycle economy. Therefore, an important trend in the development of packaging is environmental safety (Makolli, 2019). During the implementation of the program to minimize the flow of packaging waste and their release into the environment, development priorities should be structured according to the principles of 6R (Szaky, 2019):

- Reduce: reduction of used raw materials;
- Redesign: design and development (or redesign) of packaging for reuse or recycling;
- Remove: exclusion of disposable packaging from the use, where it is possible;
- Reuse: reuse or restoration / repair;
- Recycle: closed cycle recycling, where waste is used in the production of the same packaging;
- Recover: removal of useful chemical components or use as a fuel during combustion to generate heat.

The advanced packaging has to be made from the biodegradable materials, and indicate freshness, retard oxidation, prevent microbial growth, use of ethylene and CO_2 scavengers, time-temperature sensor, and release of antioxidants during storage (Majid et al., 2018).

New but with not clear future is the development of edible food packaging to reduce pollution of environment with million tons of disposed macro-plastic and micro-plastic.

The formation of packaging systems with the packaging-product interaction is carried out in packaging machines.

Modern models of packaging equipment are complex technical systems built on the aggregate-modular principle. The trend of development of packaging machines provides that the latest models of such equipment are integrated technical complexes created on the basis of mechatronic functional modules (Kryvoplias-Volodina, 2018), each of which is both functionally and structurally independent product with a large number of synergistically interconnected characteristics and parameters. implementation of packaging technologies.

In recent years, general trends in the development of technology, which provide the restructuring of all areas of human activity, include the packaging industry (Bigliardi, 2021). These trends were called the "Fourth Industrial Revolution" (Chisenga et al, 2020). Therefore, the current packaging industry is characterized by the active introduction of automated packaging.

Creating a new generation of packaging equipment which has flexible structure and is universal for different types of products and packaging materials is the main task today (Smith et al, 1990). Its solution requires a systematic approach, starting with the development of the concept of design of automated production lines of packaging and ending with the design of the working bodies of machines. Such a concept can be the concept of functionally oriented design using mechatronic modules, which allows to create clusters of functional modules, combine them, create a wide range of parametric series of packaging equipment of one functional purpose with a flexible structure of changes in processes at the automated control system and take into account the features of all stages of the life cycle of machines (Kryvoplias-Volodina. et al, 2019).

Logical design combines possible methodologies, techniques and methods of systems for designing new packaging equipment, providing the growing demands of consumers for its technical capabilities.

Trend 4. Biotechnological food processing

Use of enzymes for food processing

Microbial enzymes are used in the food processing more than 60 years but new enzymatic applications for food processing have been found every year. So, this is a developing area of food science and technology.

The hydrolases, first of all proteinases, are most applicable enzymes in food technology. Proteinases from the calve stomach – chymosin (rennin), pepsin, gastriscin – were used as milk coagulant (“calf rennet”) in the cheeses production for centuries (Moschopoulo, 2016). Proteinase from tropical fruit papaya is widely used for meat tenderization and production of protein hydrolysate for a half century (Fernández-Lucas and Castañeda, 2017). However, proteinases from bacteria and fungi have different functions and low cost so they are most widely used at present in food processing industry (Banerjee and Ray, 2017; Kamal et al., 2017; Tavano et al. 2018). Big diversity of proteases is due to their dual participation in metabolism: one part of proteinases control metabolism modifying specific proteins through the hydrolysis of specific peptide bonds, and another part of proteinases degrade proteins for turnover of aminoacids through the hydrolysis of all peptide bonds (Gotiesman and Maurizi, 1992). Specific proteinases can be used in food processing to improve texture of food, flavor

of Brie or Camembert cheeses, bitterness of food, gelation, digestibility of food, to decrease food allergenicity, for example from soybean, pea, chickpea, lentil, or peanut allergens, and to produce bioactive peptides and aminoacids for clinical or sport diet (Tavano et al. 2018). Even milk proteins can produce allergic reactions for children so properly enzymatically hydrolyzed milk protein could be used as a food supplement in these cases (Osborn et al., 2017). There are known also proteinases that are removing inflammation effect of gluten from wheat, rice, and barley. Hydrolysis of gluten by a mixture of specific proteinases, mainly prolyl endoproteases of some microorganisms, is only one way for gluten-free diet that is preventing auto-immune disorder known as celiac disease (Tavano et al. 2018).

Digestion of food proteins by specific proteinases produces bioactive peptides that can be marketed as nutraceuticals with beneficial actions on digestive, immune, or nervous systems (Tavano et al. 2018). For example, hydrolysis of milk casein by specific proteinases produces peptide with opiate-like effect (Silva and Malcata, 2005; Park and Nam, 2015; Chai et al., 2020).

Functions of proteases in food processing can be extended if the enzymes of microbes-extremophiles will be available. For example, the enzymes of thermophilic *Bacillus stearothermophilus* and can be used at processing temperature 70–80 °C, (Kumar et al., 2019), while enzymes of psychrophilic bacteria are active at 0–4 °C (Yadav et al., 2017; Kour et al., 2019) and can be used to hydrolyze fish, pork, and shrimp meat at 0 °C.

There are many commercial proteases for food industry: Alcalase, a mixture of alkaline proteases, Flavourzyme, containing a mixture of alkaline and neutral proteases, Thermolysin, a thermostable proteinase, but their spectrum in not sufficient for diverse possible applications in food processing industry (Tavano et al. 2018).

Transglutaminases are family of enzymes crosslinking glutamine of one protein molecule and lysine of another protein molecule by the formation of amid (isopeptide) bond and finally resulting in protein polymerization (Rachel and Pelletier, 2013). This ability of transglutaminases for crosslinking of protein molecules, especially collagen that was denaturated at a high temperature, is used in food industry for the meat hydrogels production and to alter the texture of meat (Savoca et al., 2018; Duarte et al., 2020). There are producing affordable microbial transglutaminase for the food industry (Wand et al., 2018).

Transglutaminases are used at present in the cheese manufacturing, meat processing, in the production of edible films from milk protein, and there are wide opportunities to use these enzymes to improve the firmness, viscosity, elasticity, and water-holding capacity of food products. For example, transglutaminase improves the quality of flour and the texture of bread or pasta, forms a texture of the minced meat, forms from gelatin low calorie food with good texture and elasticity, increases hardness of fish paste (Kieliszek and Misiewicz, 2014; Duarte et al., 2020). However, application of transglutaminase could be also a way to produce a false food from the low-quality raw materials or even from the food-processing wastes.

Use of phytases for food processing is due to the role of phytate, a dihydrogenphosphate ester of inositol, as a storage of phosphate in the major food staff such as cereals and legumes. Phosphate of phytate in this food binds calcium, iron, zinc, and other essential dietary minerals. Phytase removes phosphate from phytate thus preventing mineral starvation. Therefore, it is used in human nutrition and food processing to increase bioavailability of minerals (Herrmann et l., 2019; Handa et al., 2020). Industrially produced bacterial (Kumar et al., 2017) or fungal (Jatuwong et al., 2020) phytases are essential feed and food additives. It is especially important for vegetarian food because phytate phosphorus is not available for human (Jatuwong et al., 2020). However, applications of phytase for the food enhancement in Ukraine are still rare.

Microbial β-galactosidase is used for hydrolysis of lactose in milk because of lactose intolerance in the part of human population. This enzyme is also used for the production of lactose-based sweeteners from the effluents of cheese production. There are known also thermostable or psychrophilic β-galactosidase for the treatment of hot or cold milk (Xavier et al., 2018). Enzyme α-galactosidase is used in food industry to hydrolyze galactooligosaccharides such as raffinose, melibiose, stachyose, galactomannans and galacto-glucomannans in soymilk and before sugar crystallization process (Bhatia et al., 2020). Bacterial and fungal amylases are often used in food processing for hydrolysis of starch in alcohol fermentation, juice production, bakery. Pectinases are used mainly in wine and juice production to increase yield and quality of juices (Tapre and Jain, 2014; Habrylo et l., 2018; Sudeep et a., 2020).

The wide and increasing range of food processing applications of enzymes require the search of new enzymes and their producents. So, the trend of enzymes application in food processing will remain as actual one in 2021–2030.

Use of alive microorganisms for food processing

Food processing technologies with applications of alive microorganisms for the food and beverages fermentation originated from about 14000 years ago (Marco et al., 2021). All these fermentation technologies like beer, wine, cheese, pickled vegetables, fish and soybean sources production are existing and used at present, but they are enhancing with application of pure and starter cultures of microorganisms, probiotic strains and strains producing antimicrobial metabolites and peptides (Camargo et al., 2018). Microorganisms make different tastes during food fermentation (Tavano et al. 2018), form food preservatives, and produce bioactive peptides with numerous health effects such as antihypertensive, antioxidant, antimicrobial, opiate-like, anti-inflammatory, anticancer/antiproliferative, antithrombotic, hypolipidemic, hypocholesterolemic, etc. properties that can be used in the production of functional food and nutraceuticals (Chai et al., 2020).

Use of probiotics that are selected alive bacteria or yeasts that are used in food or as medical composition (Arevalo-Villena and Briones-Perez, 2017; Marco et al., 2021) originated about 30 years ago. The probiotics with immunomodulation properties, modulating gut microbial community, with different positive health effects is a major trend in the functional food with alive microorganisms (Jankovic et al., 2010; Bajaj et al., 2015; Voitenko and Voitenko, 2021). Modern approaches to probiotic functional food are the symbiotic combinations to stimulate the growth of probiotics (Terpou et al., 2019), production of bioactive compounds by probiotics introduced in food products (Chugh and Kamal-Eldin, 2020), addition to food both bacterial probiotics and their prebiotics such as inulin, fructooligosaccharides, galactooligosaccharides to develop functional products with improved texture (Guimaraes et al., 2020), using probiotics for mitigation of genotoxic and carcinogenic acrylamide that is formed during heating of food (Khorshidian et al., 2020), the production of postbiotics that are metabolites with beneficial functions in different human organs, for example production by probiotic lactic acid bacteria of gamma-aminobutyric acid that connected with the prevention of neural disease, diabetes, cancer, immunological disorders, and asthma (Diez-Gutiérrez et al., 2020). New functions of probiotic and food processing technologies including alive microorganisms will be developed in 2021 – 2030.

Validation of food processing wastes

Food wastes were often used as a soil fertilizer (Stabnikova et al., 2009). However, the modern trend a validation of green food processing including utilization of wastes for other food, nutraceuticals, or the mushroom cultivation. For example, grape pomace can be used for production of nutraceuticals containing antioxidants and the mushroom cultivation (Sirohi et al, 2020). Whey proteins can be used for production of bioactive peptides with the health benefits in the immune, cardiovascular, nervous and gastrointestinal systems (Dullius et al., 2018). The industrial biowastes such as peels and seeds of vegetables can be used for the production of carotenoids to enhance quality of macaroni (Martins and Ferreira, 2017). Every on-farm plant processing releases enormous quantity of wastes. For example, processing of cocoa beans from which confectionery for US$47 billion is producing, releases 80% of raw material as a waste which is disposed for soil fertilization giving putrid odors and increasing plant diseases. Meanwhile, the cocoa by-products can be transformed to the food, pharmaceuticals and cosmetics (Vasquez et al., 2019). Chicken feet can be used for enzyme-mediated production of 180–380 kg of food gelatine from 1 ton of dry waste (Mokrejs et al., 2019). Potato-processing wastes can be transformed using biotechnological methods to proteins, lipids, food-processing enzymes, and food organic acids (Javed et al., 2019; Kot et al., 2020).

Spent yeasts from beer production can be used for production of yeast extract containing a lot of edible and biologically active components (Jacob et al., 2019). There are many other examples of food waste validation (Stabnikova et al., 2010), so this trend will be just increased in 2021–2030.

New food sources

Meat is not environmentally friendly food because of the energy and material losses during the trophic chain from plants to animals and finally to human, and due to release of greenhouse gas emissions to atmosphere from livestock and poultry. However, significant part of population considers meat as the most delicious food. So, new type of food, plant-based meat that is made from the plant protein, is produced at present and the scale will be increased to satisfy the tastes and nutritional quality.

Another environmentally sustainable potential source of food is "single-cell food", *i.e.* proteins, lipids, carbohydrates, and vitamins of cultivated microscopic algae, yeasts, bacteria, and even cells of plants or animals that can produce protein from carbohydrates by hundred or thousand times faster than animals.

Micro and macroalgae are good sources of food and now biomass of *Spirulina* and *Chlorella* from some producers have GRAS ("Generally Recognized As Safe") designation. This food industry requires extraction of the healthy bioavailable components of algal biomass for the production of functional food or nutraceuticals (Wells et al., 2017; Caporgno and Mathys, 2018; Junior et al., 2020; Kusmayadi et al., 2021). Many species, especially among psychrophilic algae, contain lipids with polyunsaturated omega-3 fatty acids (Dhanya et al., 2020; Stokes et al., 2020) that can be used in nutraceuticals. Commercial biotechnological applications are known for such microalgae as biomass of *Dunaliella salina* containing up to 3.5% of beta-carotene, *Scenedesmus almeriensis* containing 0.30% of carotenoid lutein, *Chlorella vulgaris* containing 45% of protein, *Nannocholoropsis sp.* producing carotenoid astaxanthin and omega-3 fatty acids, and representatives from the genera *Botryococcus, Chlamydomonas,* and *Arthrospira* (marketed as *Spirulina*) (Caporgno and Mathys, 2018; Molino et al. 2018; Junior et al., 2020). There are still many problems in

"single-cell food", for example excessive content of nucleic acids and low digestibility of cell walls, but in every case this direction of new food production will be developed

Edible insects are another unusual source of food (de Carvalho et al., 2020; Van Huis, 2020). The market of the protein food from the edible insects will be increasing with forecast up to US$4.6 billion in 2027, especially if The European Food Safety Authority will approve the sale of insects: ground mealworms, lesser mealworms, locusts, crickets, and grasshoppers for human consumption, as it is expected by business (Meticulous Research, 2020). However, after admission of the edible insects to the market, there must be developed proper rules to assure consumers of their benefits and safety (Belluco et al., 2017). Entomophagy is not attractive for European and American cultures, so insect food can be consumed there as a nutritional powder addition to the conventional food. For example, biomass of 2000 edible species of insects can be used as a source of iron and zinc in human nutrition (Mwangi et al., 2018).

Notwithstanding the negative public perception, the food from genetically modified organisms (GMO) will be developed further because it can have higher levels of essential and valuable nutrients, and better taste. Moreover, with the new CRISPR method of gene editing it will be possible to create the genetic variants of plants and animals that will be the revolutionary sources of conventional and functional food. However, there must be also created and used the revolutionary methods of molecular-biological control for this new GMO food.

Trend 5. Personalization of food processing and distribution

Nutritional needs of the medical, racial, religious, and regional customer groups

Types of food and dietary habits are tightly connected with culture. To increase consumption of healthy food not only political or technological decisions have to be made but also optimization of the diet and related food production for the specific age, ethnical, medical, racial, religious, or regional group of the customers. Some of this topics are discussed in the above section "Trend 2. Functional food and nutraceuticals ". These differentiations will be more scientifically specified and their production and sales should be increased in 2021 – 2030.

Computerization of the personalized food production and consumption

Nutrition-related mobile applications became of common tool of the human nutrition (Flaherty et al., 2018; Ahmad et al., 2020). They are calculating right now mainly calories of the food to avoid obesity. There were screened 628 dietary guidance in China, and 75% of them were focused on energy intake and only 23% advised dietary structure. Many applications were developed for health management and some of them have social communication tools (Li et al., 2019). So, it is possible that very soon we will select the food in the supermarket that were optimized for the personal diet using mobile tool or computerized order of the food from home. Food production and retail will be totally changed due to digital short-term and long-term personalization of the food consumption.

Consumer acceptance of a new food

However, the problem of new food technologies is consumer acceptance of a new food or computerized optimization of the diet (Priyadarshini et al., 2019). To ensure commercial adoption of new food products (Santeramo et al., 2018), the consumer acceptance of new food technology and food product is the most important factor (Priyadarshini et al., 2019; Meijer et al., 2021). The consumers, usually, are hesitant to accept new food ("food neophobia") even the novel food technologies are important for the health diet, food safety and sustainability (Siegrist and Hartmann, 2020). Consumers often rely in their evaluation the naturalness of new food product of food technology due to lack of the food engineering and technology knowledge (Siegrist and Hartmann, 2020). So, the trend is the development of new food and new food technology accounting all aspects of consumer acceptance: from agriculture and farming to processing, storage and distribution of a new food, its ecological and environmental sustainability aspects, cultural and religious factors, functions in healthy or medical diet, plus some personal attractions of new food.

3D printing of food

3D printing of food could be considered as one approach in the personalization of nutrition, customized food designs, and simplification of food supply chains. It could be more expensive than conventional food products but it will satisfy personal taste, aroma, texture, diet components, a view of food, an artistic impression from the food, and a way of personal food consumption (Nachal et al., 2019). So, it is used as military and space food, and specific diet food (Liu et al., 2017).

Important and not solved yet technological points are the accuracy of printing of colorful and multi-flavor food; development of printable food materials, post-processing of food 3-D print such as cooking, drying, fast cooling technology (Liu et al., 2017; He et al., 2020). Plant-based 3-D printed food can be made by the ink of cell suspension with alginate that is cured with calcium ions after printing to form a rigid gel (Park et al., 2020). 3-D printing food based on protein, starch and fiber-rich materials showing uniformity of extrusion as well as the precision and stability of the printed pattern. The best printing precision, shape stability after printing and after post-printing oven drying shown a semi-skimmed milk powder-based paste (Lille et al., 2018). However, consumers attitude toward 3D-printed food is not clear because it is not clear yet safety and benefits of 3D-printed food (Brunner et al., 2018). This direction of food technology is just starting.

Commercial food became so diverse in 2021–2030 that the specific nutritional computer programs with the comprehensive information on this food as well as personal diet requirements will be used for the optimization of the production and delivery of the personal-specified food.

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In-vitro functional efficacy of extracts from Caucasian Rhododendron (*Rhododendron caucasicum*) and Rkatsiteli wines as pancreatic lipase inhibitors

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Keywords:

Wine
Caucasian Rhododendron
Rkatsiteli
Orlistat
Antioxidant
Pancreatic lipase

Article history:

Received 20.09.2020
Received in revised form 11.01.2020
Accepted 25.03.2021

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DOI:
10.24263/2304-974X-2021-10-1-4

Abstract

Introduction. The aim of the research is to determine the inhibitory activities of Caucasian Rhododendron *(Rhododendron caucasicum)* and Rkatsiteli wines against pancreatic lipase.

Materials and methods. The leaves of Caucasian Rhododendron were collected in the Upper Svaneti region. Wines were made of Rkatsiteli grape variety. Titrimetric method was used to determine lipase activity, total phenolic content (TPC), and Ferric reducing ability of plasma (FRAP) were determined spectrophotometrically.

Results and discussion. We could demonstrate in this research project a high correlation between TPC and antioxidant activity (AOA) in all samples. Pearson's correlation coefficient (R^2) for the Caucasian Rhododendron samples and wine samples were 0.9758 and 0.9556, respectively. The average TPC in Caucasian Rhododendron was found to vary from 13.00±0.48 to 19.48±0.84 % Gallic acid equivalent (GAE) based on dry matter content. The 3-rd sample of Caucasian Rhododendron revealed the highest TPC, 19.48±0.84 % GAE, and possessed an AOA of 16.10±0.32. No significant difference was observed between the third and first sample of 17.97±0.42% GAE and 15.35±0.74 AOA ($p<0.05$). Even though the fourth sample showed the lowest TPC and AOA, its lipase inhibitory activity closely resembled Orlistat. t seems that polyphenol, which is most responsible for anti-lipase activity of Caucasian Rhododendron is easily oxidised in the air. Consequently, similar technology to green tea processing allows retaining most of the polyphenol in the vine sample. In the rest of the samples, this substance underwent oxidation by molecular oxygen. These results indicated that the treatment of Rhododendron samples could influence the composition of bioactive compounds. The results obtained herein allow one to conclude that white wines made with Kakhetian technology are rich with bioactive compounds and possess higher antioxidant activity and Lipase inhibitory activity when compared to wines made with European technology.

Conclusion. Extracts from Caucasian Rhododendron can act as a promising natural inhibitor and reduce dietary cholesterol' absorption. Based on a dry matter content, Caucasian Rhododendron offered better inhibitory activity than white wine samples.

Introduction

The link between obesity and the intake of a lipid-rich diet (Bray and Popkin, 1998; Hariri and Thibault, 2010) raised attention towards inhibition of pancreatic lipase (P.L.) (Tian-Tian et al., 2020) P.L. is an enzyme that plays a central role in lipid digestion, it breaks down the food source's oil into fatty acids and glycerol that can be easily absorbed and digested by intestines (Lowe, 1997). Using lipase inhibitors to reduce dietary fat absorption and develop anti-obesity agent is an attractive approach, and currently, one of the main strategies in the management and treatment of obesity (Apovian et al., 2015; McCafferty et al., 2020). Lipase inhibitors have been proven to be relatively safe and have been identified as a rational and valid target of the molecular level to control obesity (Kaumar and Chauhan, 2021). Despite this fact, currently, only Orlistat® (Xenical), a hydrogenated derivative of Lipstatin, that inhibits lipase activity (Heck et al., 2000), has been approved in clinical use for the management and treatment of obesity (Bogarin and Chanoine, 2009; McClendon et al., 2009). Since the clinical utility of orlistat is limited (Cruz-Hernandez et al., 2010; Filippatos et al., 2008) and obesity remains a global health issue (Rössner, 2002; WHO, 2020), the search for new natural substances that show potent inhibitory activity against P.L. and have fewer side effects remains topical (Birari et al., 2007; De la Garza et al., 2011).

Large numbers of plants are being screened for potentially lipase inhibitory activity and a variety of phytochemicals have been identified, such as polysaccharides, polyphenols, terpene trilactones, alkaloids, saponins, and carotenoids (Bajes et al., 2020). A great deal of research showed that the class of polyphenols represents one of the most important sources of potential P.L. inhibitors (Buchholz and Melzig, 2015; Martinez-Gonzalez et al., 2017) P.L. inhibition is being reported by numerous polyphenolic compound-rich foodstuffs, including medicinal plants (Seyedan et al., 2015; Zheng et al., 2010), berries (McDougall et al., 2009, Sosnowska et al., 2018), cocoa (Gu et al., 2011), tea (Glisan et al., 2017; Gondoin et al., 2010), grape seeds ((Moreno et al., 2003, Tian et al., 2010), etc.

Additionally, Paraguariensis leaves, popularly known as Yerba Mate beverages, have been reported to have biological activities and considered as a potent anti-obesity reagent (probably, due to their high content of total phenolics) (Kim et al., 2015). Because the mate's raw material is not growing in Georgia, several studies have been conducted to discover the possibility of obtaining yerba mate from the plant *Rhododendron caucasicum*, or Caucasian Rhododendron) (Megrelidze et al., 2020; Melkadze and Kereselidze, 2010). These studies have proved that Paraguariensis leaves can be replaced by *Rhododendron caucasicum*, since they are similar in chemical composition and health effects (both positive and penalty ones). However, there have been no data published regarding anti-lipase activity, total phenolic content (TPC) and antioxidant activity (AOA) of Caucasian Rhododendron itself.

In this regard, the aim of the present study is to investigate a new agent (Caucasian Rhododendron) for its ability to impair digestion and assimilation of dietary fat and to determine TPC and AOA of it. According to the published data, Georgian wines have also displayed a relationship between total phenolic content and their inhibitory activity against P.L. (Gulua et al., 2018). Therefore, this research aims to compare the anti-lipase activity and bioactive compound contents between Caucasian Rhododendron's extracts and white wines made from Rkatsiteli, which is the leading white grape variety in Georgia (Robinson et al., 2012) and to assess their potential use in the management of obesity compared to Orlistat.

Materials and methods

Chemicals

Ascorbic acid, Olive Oil, Sodium Hydroxide, Potassium dihydrogen Phosphate, Folin-Ciocalteu reagent, Detergent Tween 80, Sodium carbonate, Ethyl acetate and methanol were purchased from Sigma – Aldrich (Steinheim, Germany), TPTZ-2,4,6-Tris (2-pyridyl)-s-triazine (Sigma – Aldrich, Switzerland), hydrochloric acid, formic acid and phosphoric acid were provided by Merck (Darmstadt, Germany), Lipase concentrate – H.P. was purchased for Integrative Therapeutics, LLC (USA). Orlistat® (trade name Xenical) by Roche (Italy) was purchased at the local pharmacy. All other reagents were commercially available at the local market and were of analytical grades.

Materials – Sample collection

Wine samples

Four commercially produced white dry wines (see table 1), made from autochthonous and leading white grape variety (Rkatsiteli) grown in the region of Kakheti, were chosen. The wine samples for the experiment were chosen at random. The wines, packed in glass bottles, were purchased from the local supermarket and stored at room temperature until being analysed. We did that because it is as consumers would do, promising in vivo potent lipase inhibitory activity can be the definite factor behind consumer decision making.

Table 1

Rkatsiteli Wine samples

Name of the bottle	Vintage	Alcoholic strength %	Technological treatment
Vine Ponto – Rkatsiteli white dry	2016	12.5	Qvevri and Oak Barrel technology
Glekhuri – Rkatsiteli Qvevri;	2017	13	Qvevri technology
Vaziani, Rkatsiteli	2016	12.5	Classic and Oak Barrel technology
Kindzmarauli Marani Rkatsiteli	2018	13	Classic technology

Caucasian Rhododendron samples

Caucasian Rhododendron (Rhododendron caucasicum) samples were collected in the Upper Svaneti region, in the village of Ushguli (42.917797°N 43.015672°E), at an altitude of 2100 m. The samples were picked during the harvest-time, June 10-20, 2020; mainly 3-4th leaves were collected.

Caucasian Rhododendron sample preparation

The samples were treated with four different processing methods, as follows:

1. Sun-dried: samples were drying for 5 days and nights at the average daytime temperature 27–28 °C;

2. Shade-dried: drying lasted for 12 days and the average daily temperature was 16–17 °C;
3. The Classical technological scheme of black tea processing, including withering at room temperature, rolling, fermentation and drying (Samarasingham, 2009).
4. The classical technical method of green tea processing, including fixation with roasting, thermal treatment rolling and drying (Singh et al., 2014).

Preparation of Caucasian Rhododendron extracts

The Caucasian Rhododendron extracts were prepared by extracting 3 g of dried samples in 300 mL of boiling deionised water and infusing for 15 min. Subsequently, the extract was gently stirred, filtered under vacuum, cooled down to room temperature, and the final volume was brought up to 500 mL with cooled deionised water. Extracts were stored at +4 °C for the analyses.

Determination of moisture content (%)

Moisture content in dried leaves of Caucasian Rhododendron was determined with a drying subsample (2g) at 105°C to constant weight. SFY-20 infrared rapid moisture tester (Hangzhou Hengqing Technology Co., LTD, China) was used for quick and reliable determination of the moisture content of samples.

Total dry matter

For measurement of non-volatile dry matter, a 50 mL sample of wine and tea extracts were aliquoted into a porcelain dish. Extracts were filtered initially. The dish was then placed onto a boiling water bath until the evaporation of water, alcohol (in case of wine), and other volatile compounds had occurred. The residual moisture was then evaporated from the samples by oven drying at 105^0C for 16h. Total dry matter was determined gravimetrically as the residue remaining after drying.

Determination of total phenolic content (TPC)

The total phenolic content (TPC) was determined spectrophotometrically (UV 1609, A&E Lab Co LTD, U.K), using gallic acid (G.A.) as standard, according to the method described by the International Organization for Standardization ISO 14502-1 (ISO, 2005).

Briefly, the diluted sample extract (1 mL) and diluted G.A. working standard solutions (10-50 μg mL^{-1}) were pipetted into separate disposable test tubes. Additionally, 5 mL of (1/10) diluted Folin-Ciocalteu phenol reagent in water was added into each tube. 8 min after, 7.5% (w/v) of Sodium Carbonate solution (4mL) was added into each test tube. The mixtures were mixed well, and the tubes were allowed to stand for another 60 minutes at room temperature. Then their optical densities against the water were measured at 765 nm, with a 10 mm path length cell.

The calibration curve of absorbance vs concentration of a standard solution (Pearson's correlation coefficient: r^2 = 0.9918) was used to quantify TPC content. Results were expressed as gallic acid equivalents (GAE) in mg/L of wine and in g/100 g of the dried matter of Caucasian Rhododendron.

Ferric reducing ability of plasma (FRAP) assay for total antioxidant activity

Ferric reducing ability of plasma (FRAP) assay has been applied for the evaluation of the total antioxidant activity (AOA), according to Benzie and Strain, 1996, with slight modifications. The working FRAP reagent was prepared freshly by mixing acetate buffer (300 mM, pH 3.6), 2,4,6- tripyridyl-s-triazine (TPTZ) solution (10 mM, dissolved in 40 mM of HCl) and Ferric Chloride solution (20 mM) in the ratio 10:1:1. The FRAP reagent and vitamin C (1mM) were separately incubated for 15 min at 37 ^{0}C. 3 mL of working reagent was mixed with 100 microliters of the diluted sample. Ascorbic acid was used as a standard. The reduction was monitored at 593 nm, and the absorbance was recorded after 4 min. FRAP values of samples were compared to that of ascorbic acid and expressed as vitamin C equivalents per 100 g of dry matter of Rhododendron and mg per 1 litre of wine.

Determination of Lipase inhibitory activity

Titrimetric assay method was used to determine lipase activity as reported by Stoytcheva et al., 2012, with minor modifications.

Briefly, the initial reaction mixture consisted of 2.5 mL of deionised water, 1 mL 200 mM Tris HCl buffer (pH 7.2), 3 mL of olive oil, and 0.5 mL of detergent (Tween 80). To obtain a good result, the solution was vigorously mixed on a magnetic stirrer for 15 min. Subsequently, 110 mg of the lipase concentrate was then added to the emulsified mixture, which was then incubated at 37 ^{0}C for exactly 30 min. At the end of the incubation, 3 mL of 95% ethanol was added, and the final reaction mixture was titrated with 50 mM NaOH until the value of pH 9 at automatic titrator (ZDJ-4A, INESA Scientific Instrument Co., Ltd, Anting Shanghai, China) was achieved. Blank titration was carried out as above, but without lipase, in test samples potent inhibitors were involved. One unit of lipase activity is defined as the amount of enzyme that hydrolyses 1.0 micro equivalent of fatty acid from a triglyceride in one hour at pH 7.2 at 37 °C. Lipase activity was calculated using following equation:

$$\text{Lipase Units} = \frac{(A - B)\ (1000)\ (2)\ (DF)}{(1)}$$

where A = volume of 50 mM NaOH consumed by the test sample in mL;
B = volume of 50 mM NaOH consumed by the blank sample in mL;
1000 = conversion factor from milli equivalents to micro equivalents;
2 = time conversion factor from 30 min to 1 hour;
DF = dilution factor
1 = Volume (in millilitre) of enzyme used

The percentage of inhibition was calculated in the presence and absence of inhibitors. Orlistat was used as a standard inhibitor. Lipase activity was measured in the presence of Orlistat (10mg) and the percent of inhibition was calculated per 1 mg of Orlistat.

To measure the percentage of lipase inhibition 1 mL of potent inhibitors (Caucasian Rhododendron extracts and Rkatsiteli wines) were added separately to the initial mixture and the following procedures were identical to those described previously. The effect of inhibition of the samples was calculated as the percent of Orlistat inhibition value.

Statistical analysis

The data represents the mean of a minimum three replicates ± standard deviation (S.D.). Data were subjected to the one-way ANOVA and Tukey's HSD tests. One-way analysis of variance (ANOVA) was done to analyse the significance in the variation of the means between the experimental samples. Tukey's HSD test was used to differentiate between the mean values. All calculations were performed with Microsoft Excel for Microsoft 365 MSO with PHstat 2 version 3.11add-in assistance.

Results and discussion

Moisture content (%) of the Caucasian Rhododendron dried samples

The moisture content of the Rhododendron samples was varying from 7.82±0.52 up to 8.71±0.65 %. In particular, the moisture content of the sun-dried sample was equal to 7.82% ±0.52, shade-dried – 8.63% ±0.18. The moisture content of the sample obtained by black tea making technology was equal to 8.21% ±0.74, while that of the sample obtained by green tea technology was 8.71% ±0.65 (Table 2).

Table 2

Moisture content (%) of the Caucasian Rhododendron dried samples

Sample name	Moisture content (%)
1. Sun-dried	7.82 ±0.52
2. Shade-dried	8.63 ±0.18
3. Black tea like processing technology	8.21±0.74
4. Green tea like processing technology	8.71±0.65

Despite the different treatments, as shown in Table 2, there was no statistically significant difference between the samples' moisture contents.

Total phenolic content

As shown in Figure 1, the average total phenolic content in Caucasian Rhododendron was found to vary from 13.00±0.48 to 19.48±0.84 % GAE based on dry matter content. The highest TPC 19.48±0.84 % GAE was found in Caucasian Rhododendron, which was processed with the classical technological scheme of black tea. Rhododendron treated with green tea like processing technology showed the lowest TPC 13.00±0.48 % GAE, Sun-dried and shade-dried Rhododendron obtained 17.97±0.42 and 15.32±0.55 % GAEs respectively.

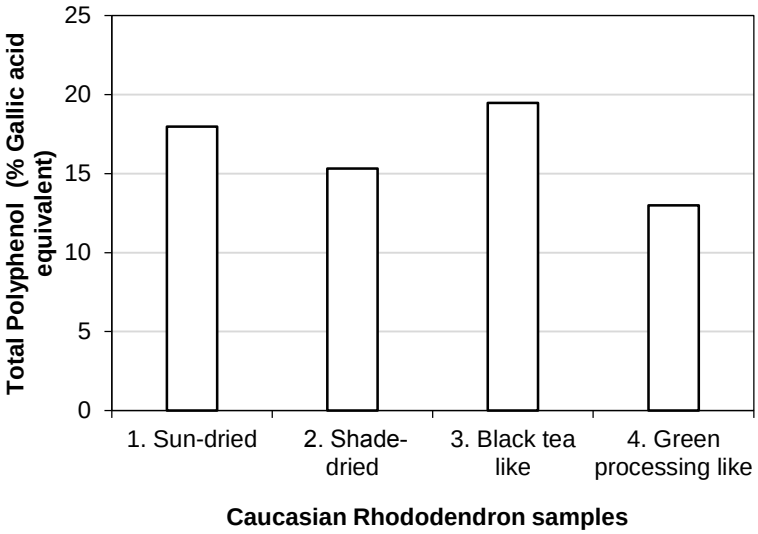

Figure 1. Total phenolic content of Rhododendron dried samples based on the dry matter content

The phenolics have been oxidised during the drying process. Although fewer phenolics have been oxidised during sun drying than shade drying. This can be explained by the difference in the drying time duration. The roasting process destroyed more phenolics than by enzymatic oxidation in the black tea like processing. The results reported by Bastos et al., 2007 and Prasanna et al., 2018 showed that the roasting process leads to a significant alteration of major bioactive and antioxidant activities in all leafy vegetables and yerba mate beverages tested.

Most phenolics were probably lost due to insufficient inactivation of the enzyme phenolic oxidase (PPO) during the fixation process. Also, part of the phenolics was non-enzymatically oxidised during the processing. The amount of phenolics oxidised by PPO in black tea like processed leaves was lower than in green tea like processed Rhododendron leaves. There was no significant difference between the 1st and 3rd samples ($p < 0.05$).

Total phenolic content in wine

As Figure 2 shows, phenolic content in wines was statistically significantly different. The highest phenolic content was found in the Rkatsiteli sample from brand Qvevri, 2901.626±34.648 mg/L GAE. The phenolic content of brand Vine Ponto (2515.447 (± 137.972) was higher than that obtained from brands Vaziani and Qindzmarauli Marani, 489.577 (± 36.112) and 190.243 (±11.498) mg/L GAE respectively.

As seen from the table 1, wines differed by processing technology, they were processed by classic (European) technology and Kakhetian Qvevri technology. According to the Kakhetian Qvevri technology, grapes along with other parts i.e., cluster (stem, skin, seeds) are crushed in a juicer, then placed and sealed in a fermentation vessel called Qvevri, which is dug in the ground (UNESCO, 2013).

During fermentation, phenolic compounds are extracted in large quantities from the stem, peel and grapes, which explains the reason why wines of Kakhetian type showed the highest phenolic content than those of European type. Similar results were reported by A. Shalashvili et al., 2010.

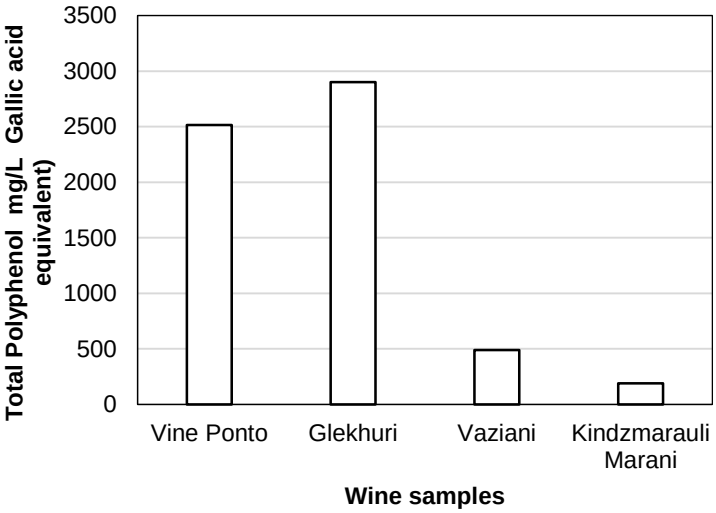

Figure 2. Total phenolic content in wine samples mg/L Gallic acid equivalent

Ferric reducing ability of plasma FRAP

FRAP assay (Figure 3) showed that a 3-rd sample of Caucasian Rhododendron revealed the highest AOA 16.10±0.32. However, no significant difference was observed between the third and first sample of 15.35±0.74 ($p < 0.05$). These samples were followed by the 2nd sample 11.03±0.53 and the 4th sample had the lowest antioxidant activity (AOA) 8.93±0.19.

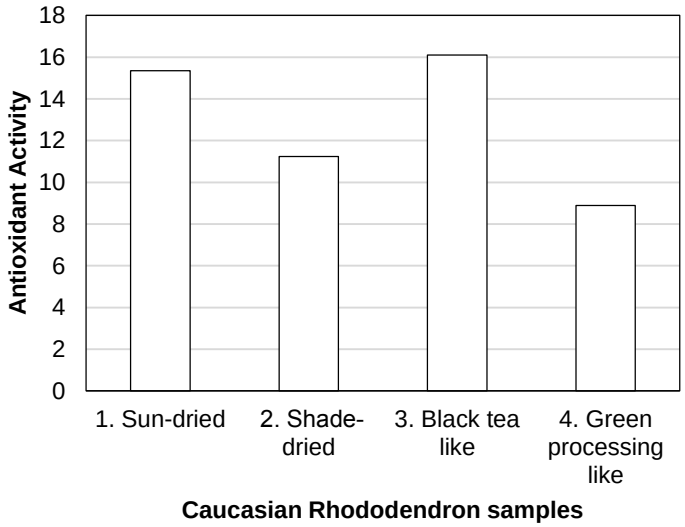

Figure 3. Antioxidant activity of Caucasian Rhododendron samples based on the dry matter content (g/100 g)

For the wines studied herein, Wines made with Kakhetian technology possess noticeably higher antioxidant activity compared to those made with European technology (Figure 4). The highest AOA was found in the Rkatsiteli sample from brand Vine Ponto, 2413.275±53.247 mg/L. The antioxidant activity of brand Qvevri (2177.584 (±130.730) was higher than that obtained from brands Qindzmaraulis Marani and Vaziani, 199.825 (±53.247) and 179.330 (±62.121) mg/L, respectively.

The wines fermented in qvevri and then moved to oak barrels showed the highest AOA, compared to those that have been fermented and stored in Qvevri. These results are in good agreement with results published by Shalasvili et al., 2010 and Tauchen et al., 2015. Tauchen et al. compared the Antioxidant effect and phenolics content of different wines. According to this research, among white wines, Georgian wines possessed significantly higher antioxidant activity in comparison with white wines prepared by the standard European method. This also can be explained by different processing technology.

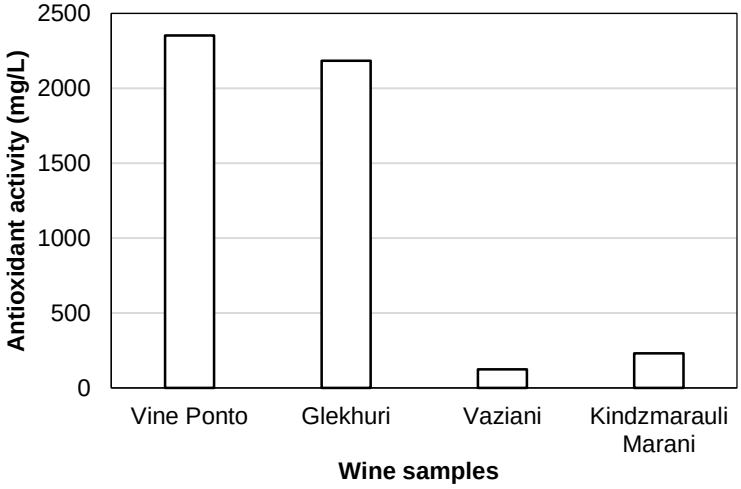

Figure 4. Antioxidant activity mg/L, by brand of Rkatsiteli

Anti-lipase activity of samples

The anti-lipase activities (effect of inhibition) of 1 mg dried samples of Caucasian Rhododendron are shown in Table 3. The anti-lipase activities (effect of inhibition) were calculated as the percent of 1mg Orlistat inhibition value. Orlistat® itself (10 mg) showed 75.84% inhibition of lipase activity.

Table 3

Effect of inhibition per 1 mg dry matter of Caucasian Rhododendron samples as the percent of Orlistat inhibition value

Treating technology of Caucasian Rhododendron samples	**Effect of inhibition as the percent of Orlistat inhibition value**
Sun-dried	69.9
Shade-dried	85.35
Black tea like processing technology	86.15
Green tea like processing technology	99.63

As it can be seen from Table 2, green tea-like processing technology treated samples showed the highest anti-lipase activity. Moreover, the effect of inhibition was almost equal to the one of Orlistat. It seems that phenolic, which is most responsible for anti-lipase activity of Caucasian Rhododendron, is easily oxidised in the air; therefore, most of this substance was retained in the sample made by green tea like processing technology. Regarding the other samples, this substance underwent oxidation by molecular oxygen.

The effects of inhibition of 1 mL wine samples depicted as the percent of 1 mg Orlistat inhibition value are shown in Table 3.

Table 4*

Effect of inhibition of white wine samples as the percent of Orlistat inhibition value

Wine producer	Effect of inhibition as the percent of Orlistat inhibition value*
Vine Ponto	26.75
Qvevri Glekhuri	20.5
Vaziani	4.91
Kindzmarauli Marani	14.91

*- Inhibition by wines was calculated as per 1 mL, inhibition by Orlistat was calculated as per 1 mg.

As it is seen from Table 4, wines made with Kakhetian technology showed a higher inhibition effect than those made with European technology. In overall, these values were statistically significant ($p > 0.05$).

A high correlation was demonstrated in this work between the TPC and AOA in all samples. Pearson's correlation coefficient (r2) for the Caucasian Rhododendron samples (Figure 5) and white wine (Figure 6) samples was 0.9758 and 0.9556, respectively. Several data have been published regarding the relationship between antioxidant capacity and total phenolic content of different wines (Gulua et al., 2018), (Paixao et al., 2007).

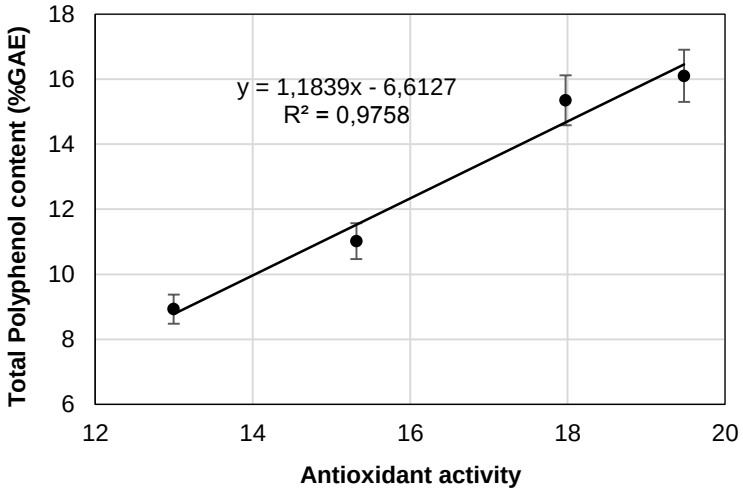

Figure 5. Correlation between the total phenolic content and antioxidant activity of Caucasian Rhododendron samples

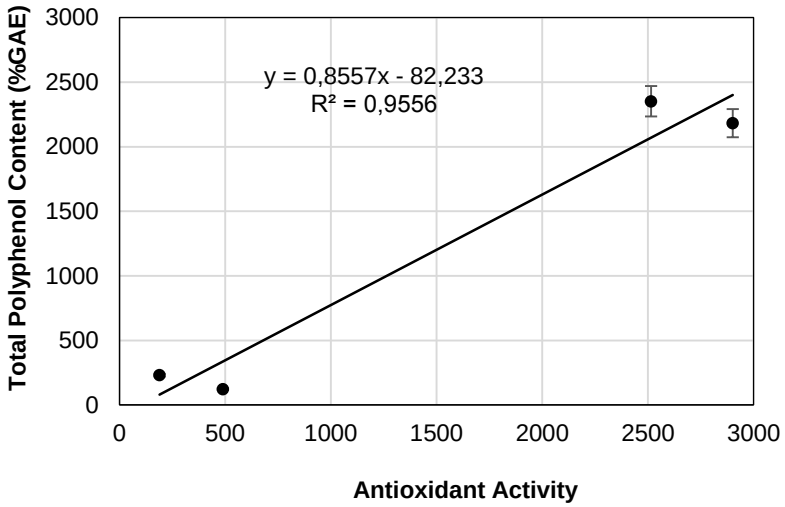

Figure 6. Correlation between the total phenolic content and antioxidant activity of Rkatsiteli wine samples

Lipase inhibitory activity of Rkatsiteli samples displayed a higher correlation with AOA (Pearson's correlation coefficient (r^2) 0.88, than with total phenolic content (Pearson's correlation coefficient (r^2) 0.78).

Tested wine samples were differentiated according to production technology, vintage, and alcohol content (see table 1). Probably due to these reasons, this experiment showed the range of results in wine and furthermore in-depth investigations are needed.

Conclusion

1. Caucasian Rhododendron can be used as a potent lipase inhibitor; it showed better inhibitory activity than white wine samples. Thus, Caucasian Rhododendron could be a reasonable natural resource for the preparation of ingredients with lipase inhibitory activity. However, there are further studies needed to obtain detailed information regarding the influence of treatment methods on bioactive compounds and lipase inhibitory activity.
2. Extracts from Caucasian Rhododendron can act as a promising natural inhibitor of pancreatic lipase and reduce dietary cholesterol' absorption.
3. The treatment of Rhododendron samples could influence the composition of bioactive compounds.
4. The Caucasian Rhododendron sample treated with the classical technical method of green tea processing showed the showed the highest inhibitory activities against pancreatic lipase. Moreover, the effect of inhibition was almost equal to the one of Orlistat.
5. Winemaking technology effects on phenolic composition in Rkatsiteli wine samples. White wines made with Kakhetian technology are rich with bioactive compounds and possess higher antioxidant activity and Lipase inhibitory activity than wines made with classical (European) technology.

Acknowledgment
The authors gratefully acknowledge the financial support provided by the Shota Rustaveli National Science Foundation of Georgia (SRNSFG) (grant number FR-18-566. The Biotechnological bases for making Mate type tea) and Volkswagen (VW) Foundation (Volkswagen Stiftung). Zhuzha Khatchapuridze is the PhD student supported by Sustainable Agriculture and Food Sytems (SAFS) program.

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Sodium chloride substitution in industrial white slice diary bread

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Keywords:

Bread
Sodium chloride
Rheology
Mixolab
Texture

Article history:

Received 15.07.2020
Received in revised form 12.11.2020
Accepted 25.03.2021

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DOI:
10.24263/2304-974X-2021-10-1-5

Abstract

Introduction. The effect of sodium chloride replacement was studied in industrial white slice diary bread, promoting a technological approach to decrease the sodium content from bakery products in order to respond to the World Health Organization´s recommendation to reduce dietary salt intake.

Materials and methods. Granolife CV Sustisal 100 (GCVS100) was evaluated as sodium chloride substitute analyzing the dough fermentative properties by Rheofermentometer, and the dough behaviour properties on mixing–heating–cooling by Mixolab. Additionally, loaf specific volume and texture profile were considered as baking quality parameters.

Results and discussion. The addition of GCVS100 or NaCl to wheat flour dough led to decrease gas production during fermentation stage. However, they significantly increased the coefficient of gas retention, promoting the improvement of the gluten network and allowing to get a dough development curve similar to dough flour. Additionally, both ingredients changed several flour dough parameters in Mixolab. Water absorption was decreased, dough stability was prolonged, gelatinization process (C3-C2) was reduced, stability of the starch gel when heated (C4-C3) was improved and retrogradation of the starch was increased.

GCVS100 assessed in WSDB formula showed similar effects than NaCl. The addition of GCVS100 or NaCl to WSDB caused reduction of gas production during fermentation. Meanwhile, the coefficient of gas retention did not show significant differences between the treatments, due to WSDB formulation include compounds promoter of strengthening of the gluten structure of the dough that masked NaCl and GCVS100 effect. In this way, NaCl and GCVS100 led to decrease dough development according to less gas production.

WSDB baking parameters revealed that bread loaf specific volume was significantly higher for WSDB without NaCl or GCVS100, in agreement with fermentation results. Texture profile analysis of WSDB did not showed changes in crumb firmness and springiness when NaCl or GCVS100 is added.

Conclusions. The addition of GCVS100 in WSDB caused a similar effect to NaCl. The results of the present study suggest that GCVS100 exhibits a potential use to obtain sodium-free WSDB.

Introduction

According to the World Health Organization, it is recommended to consume 5 g of sodium chloride (or 2 g of sodium) per day (WHO, 2012). Globally, average daily dietary salt intake is much higher than this recommendation and the majority of salt intake comes from commercially manufactured foods (Brown et al., 2009; Newson et al., 2013).

Salt reduction programs have been shown to be highly cost-effective (Cobiac, 2010), hence the urgency to implement strategies and policies to address the issue of reducing salt intake. To carry out this approach certain topics should be taken in account. First, salt as an ingredient influences textural and stabilities properties of commercial food, hence salt reduction requires advances in food technology (Doyle and Glass, 2010). Second, salt affects food flavor, so it is important to work on consumer perception such as sensory reeducation, unaccustomed the palate to excessive salty taste and re-feeling the original flavors of food, likewise, modify behaviors in the domestic habit, method of shopping and food preparation (Zandstra et al., 2016; Ding et al., 2020). The successful reduction of sodium chloride in food is a long process that depends on solving technological issues in each of the food industries, awakening the healthy conscience of consumers, and an adequate complement of the food industry with government programs (FSA, 2012; FDA, 2016].

Bread is one of the major contributors to dietary sodium intake (Ni Mhurchu et al., 2010). Sodium content of bread is relatively low, however people consume highly this group of foods which contributes 35% to 50% of the sodium consumption (Beer-Borst et al., 2009; Quilez and Salas-Salvado, 2012). As the consumption of bread is approximately 70 Kg / Hab / year, it provides 3.2 g of sodium chloride per day, around 40% of the total salt ingested (Conde Molina et al., 2020). This estimation clearly demonstrates the relevance of reducing sodium content in breads.

Sodium chloride replacement is not an easy task to do on bread. Since sodium chloride contributes three main functions in the dough: enhance flavor, reduce gas production by inhibiting yeast activity, strengthen gluten structures of dough producing larger protein network (Miller & Hoseney, 2008; Silow et al., 2016). In many cases, taste is one of the most important challenge associated with substitution. This aspect can be achieved with combinations of: salts (KCl, calcium salts, magnesium salts), amino acids (lysine) and flavor enhancers (monosodium glutamate, nucleotides, yeast extract) (Bassett et al., 2014; Rafo et al., 2018). Moreover, sodium chloride has a significant impact on the bread making process. In order to understand the impact of sodium chloride replacement on the bakery products' technological process, its influence on dough processing has to be known.

Several studies have reported changes in dough rheology and bread quality properties when sodium is substituted in breads (Nogueira et al., 2015; Pasqualone et al., 2019), however such characteristics depend on bread recipe and process. Thus, the aim of this work was to investigate the sodium substitution in white slice dairy bread (WSDB), which represents to be the most important industrial bread consumed in Argentina. For this purpose, Granolife CV Sustisal 100 substitute was assessed in order to evaluate the influence of sodium chloride replacement on fermentative and rheological properties on flour and then on WSDB formulation.

Materials and methods

Materials

Granolife CV Sustisal 100 (GCVS100, blend of KCl and flavor enhancers) (Granotec Argentina) was studied as NaCl substitute.

The Argentinian wheat flour analyzed presented the following values: humidity 14.20 % (ISO 712), ashes 0.64 % (AOAC 923.03), wet gluten 28 %, index gluten 99 %, dry gluten 10 % (AACC 38-12), falling number 410 s (AACC 56-81B), damaged starch 9 % (AACC 76-33). The alveograph parameters were tenacity/extensibility (P/L) 1.2 and deformation work (W) 310 10^{-4} J (AACC 54-30A). Stability of dough was 13.5 min (modified method AACC 54-60.01).

Rheological properties of wheat flour dough

The effect of NaCl replacement was studied in wheat flour dough. To do this, three conditions were evaluated: flour (F) as control, flour + NaCl (2% flour weight basis) (F+NaCl) as reference, flour + GCVS100 (2% flour weight basis) (F + GCVS100) as substitution.

Doughs (55% hydration) were kneaded for 1 min at slow speed and 4 min at medium speed in a bakery mixer (model A-120T, Hobart, USA). Then, 315 g of doughs were tested in the Reofermentograph, applying 2 kg weights over dough, at 28 ºC for 3 h. Fermentation assays allowed to obtain gas evolution and dough development curves. Additionally, 75 g dough was analyzed in Mixolab (Chopin, France) to determine instant dough consistency (C1, Nm) at 100 rpm (Chopin, 2012).

The rheological characteristics of dough were measured using Mixolab according to modified AACCI Approved Methods 54-60.01. Results were analyzed by Chopin Mixolab software (Version 3.14, Chopin, France).

Rheological and baking properties of white slide diary bread

GCVS-100 was assessed in WSDB. Formulation for WSDB was: 1 kg flour, 12 g dry yeast, 20 g NaCl, 75 g sugar, 30 g of vegetable oil, 20 g milk powder, 10 g wheat gluten, 3.5 g calcium propionate, 15 g Toler Miga Bollo Directo (blend of ascorbic acid and enzymes (alpha-amylases, xylanases, lipases), Granotec Argentina), 650 ml water. Test conditions were: WSDB without NaCl as control, WSDB with NaCl as reference, WSDB with GCVS-100 as substitution. Doughs were prepared using a bakery mixer (model A-120T, Hobart, USA). They were kneaded for 1 min at slow speed, 2 min at medium speed and 3 min at fast speed.

In order to study fermentation stage, 100 g of doughs were analyzed in the Reofermentograph, applying 2 kg weights over dough, at 28 ºC for 3 h (Chopin, 1996). Instant dough consistency was also determined in Mixolab (Chopin, France) as described above for flour dough.

Otherwise, breads were prepared. Doughs obtained were divided into 500 g portions of spherical shape and rest for 10 min. Then, doughs were passed through a dough pressing machine (model 0203, Indupan, Argentina). Subsequently, pieces were rolled down like tube shape and placed into pans (20 cm length, 10 cm width, 10 cm height). For each formulation, two sequence of three pans were placed in the fermentation camera at 36 ºC for 90 min, RH = 80%. Three loaves of bread were baked in an oven (RPO4A10-2, Eurofours, France) at 150

ºC with lidded pans for 35 min and another three loaves of bread were baked without the lids for 40 min. Breads with lid reached their baking time 5 min earlier because the lid accelerated the baking time. Loaves baked without the lids were left to cool to determine specific volume. Otherwise cool loaves baked with the lids were packed and stored at room temperature until texture analysis.

Bread baking quality was evaluated by loaf specific volume, crumb firmness and springiness textural parameters. Loaves volume was measured by rapeseed displacement according to AACC 10-05 method, using bread loaf volumeter equipment (Chopin, France). Specific volume of the loaves was calculated from the measured volume and weight, obtained by direct measure. Texture profile analysis was analyzed in order to study the structure of the crumb. It was carried out using QTS Farnel Texture Analyser (Brookfield). Crumb firmness was determined according to the method AACC 74-09. Slices (25 mm-thickness) were compressed with a 36 mm diameter cylindrical probe at a speed of 2 mm/s until a deformation, to a total deformation of 10 mm and a trigger force of 4 g were the selected settings. Springiness parameter was determined by texture profile analysis (TPA). Bread slices (50 mm-thickness) were compressed twice using a 25.4 mm diameter cylindrical probe (TA 11) and a test speed of 1.0 mm/s; to a total deformation of 15 mm and a trigger force of 4 g were the selected settings. Bread slices (50 mm-thickness) were compressed twice to give a TPA from which springiness textural parameter was obtained (Bourne et al. 2002). Crumb firmness and springiness textural parameters were obtained through Textute Pro v. 2.1 software. The test was carried out at different times of storage (5, 10 and 15 days) in order to evaluate bread aging.

Statistical analysis

Data were expressed as means ± standard deviations for triplicate determination. Statistical analysis was performed using Microsoft Excel 2010. Significant differences were determined at $p < 0.05$ by analysis of variance (ANOVA) and Tukey's HSD test. The analyses were carried out using the software Statgraphics Centurion XVII (Statpoint Technologies, USA).

Results and discussion

Flour dough rheology evaluation

Initially, GCVS100 substitute was evaluated in an Argentinian wheat flour dough (F). To point view, this type of flour presents some high quality parameters as wet gluten 28 %, P/L 1.20, W 310 10^{-4} J, stability of dough 13.5 min, being suitable to produce industrial WSDB.

Dough rheological properties in F+NaCl and F+GCVS100 presented significantly differences during fermentation in comparison to F (Table 1). The volume of gas produced (V_T) in both cases was inferior ($p<0.05$) than F, evidencing the reduction of yeast activity caused by the presence of this type of salts. Chloride salt influences on yeast metabolism by its osmotic pressure, inhibiting yeast growth and leading to lower CO_2 production (Jekle et al., 2019). Furthermore, maximum height of gas production curve (H'_m) significantly decreased ($p<0.05$) in F+NaCl and F+GCVS100 in relation to F, as consequence of the fact that the CO_2 produced by the yeast during fermentation was reduced. Similar influences on H'_m value by salt addition have also been previously reported (Miller and Jeong, 2014;

McCann and Day, 2013). Although NaCl and GCVS100 led to reduce V_R and V_T values, they significantly increased ($p<0.05$) the coefficient of gas retention (V_R/V_T) value. This fact indicated that an improvement of the gluten network occurs with the addition of NaCl or GCVS100. NaCl produces a strengthening effect on the gluten network, which makes the dough more capable of retaining the gas released by fermentation (Mohammed et al., 2012).

Table 1

Effect of NaCl (F+NaCl) and GCVS100 (F+GCVS100) on flour (F) dough rheological properties during fermentation

Rheofermentograph	**F**	**F+NaCl**	**F+GCVS100**
Curve of gas			
V_T (volume of gas produced, mL)	$(1604\pm8)^a$	$(1204\pm12)^b$	$(1227\pm13)^b$
V_R (volume of gas retained, mL)	$(1256\pm10)^a$	$(1058\pm8)^b$	$(1057\pm7)^b$
V_L (volume of gas lost, mL)	$(348\pm9)^a$	$(146\pm8)^a$	$(170\pm9)^c$
V_R/V_T (coefficient of gas retention, %)	$(78\pm1)^a$	$(89\pm2)^b$	$(86\pm2)^b$
H'_m (maximum height, mm)	$(54\pm1)^a$	$(40\pm2)^b$	$(42\pm2)^b$
T_x (time needed to start losing gas, min)	$(82\pm1)^a$	$(82\pm2)^b$	$(84\pm3)^b$
Curve of dough development			
H_m (maximum dough height, mm)	$(33\pm1)^a$	$(30\pm2)^a$	$(29\pm2)^a$
H (dough height after 3 h, mm)	$(27\pm1)^a$	$(30\pm2)^a$	$(29\pm2)^a$
Dough consistency (Nm)	$(2.87\pm0.15)^a$	$(2.84\pm0.09)^a$	$(2.77\pm0.11)^a$

Means with different letters in each row are statistically different ($P<0.05$).

Maximum dough height of the dough development curve (H_m) were similar for all cases. This suggests that the effect the salts to increase V_R/V_T compensate the low V_T value, thereby leading to similar H_m.

Mixolab was used in order to analyze the replacement of NaCl on flour dough by GCVS100 during mixing–heating–cooling. Therefore, changes associated with dough during mixing due to ingredients hydration, heating due to protein weakening and starch gelatinization, and cooling due to starch gelling, were registered by following dough consistency (Figure 1, Table 2). Results showed that NaCl and GCVS100 significantly decrease ($p<0.05$) water absorption (WA). In fact chloride salt, due to its ionic nature, interacts with water and macromolecules from the dough complex reducing the WA of wheat flour. As consequence of higher hydrophobic interactions, gluten proteins interact to a higher extent leading to a reduced water uptake capacity (Voinea et al., 2020; Lopes et al., 2017).

Concerning dough stabilities, values significantly increased ($p<0.05$) with NaCl or GCVS100 addition. Although F+NaCl showed higher stability than F+ GCVS100, 22 Vs. 18 min, they are both considered to be high stability parameter to WSDB making process. This effect may be attributed to the fact that greater hydrophobic interactions between gluten proteins leads to a closer molecular structure between these proteins. The strengthening effect of wheat flour dough has been reported due to addition of chloride salts in dough (Mohammed et al., 2012).

Protein weakening pattern (C2) showed no significant difference when NaCl or GCVS100 was added to the flour dough, during heating. Meanwhile with the increase in temperature, the incorporation of NaCl or GCVS100 to the dough exhibited lower values ($p<0.05$) of the starch gelatinization range (C3–C2) compared to F. These data may be related

that the presence of these types of salt may affect swelling starch, remaining intact the starch granules for a long time before fragmentation (Nogueira et al., 2015). Furthermore, parameter C4-C3 had higher values ($p<0.05$) for F+NaCl and F+ GCVS100 than F, indicating that the addition of NaCl or GCVS100 in dough improved the stability of the starch gel when heated.

Significant differences ($p<0.05$) were observed in the dough consistency at cooling (C4-C5) in the presence of NaCl or GCVS100. In these cases, retrogradation of the starch was increased. It was reported (Krupa-Kozak et al., 2012) that retrogradation of the starch changes when calcium salts were added to fortified formulas in Mixolab. This study suggests that anions modified the starch chains' recrystallisation. Other works reported that NaCl reduces the retrogradation of starches during storage (Baker and Rayas-Duarte, 1998; Beck et al., 2012), however retrogradation of the starch effect caused for NaCl in wheat flour dough was not reported previously in Mixolab. In our case of study, the effect of increase of retrogradation of the starch due to NaCl or GCVS100 addition may not be remarkable, as WSDB formula contains Toler Migo Bollo Directo (which includes enzymes) that promotes the mean anti-staling characteristic in the final product.

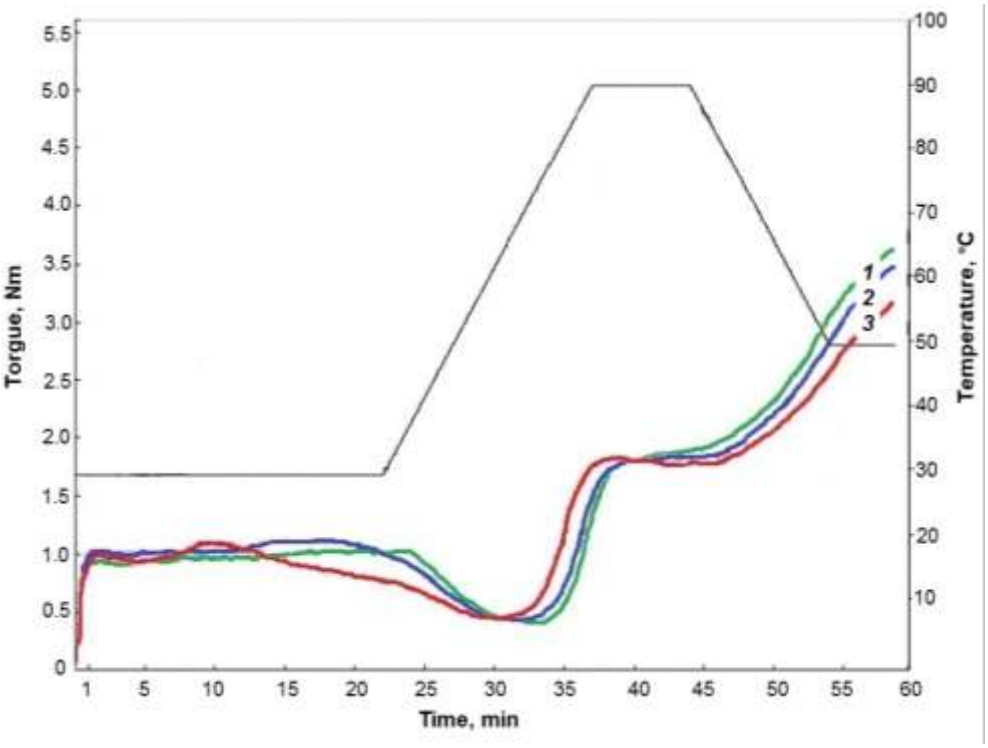

Figure 1. Effect of NaCl (F+NaCl) and GCVS100 (F+GCVS100) on flour (F) dough consistency determined by Mixolab:
1 – F, 2 – F+NaCl, 3 – F+ GCVS100

Table 2

Effect of NaCl (F+NaCl) and GCVS100 (F+GCVS100) on flour (F) dough rheological properties during Mixolab analysis

Mixolab	F	F+NaCl	F+GCVS100
WA (water absorption, %)	(58.0±0.1)[a]	(56.3±0.3)[b]	(56.1±0.1)[b]
Stability (min)	(13.5±0.7)[a]	(22.3±0.5)[b]	(18.2±0.4)[c]
C2 (protein weakening, Nm)	(0.45±0.02)[a]	(0.41±0.02)[a]	(0.44±0.02)[a]
C3 (starch gelatinization, Nm)	(1.83±0.01)[a]	(1.73±0.02)[b]	(1.76±0.02)[b]
C3-C2 (starch gelatinization range, Nm)	(1.38±0.02)[a]	(1.32±0.02)[b]	(1.32±0.02)[b]
C4 (hot gel stability, Nm)	(1.76±0.04)[a]	(1.89±0.02)[b]	(1.86±0.04)[b]
C4-C3 (cooking stability range, Nm)	(-0.07±0.04)[a]	(0.16±0.02)[b]	(0,10±0.04)[b]
C5 (starch retrogradation in the cooling phase, Nm)	(3.21±0.02)[a]	(3.65±0.02)[b]	(3.52±0.02)[c]
C5-C4 (gelling, Nm)	(1.45±0.02)[a]	(1.76±0.02)[b]	(1.66±0.03)[c]

Means with different letters in each row are statistically different ($P<0.05$).

WSDB dough rheology and bread evaluation

GCVS100 was assessed in WSDB formula in order to evaluate dough rheology and bread quality. NaCl and GCVS100 caused changes in the fermentative properties of the dough (Table 3). V_T parameter significantly decreased ($p<0.05$) when NaCl or GCVS100 was added to WSDB. It had already been observed and discussed in the wheat flour assays that the presence of NaCl or GCVS100 changes the yeast growth environment to be less favourable, thus reducing the amount of CO_2 produced. The influence of NaCl in WSDB was even greater than that of GCVS100, being V_T of WSDB with NaCl lower than V_T of WSDB with GCVS100.

H'_m of gas production curves were significantly lower ($p<0.05$) for dough WSDB with NaCl or GCVS100 comparing to WSDB without salt. These results correlated with less quantity of gas produced. H_m of the dough development curve also decreased due to NaCl and GCVS100 effect.

Additionally, V_R/V_T did not show significant differences between the treatments. This may be to the fact that WSDB formulation has ingredients, such as gluten, oxidizing agents and enzymes, which promote strengthening of the gluten structure of the dough (Aamodt et al., 2003; Steffolani et al., 2010), leading to retain the gas released during fermentation. As consequence, it was noticed that WSDB without NaCl presented significantly higher H_m value ($p<0.05$) than in the case of WSDB with NaCl or GCVS100. Thus, WSDB ingredients probably lead to strengthening of the dough mitigating the absence of NaCl and GCVS100 on the gas retention effect. On the other hand, the lack of this salts in WSDB produces higher gas production, thereby increases development of the dough.

Table 3

Effect of NaCl and GCVS100 on WSDB dough rheological properties during fermentation

Rheofermentograph	**WSDB without NaCl**	**WSDB with NaCl**	**WSDB with GCVS100**
Curve of gas			
V_T (volume of gas produced, mL)	(1029±8)^a	(671±12)^b	(721±13)^c
V_R (volume of gas retained, mL)	(988±10)^a	(665±8)^b	(715±7)^c
V_L (volume of gas lost, mL)	(31±9)^a	(6±8)^b	(6±9)^b
V_R/V_T (coefficient of gas retention, %)	(97±1)^a	(99±2)^a	(99±2)^a
H'_m (maximum height, mm)	(55±1)^a	(41±2)^b	(40±2)^b
T_x (time needed to start losing gas, min)	(139±3)	-	-
Curve of dough development			
Hm (maximum dough height, mm)	(58±1)^a	(38±2)^b	(35±2)^b
H (dough height after 3 h, mm)	(58±1)^a	(38±1)^b	(35±2)^b
Dough consistency (Nm)	(1.91±0.12)^a	(1.79±0.09)^a	(1.85±0.07)^a

Means with different letters in each row are statistically different ($P<0.05$).

The effect of NaCl and GCVS100 on bread quality indicators is shown in Table 4. When substitute GCVS100 was evaluated in WSDB preparation, baking results showed that bread loaf specific volume was significantly higher ($p<0.05$) for WSDB without NaCl than for WSDB with each salt, in agreement with the data analyzed above concerning to the fermentation parameters of the WSDB tests. This is agreeing to other works which reported that bread with less salt has been found to have higher volume of dough (Lynch et al., 2009; Beck et al. 2012).

Textural parameters were measured at 0, 5 and 15 days, in order to evaluate the evolution of the crumb during 15 days, which is the normal shelf life and sale of WSDB. It was noticed that significantly differences were not found in crumb firmness and springiness between the three bread treatments. WSDB formulation includes enough ingredients that contribute to stand suitable textural properties of bread, irrespective of presence or absence of NaCl. Previous report (Lynch et al., 2009) noticed that NaCl helps to strengthen and improve the gluten network of the dough, leading to produce uniform crumb structure. In our study, this effect was not observed as consequence of the integral WSDB formulation.

Furthermore, crumb firmness was similar between treatments along 15 days, indicating that NaCl and GCVS100 did not influence the retrogradation of the starch during WSDB storage. It was reported (Baker and Rayas-Duarte, 1998) that NaCl reduced the retrogradation of starches during bread storage, due to the fact that large ions, as Na^+, are entrapped in the molecules compared to the small H^+ ions. However, this research suggests that retrogradation of starch caused by NaCl may be masked by others ingredients in the bread recipe. Therefore, the effect of NaCl or some substitute on bread shelf life should be analyzed according each bread formulation.

Table 4

Effect of NaCl and GCVS100 on indicators of bread quality

Rheofermentograph	WSDB without NaCl	WSDB with NaCl	WSDB with GCVS100
Bread weight (g)	(429±1)[a]	(428±2)[a]	(431±2)[a]
Bread volume (mL)	(2680±4)[a]	(2500±7)[b]	(2530±4)[c]
Bread loaf specific volume (mL/g)	(6.25±0.03)[a]	(5.84±0.02)[b]	(5.87±0.02)[b]
Crumb firmness (g)			
5 (days)	(475±9)[a]	(480±6)[a]	(479±7)[a]
10 (days)	(927±7)[a]	(832±6)[a]	(828±4)[a]
15 (days)	(1395±12)[a]	(1401±11)[a]	(1405±9)[a]
Springiness			
5 (days)	(0.93±0.01)[a]	(0.93±0.01)[a]	(0.93±0.01)[a]
10 (days)	(0.92±0.01)[a]	(0.93±0.01)[a]	(0.92±0.01)[a]
15 (days)	(0.92±0.01)[a]	(0.93±0.01)[a]	(0.92±0.01)[a]

Means with different letters in each row are statistically different ($P<0.05$).

This work does not include sensory traits to study the influence of the substitute on the taste of WSDB. Perhaps a dosage of 20 g of GCVS100 / 1 kg of flour (as indicated in the recipe) will not be enough to affect the flavor of the bread. Moreover, GCVS100 includes flavor enhancers that masked "metallic" and "bitter" after-taste imparted by potassium ions. Further research will be necessary to evaluate sensory characteristics.

Conclusion

The current research has shown that GCVS100 fulfits its function as NaCl substitute adequately for industrial WSDB production.

The addition of GCVS100 in WSDB caused a similar effect to NaCl. Both ingredients lead to decrease gas production and dough development during fermentation stage. Consequently, baking test showed that bread loaf specific volume decreased when NaCl or GCVS100 is added.

Texture profile analysis of WSDB did not showed changes in crumb firmness and springiness when NaCl or GCVS100 is added. WSDB contains several ingredients, as gluten, oxidizing agents and enzymes, which confer a preponderant effect to textural characteristics.

The results of the present study suggest that GCVS100 exhibits a potential use to obtain sodium-free WSDB.

Acknowledgements. The authors would like to express their appreciation to Granotec Argentina for financial support of this study. Data collection for this study was undertaken while DCM worked for Granotec Argentina (GA). VA works for GA. CQ has no conflicts of interest to declare.The views expressed are entirely those of the authors and do not necessarily reflect GA policy.

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Antioxidant effectiveness of plant cultures

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Keywords:

Antioxidants
Oxidation
Berries
Herbs
Extraction

Article history:

Received 18.06.2020
Received in revised form 17.12.2020
Accepted 25.03.2021

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DOI:
10.24263/2304-974X-2021-10-1-6

Abstract

Introduction. Since the natural antioxidants do not cause any undesirable side effects, they have more preferences in comparison to those synthetic. The objectives of this article are to reveal the plants to be determined as antioxidants' concentrators among the berries and herbs in order to highlight the ways of their practical usage in food technologies.

Materials and methods. Four sorts of cultivated berries and eight species of wild herbs were studied to define their general antioxidant effectiveness. Ten specimens of herbs were examined to reveal the amount and level of bioflavonoids in water-and-alcohol extracts experimentally obtained. The amount of ascorbic acid, bioflavonoids, and carotenoids was determined by traditional methods.

Results and discussions. The maximal amount of ascorbic acid (mg/100 g) was revealed in black currants – 234, cherries – 62.2, chokeberries – 129, wild strawberries 104, blackberries 68.8. In addition, all the berries listed differ with high amount of bioflavonoids (mg/100 g) – correspondingly, 1858 in black currants; 1340 in cherries; 2460 in chokeberries; 1978 in wild strawberries; 2447 in blackberries. There was observed the natural correlation between the amounts of these two groups of antioxidants in the raw materials researched. This would allow proving the expedience to use them in obtaining the foodstuffs with antioxidant targeting. The ranking of berries alleged as the most utile to correct the amount of ascorbic acid, bioflavonoids, and carotenoids in accordance with recommended daily intakes, looks like this (mg/100 g): chokeberries – 100, blackberries – 2514, bilberries – 2199 black currants 2096, wild strawberries – 2084, cherries – 1405.

Relatively high amount of bioflavonoids that act together and henceforth serve as buffer antioxidant system was found in herbs (mg/100 g): St. John's wort – 3.89, oregano – 2.98, immortelle – 2.638, melissa – 1.685, and thyme – 1.470. Under previously determined indices of the main extraction parameters, 85 percents of bioflavonoids diffused into the extract from St. John's wort; more than 60 percents from black currants, melissa, thyme, salvia, and immortelle; ca 40 percents from oregano; less than 30 percents from nettle and birch. This can be explained by many factors such as different amount of food cellulose, which, in general, affects the bioflavonoid diffusion coefficient during extraction.

Conclusions. The plant raw materials which are endemic for moderate climatic zone – cultivated and feral berries as well as herbs – with high antioxidant content should become the integrate part of foods and drinks elaborated to protect the human organism from harmful free-radical impacts.

Introduction

The low-quality foodstuffs eaten by humans, as well as the polluted environment, essentially influence the apparition of free radicals and further proliferation of free-radical processes.

The high reaction ability of free radicals can accelerate the oxidation processes in a live organism and, consequently, lead to the collapse of cellular membranes and their molecular base. This would finally result in numerous pathological states such as oncology diseases (Menshieva et al., 1994), genetically conditioned diseases (Armstrong, 2002), second type diabetes, atherosclerosis, cardiologic insufficiency (Miwa et al., 2008). Therefore, the problem of free radicals and the reaction-capacious oxygen-containing substances is topical for both academic institutions and the society as a whole.

Analysis of the recent scientific works

The antioxidant substances of various chemical natures (bioflavonoids, ascorbic acid, carotenoids and so on) contained by foodstuffs in different concentrations (Toor et al., 2006; Ishiguro et al., 2007) are believed to resist the expansion of free-radical processes. Whenever such foodstuffs are consumed, the aforementioned substances help to avoid accumulating of free radicals in cells (Backer et al., 2004). Unlike the synthetic pharmacological remedies, antioxidants of biological origin are easily and organically involved into metabolic processes in the organism and, in turn, do not cause undesirable side effects (Simakhina, 2011).

The results of the recent researches in the outlined trend evidence the perspectives of selection of fruit and vegetable cultures or herbs as the sources of antioxidants, regarding the proved ability of the latter to support the immune functions of human's natural antioxidant system (Saura-Calixto et al., 2006), to retard all the stages of free-radical reactions (Van der Sluis et al., 2000), and to provide stabilization of lipids contained by cellular membranes (Samotyja et al., 2007).

Up to nowadays, the majority of scientific works were dedicated to elucidation of antioxidant activity of ascorbic acid, vitamins A and E, and carotenoids. For instance, there were established the newest facts about the mechanism of biological influence by ascorbic acid (Timirkhanova et al., 2007): particularly, it is the zero C-hypervitaminosis even after its excessive intake in treatment and prevention of many diseases characterized by enhancement of free-radical processes as a consequence of exhaustion of human's natural antioxidant system.

Today, the scientists' attention is more and more drawn to P-active substances of phenolic origin (bioflavonoids, in other words): catechinins, anthocyanins, leucoanthocyanins, flavone glycosides, chlorogenous acid and others. There are many evidences advocating this fact.

Firstly, this group of phenolic compounds is the most wide-spread and represented in maximal concentrations in the certain species of plants. In particular, the authors (Sun et al., 2002) determined high antioxidant activity of feral berries – bog whortleberries, wild strawberries, and bilberries.

Secondly, it is bioflavonoids that are now being studied as the most essential plant-originated biological regulators; in addition, there was proved that fruit or berry pulp, for instance, contains more flavonoids than juices do (Kjersti, 2004).

Finally yet importantly, the key property of bioflavonoids is their ability to regulate the peroxidation syndrome development that is, unfortunately, a universal factor of the pathogenesis of practically all the diseases known and can be activated in any stress or intoxication (Menshieva et al., 2004).

As the bioflavonoids were predominantly researched in terms of their capability to normalize the capillary penetrability in earlier works (Kjersti, 2004), nowadays, due to the expansion and diversification of the studies, they are going under revelation of the great deal of biological properties. Some of them, particularly, are the ability to regulate the oxidation and restoration processes in the organism (Levitskyi, 2001), to stabilize the cellular membranes (Gordiyenko, 2000), to conduct the preventive action since included into foodstuffs (Voskresensky et al., 2002), to correct the cholesterol amount in the organism (Hässig et al, 1999), and to boost the resistance of live organisms to the malignant environmental factors (Yordanov et al, 2005).

There are many convincing proofs that all of the bioflavonoids' effects listed above are the consequence of their antioxidant activity (Nagendran et al., 2006; Kähkönen et al., 1999), which, in turn, can be determined by the specifications of their composition, precisely the presence of two or more hydroxyl groups in the benzole nucleus of a molecule (Van Acker et al., 1998).

The unique fact is that a great deal of bioflavonoid substances to amplify the biological antioxidant action of each other is contained in the tissues of fruit, berries, vegetables, and herbs (Shkarina et al., 2010).

Antioxidant activity, which is one of the important characteristics of either natural or synthetic compounds, reveals in interception and neutralization of free radicals that appear as a result of physiological processes and hence become capable of attacking the vital targets (Alves et al., 2013). The essential position among the natural sources of biologically active substances belongs to raw fruit and berries (Shestopal, 2011; Simakhina et al., 2016) rich of antioxidant compounds, primarily ascorbic acid and bioflavonoids. Crucially powerful antioxidants are anthocyanins (Mazza et al., 1993; Harborne et al., 2001): upon interaction with the separate free radicals, an anthocyanin imparts the latter a proton and therefore transforms them into a molecular product; in turn, it becomes a weak radical unable to continue the chain reaction (Lashen et al., 2007).

According to the data obtained by Vira Petrova (Petrova, 1986), it is the anthocyanins that contribute to the polyphenolic compound of berries (feral especially), whose main representatives are pelargonidin, cyanidin, and delphinidin. The authors (Stetsenko et al., 2016), having used the HyperChem software (Solovyov et al., 2005) and acquired results of quant-chemical half-empirical calculations (Butyrskaya, 2011), investigated the connections between the electronic structure of anthocyanins and their ability to initiate the mono-electronic reactions with the free radicals. As a result, there was shown that the pelargonidin molecule appears to be the most probable to have a proton split from a hydroxyl group, which would provide the highest antioxidant activity of the substance noticed.

Apart from fruit and berries, the prospective sources of natural antioxidant are herbs that contain the significant amounts of biologically active substances (hereinafter named BAS) capable of variously affecting all the human organs and systems. For instance, they can mobilize the immune system to fight with many harmful factors such as the small radiation doses, stresses, free-radical injuries (Gordon et al., 1994).

Due to the specific biochemical functions of one's body, a human is unable to synthesize the sufficient portions of these substances or at least possesses them in a limited amount. Thenceforth, the main antioxidants (like ascorbic acid, bioflavonoids, carotenoids and so on) should be consumed with food – raw fruit and berries or the final foodstuffs fortified with BAS complexes extracted from herbs. The plant-originated ingredients are able to act in synergy, which fact proves the expedience to extract not a single component, but a complex of BAS from herbs. The scientific experience shows (Chekman, 2000) that it is more facile for the complexes, unlike for the simplex substances, to harmonize the system of active and auxiliary substances to increase their

antioxidant activity and, correspondingly, to empower the functions of human natural antioxidant system.

The topicality of the problem under discussion can be confirmed by the results of numerous researches on elaboration of the methods to obtain the natural antioxidants, which may be categorized into two large groups.

The **first group scientists** aim their work at the extraction of the certain single substances (ascorbic acid, rutin, quercetin, dihydroquercetin, chlorogenous acid and others).

The **second group scientists** tend to obtain some other BAS in the complex with phenolic substances (ascorbic acid, organic acids, carotenoids etc.)

The first trend can be epitomized by the method of rutine obtaining proposed by A. Kosyan (2006, Ukrainian patent UA 12544). This method belongs to glycoside chemistry, particularly to separation and purification of vitamin preparations from plant raw materials. What make it attractive to the scientists are its ecological purity, safety, relative cheapness and technological expedience.

Besides, there is also an interesting method to obtain the flavonoids from plant raw materials proposed by A. Sampiyev et al. (Sampiyev et al., 1999). It is intended to use the following species of herbs: *Herba polygoni, Herba leonuri, Sofora iaponica, Scutellaria baikalensis*.

What appears to be quite expedient is the method by L. Igrunov (2005, Ukrainian patent UA 10365), elaborators of which propose to use oat straw or hull for the raw material to be further extracted by water-and-alcohol solution during 1–3 hours with a temperature of 40–98 °C.

The usage of the complex extracts makes possible to intensify the process, to increase the purity grade of the target products, and to raise the output of biologically active substances. For instance, the method to obtain an antioxidant from bearberry leaves (N. Bila, 2006, Ukrainian patent 16774) is based on the raw material extraction in the direct electric field by 1-percent water solution of acetic acid with addition of Twin-80 surfactant.

N. Hrybova et al. (2008, Ukrainian patent 33578) proposed the method to obtain an antioxidant from bearberry leaves by ultrasound extraction with a constant impact of ultrasonic waves (frequency of 60 kHz) during 100 minutes in a room temperature. This method is believed to increase the general flavonoid output and reduce the time of extraction process.

On the other hand, the usage of ultrasound may have some negative consequences due to destruction of phenolic compounds, resulting from distribution of ultrasonic waves within a system.

The method to obtain the biologically active extraction from plant raw material under low temperatures (O. Osetsky et al., 2000, Ukrainian patent 32028) is considered interesting and relevant in terms of theoretical substantiation.

There has been already noticed that the range of authors set the objectives to extract some other substances together with phenolic complexes (for instance, vitamin C, organic acids, amino acids etc.). This can be epitomized by the method to obtain the natural antioxidant from oak bark (L. Danilova et al.) (Danylova et al., 2016). The authors of this work widened the array of plant raw materials from which the antioxidant complexes may be attained, and their main conclusion is that the target products (as mono compounds) are expedient to be used in pharmacy, and the BAS complexes in food industry, regarding their synergistic action towards each other.

Despite the fact that the researches on the BAS of fruit, berries and herbs initiated in 1960-1970s (Chekman, 2000; Petrova, 1986), the authoritative data about their antioxidant activity and the amounts in various sorts of plants have not been systematized yet. This may limit the range of their usage on food technologies and elaboration of the new food products with antioxidant trend. Therefore, blocking of free radical processes, which are triggered with active oxygen forms, on the starting stages would finally abate. Finally yet importantly, the effective methods to obtain the

BAS complexes with antioxidant action, oriented at involvement of cheap raw materials and accessible technological equipment, are still under design.

The *objectives* of this research are to reveal the concentrators of antioxidants (ascorbic acid, bioflavonoids and carotenoids) among fruit, berries and herbs, to study the grade of bioflavinoid transition from raw herbs into water-and-alcohol extract, and to determine the trends of their practical usage in food technologies.

Materials and methods

Plant raw materials

Cultivated sorts: cherries, raspberries, black currants, red currants. Feral species: chokeberries, bilberries, blackberries, guelder, cranberries, cornel, gooseberries, wild strawberries.

Herbs: nettle (*Urtica*), oregano (*Origanum vulgaris)*, melissa (*Melissa officinalis*), thyme (*Satureja hortensis*), salvia (*Salvia officinalis*), blossoms of St. John's wort (*Hypericum perforatum l.*), leaves of birch (*Betula pubescens*), blossoms of chamomile (*Chamomilla recutita*), leaves of salvia (*Salvia*), dead nettle (*Herba leonuri*), blossoms of immortelle (*Helichrysum arenarium l. Moench*.).

All the berries selected were assessed to define the amount of ascorbic acid, bioflavonoids, carotenoids and, correspondingly, the general antioxidant activity of each of the culture in terms of their further usage in antioxidant foodstuffs production. Owing to the fact that cultivated berries and their feral analogues differ with the correlations of essential biocomponents as a consequence of the conditions of their growth and far higher resistance of feral plants to malignant environmental factors, the mentioned raw materials were studied separately.

Obtaining the extractions

The dried raw materials (leaves and blossoms) with humidity of 10–12 percents were used to obtain the herbal extractions (Sampiyev et al., 1999). The water-and-alcohol extractions were obtained by counter-flow extracting until the amount of dry substances reached 15–18 percents, depending on the sort of raw (Chuyeshov et al., 2002).To establish the optimal indices of the main parameters of herb extraction process, the impact of the factors like dispersion level, extraction duration, correlation between raw material and the extracting substance, and alcohol concentration in the extracting substance on bioflavonoid output was studied. Since the herbal raw material was represented only by leaves and blossoms (solid parts like roots and stems were not taken into account), the selection of the optimal extraction conditions was based on the analysis of St. John's wort blossoms (Sampiyev et al., 1999).

Vitamin C determination

Vitamin C is one of the main antioxidants in fruit and berries (Petrova, 1986). The method of its extraction is traditional, based on the usage of sodium 2.6-dichlorphenolindophenolate (Dadali et al., 2003)

Bioflavonoids determination

The amount of bioflavonoids was determined by the general colorimetric method based on formation of flavonoid-and-aluminum complex (Wang et al., 2007).

β-carotene determination

The amount of carotenoids was determined by the general method based on the extraction of carotene with addition of organic solvents, and further measuring optical density of the solution on the spectrophotometer (Juntachote et al., 2005).

General antioxidant effectiveness index

This index (hereinafter GAE) was determined regarding the general amount of ascorbic acid, bioflavonoids and carotenoids (Santos et al., 2019).

Results and discussions

General antioxidant effectiveness of fresh berries

Fresh fruit and berries as well as the frozen half-products on their base are the main source of essential BAS, first of all the vitamins – antioxidants, bioflavonoids and carotenoids (Ukrayinets et al., 2019) The results of estimation of the raw materials in terms of antioxidant amounts are presented in Tables 1.

Table 1

General antioxidant activity of cultivated berries, mg/100 g

Specimens	Ascorbic acid			Bioflavonoids			Carotenoids			AE, mg/100 g
	Total amount	RDI, mg	Satisfaction, %	Total amount	RDI, mg	Satisfaction, %	Total amount	RDI, mg	Satisfaction, %	
Cherries	62.2	200	31.1	1340	500	268	2.4	6	40	1405
Raspberries	51.4	200	25.7	1285	500	257	1.2	6	20	1338
Black currants	234	200	117	1858	500	372	2.8	6	63.3	2096
Red currants	49.4	200	24.7	1305	500	261	1.15	6	23	1356
Chokeberries	129	200	64.5	2466	500	493.2	4.9	6	81.6	2600
Bilberries	54.6	200	27.3	2143	500	428.6	1.4	6	23.3	2199
Blackberries	68.8	200	34.4	2447	500	489.4	1.57	6	26.4	2517
Guelder	39.4	200	19.7	1345	500	269.0	1.7	6	28.3	1386
Cranberries	36.6	200	18.3	1076	500	215.0	0.56	6	9.3	1113
Cornel	31.2	200	15.6	373	500	74.6	1.1	6	18.4	405
Gooseberries	55.8	200	27.9	876	500	175.2	1.18	6	19.7	933
Wild strawberries	104	200	52	1978	500	395.6	1.35	6	22.5	2084

Notes: RDI – recommended daily intake (MOZ, 2017); AE – antioxidant effectiveness.

The researched cultivated sorts and wild species have quite high amount of ascorbic acid and bioflavonoids. As it was expected (Petrova, 1986), the bioflavonoid content in feral berries is far higher than in those cultivated. For instance, the grade of satisfaction the daily need in bioflavonoids is from 257 до 372 percents for cultivated plants (MOZ, 2017) whereas in feral berries this index oscillates from 74.6% to 493.2 percents (MOZ, 2017). Therefore, all of the cultures researches were taken into consideration as the raw materials rich in bioflavonoids, meantime cornel and gooseberries were omitted from this list due to low bioflavonoid content.

With a few exceptions, the correlation between the amount of ascorbic acid and bioflavonoids is different for each sort of raw materials. As Vira Petrova asserted (Petrova, 1986) the dynamic balance in such a system may remain stable only under the certain concentration correlations between flavonic compounds and ascorbic acid. Whenever the concentration of any compound changes, it would cause the shift of the balance into one or another side to weaken the stabilizing factor of both vitamins relatively to each other and, in turn, to lower their antioxidant effect.

The third factor to evaluate the antioxidant activity of raw berries was the content of carotenoids. Generally, berries cannot be related to plentiful carotenoid sources, except of some sorts of eglantine, hawthorn, chokeberries, rowan and sea-buckthorn. The author of the monograph (Petrova, 1986) indicate that the maximal amount of carotenes is contained in ripen berries; what is more, this group of BAS is accumulating unevenly. This can be explained by the fact that the berries have a certain concentration of BAS formed at the first stage of growth; from thence, it slightly lowers and thereby sharply flashes up to the time of full ripening.

According to data obtained in our researches, most of carotenoids is contained in chokeberries (4.9 mg/100 g), black currant (3.8 mg/100 g), cherries (2.4 mg/100 g), guelder and blackberries (1.7 mg/100 g and 1.57 mg/100 g correspondingly).

Regarding the tables 1 and 2, feral berries (apart from cranberries, cornel and gooseberries) have their GAE higher than in cultivated sorts. Particularly, the maximal index of activity is 2,096 mg/100 g of the final product for black currants, whereas for wild-grown chokeberries it reaches 2,600 mg/100 g of the final product. This tendency allows confirming the results obtained by other scientists (Petrova, 1986; Mazza et al., 1993). Overall, the descending sequence of GAE in cultivated berries looks like this:

Black currant > Cherries > Red currants > Raspberries.

For feral berries, the sequence is the following:

Chokeberries > Blackberries > Bilberries > Wild strawberries > Guelder > >Cranberries >Gooseberries >Cornel.

The recommended daily intakes of the antioxidants researched are 500 mg for bioflavonoids (MOZ, 2017), 200 mg for ascorbic acid (MOZ, 2017), 6 mg of carotenoids (MOZ, 2017); total amount is 706 mg (MOZ, 2017). Besides, all the substances should be consumed altogether because only in combination can they make a positive effect (Spirichev et al., 2003). Therefore, quite important is the early substantiated presence of the studied antioxidants in the amounts manifold exceeding the RDI: namely, 100 g of chokeberries – in 3.68 times, 100 g of black currants – in 2.97 times.

Starting from the RDI of the studied antioxidants as well as the grade of their accumulation in berries, it is decidedly expedient to relate some of them, which have their

GAE of 1400 mg/100 g of the final product and more (to twice exceed the RDI), to natural antioxidant concentrators, and thereinafter to select the berries for obtaining the foodstuffs of antioxidant destination in both the various branches of food industry and restaurant households, according to the principles presented in Figure 1.

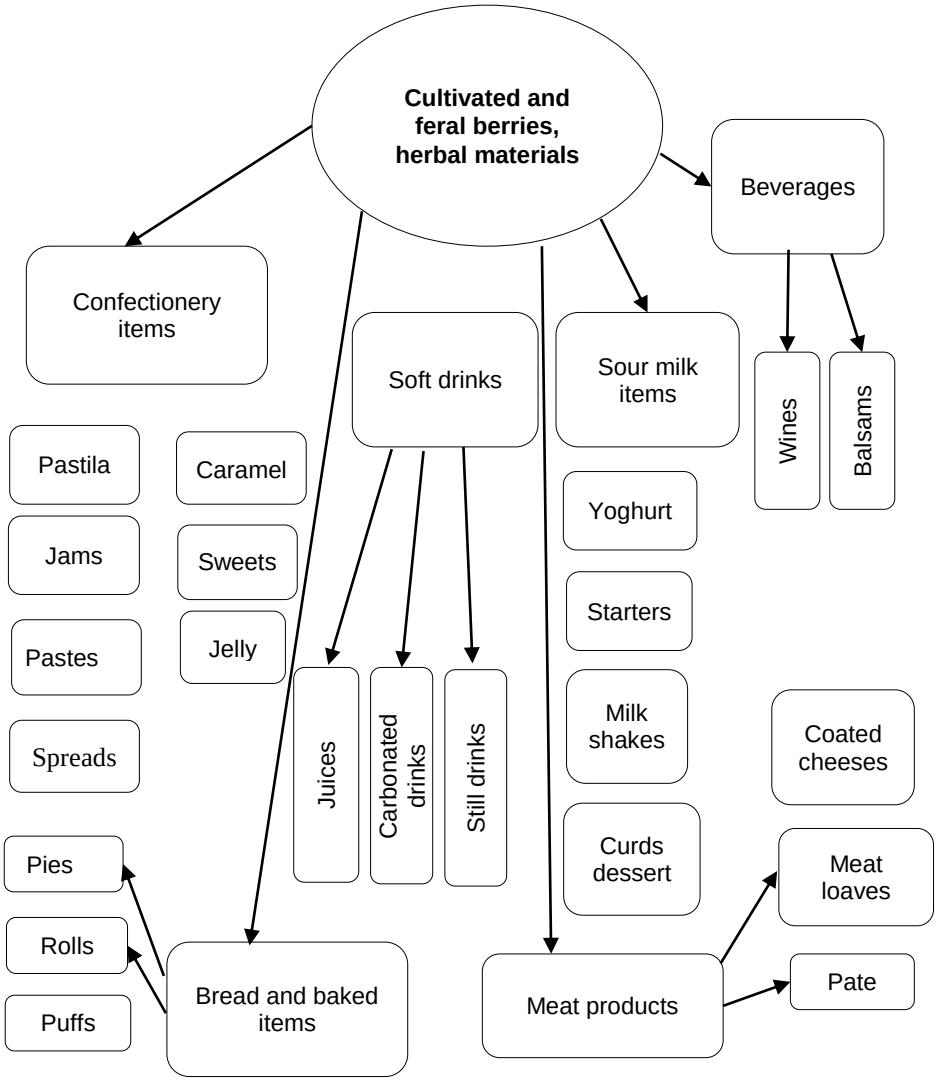

Figure 1. Assortment of foods and drinks with antioxidant destination using the plant raw materials

Certainly, the proposed trends of natural antioxidant usage cannot be limited by the listed options. For instance, the optimal solution to the problem of exploiting the antioxidant

properties of plant raw materials was implemented by the authors (Robert et al., 2010), which is incapsulation of pomegranate juice alcohol extractions into malt-dextrin capsules with a help of spray drying technology.

The authors (Rozek et al., 2010) proposed the method of adding the industrially produced phenolic extraction from grapes, which were obtained by osmotic procession of grape seeds and white grape mash, into different food bases.

Lipophilic bioflavonoid (rutine) derivatives, due to their capability of capturing the free radicals and retard the lipid peroxide oxydation (in other words, revealing the antioxidant properties) are also recommended to protect the foodstuffs from oxidation. Bioflavonoids (namely quercetine) can be extracted from onion husk in the supercritical state (Ko Min-Jung et al., 2011), on the basis of which the possibility to substitute the organic solvents by water in food technologies is proved properly.

Henceforth, selection of the raw materials with high antioxidant activity, methods of natural antioxidant obtaining and spheres to use them are being elaborated and improved, regarding the constant threat of uncontrolled free-radical processes and the necessity to neutralize them.

Process of bioflavonoid extraction from herbal raw materials

Herbs are used to treat the diseases in humans and animals either directly or as a raw material for chemical and pharmaceutical industry (Chekman, 2000). Among 100,000 medicines well-known in the world, circa 30,000 are produced from plants (WHO, 1998). According to the WHO, 10,000 species of herbs are exploited with medicinal purposes in 73 high economically developed countries (WHO 2004). Scientists are discovering the new BAS, widening the nomenclature of their existing groups, searching the possibilities to complexly use both the entire plant and its separate parts. The great deal of plant-originated substances reveals their ability to synergistic action (Pezzani et al., 2019). Along with that, the usage of herbs in production of foods for mass and special consumption is rapidly proliferating (Dadali et al., 2003). For example, it has become a tradition to add the various made of fruit, berry and herbal half products into confectionery items; on the other hand, many recipes with additives of herbs – like ginseng, chamomile, marigolds etc. – in different aggregate states (powders, water, alcohol, ether extractions, purees) for fortification of food bases (Spirichev, 2003). Herbal BAS are confirmed to be congenial to human organism; therefore, as a constituent of easily absorbed food complexes, they are the essential link of the structure and effective functioning of human organism systems, including the one of antioxidant protection (Levitskyi, 2011).

While fruit and berries may be consumed directly or as half products to enrich many foodstuffs, herbal raw materials is being prepared in the form of water and water-and-alcohol extractions, condensed and pastous concentrates etc. (Dekebo, 2021). However, the antioxidant properties and antioxidant effectiveness of herbs is still studied sporadically (Gromovaya et al., 2008). Hence, this part of the article is dedicated to researches on extraction of herbal raw materials and elucidation of the conditions for maximal diffusion of bioflavonoids into the extract as the factor to characterize the antioxidant effectiveness of the latter (Shkarina et al., 2010). In studying the herbal materials, the main attention was paid to phenolic substances regarding their curative effect, particularly because they can easily create the complex compounds of flavonoids and metal ions (Simakhina, 2011).

Dispersion analysis showed the impact of the researched factors to define the effectiveness of extraction on the bioflavonoid output (Table 2).

Table 2

Influence of conditions to extraction of St. John's wort blossoms on bioflavonoid amount in the extract, mg/100 g

№	Extraction conditions	Bioflavonoid amount, mg/100 g
1	Dispersion of the material, mm	
	1	3.22
	3	3.26
	5	2.87
	7	2.46
2	Alcohol percentage in a water-and-alcohol solution	
	0	0.64
	30	2.75
	50	2.92
	70	3.24
	95	1.22
3	Proportion between raw material and an extracting substance	
	1 : 5	2.59
	1 : 10	3.28
	1 : 15	3.21
4	Extraction duration, minutes	
	30	2.44
	60	2.62
	90	3.26
	120	3.28

Since BAS of herbs mostly belong to thermo labile substances (Wills et al., 2000) extraction was conducted with a temperature of 35–40 °C. There was confirmed that the bioflavonoid amount in extractions from raw materials with particle dispersion of 1–3 mm is practically identical, but in case of larger particle size it lowers by 12 percents. The optimal concentration of alcohol in water-and-alcohol solution is averred 70 percents, on account of the fact that in 30 percents and 50 percents the rate of extracted bioflavonoids is lower, correspondingly, by 15 percents and 11 percents.

In water extraction, it was only 6 percents of bioflavonoids to diffuse into the extract. The mitigation of bioflavonoid output after extraction by pure alcohol can be explained by the fact that the significant amount of bioflavonoid substances transformed into constrained state. The largest part of bioflavonoids was extracted in proportion between the raw material and extracting substance of 1:10 and 90-minute duration of the process.

These results complement the information presented in (Inglett et al., 2011), which affirmed that far higher output of bioflavonoid substances is provided after extraction of plants by 70-percent water-and-alcohol solution than by water or absolute alcohol.

Statistical procession of the attained results evidences that the average relative error with probability of 95 percents is defined as 3.6 %. Therefore, the further researches were accomplished with the following parameters: dispersion of herbal particles – 1–3 mm, alcohol concentration in water-and-alcohol solution – 70 percents, Proportion between raw material and an extracting substance – 1:10, duration of the process – 90 minutes, temperature of the process – 35–40 °C.

The results of our researches are presented in Table 3 according to the descendant index of bioflavonoid extraction from 10 kinds of herbs.

Table 3

Percentage of extraction of bioflavonoids from plant raw materials

Raw materials	Optical density, units	Bioflavonoid amount/100 g		Bioflavonoid extraction percentage
		In raw	In potions	
1. St. John's wort blossoms	0.38	3.890	3.286	84.48
2. Black currant leaves	1.68	1.281	0.885	69.06
3. Melissa leaves	0.49	1.685	1.159	68.81
4. Thyme leaves	1.53	1.470	0.996	67.74
5. Salvia leaves	1.42	0.634	0.409	64.62
6. Immortelle blossoms	1.88	2.638	1.702	64.52
7. Oregano blossoms	1.72	2.980	1.175	39.44
8. Chamomile blossoms	1.44	0.472	0.139	29.46
9. Dead nettle herb	1.44	0.462	0.132	28.63
10. Birch leaves	0.47	0.825	0.203	24.62

As a result of the researches, there were determined the amount of bioflavonoids in raw herbs and the expedient concentrations of their extractions (potions). Additionally, it was stated that in case of optimal conditions of the process, the bioflavonoids are believed to diffuse completely into the extraction. For example, bioflavonoid extraction percentage is 84.46 for St. John's wort blossoms, and about 69 for leaves of black currant, melissa, thyme, salvia, and for immortelle blossoms. Talking about the other species of plants, the bioflavonoid output lowers to 24 percents (birch leaves) Such a range of the absolute indices of bioflavonoid amount in extractions may be explained to the certain extent by differences in tissue structure and biocomponent composition of various plants, which fact can impact the diffusion coefficient. All of the researched plants have different grade of antioxidant effectiveness that can be conditioned by bioflavonoid amount. According to this index, we composed a scale of their comparative assessment:

St. John's wort > Oregano >Immortelle > Melissa > Thyme *> Black currants > > Birch > Salvia > Chamomile > Nettle*

Thenceforth, the first five of this scale are alleged to be the most effective out of the herbs researched, so they would be widely and successfully used in production of foods and drinks with antioxidant action as the constituents of a diet minimizing toxic influence of free-radical processes on human organism (Kähkönen et al., 1999). Last but not least, these plants are expected to occupy their place in medical practice, particularly in antioxidant therapy (Menshieva et al., 1994).

Conclusions

1. All the biologically active substances, which are necessary for normal vital activity of a human, are consumed with food, drinks and herbal remedies and are further biologically transformed, digested and absorbed. In transformations into structural and functional cellular elements during metabolic processes, BAS provide the mental and physical endurance of the organism; determine one's state of health and workability. The lack of certain biological components in a diet inevitably leads a human to detrimental consequences.
2. Within the variety of biologically active substances, the significant group is comprised by antioxidants such as ascorbic acid, bioflavonoids and carotenoids, which are able to block the harmful free-radical processes in human organism. The latter are usually triggered by the excessive amount of active oxygen forms damaging the molecules of proteins, nucleic acids, cellular membranes etc. and therefore causing different pathologies.
3. The raw berries and herbs are the plentiful natural source of antioxidants, which statement may be proves by our results of researches on their biological composition. In our insight into biological systems, human organism primarily, we can predict that foods produced with the usage of berries and herbs (the natural antioxidant concentrators) would demonstrate the proper antiradical activity by decreasing of the level of hydroxyl radicals that are the most reactively capable intermediates of oxygen restoration in the system; along with that, they would reveal the antioxidant properties in blocking the peroxide oxidation processes. In a nutshell, quite a topical, prospective, and precisely oriented at amelioration of human health is the problem of elucidating the chemical composition of widespread and unknown kinds of domestic plants, inquiries of the new sources of antioxidants with their further application to obtain the wide array of antioxidant foodstuffs.

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Influence of sous-vide thermal treatment, boiling, and steaming on the colour, texture and content of bioactive compounds in root vegetables

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Keywords:

Sous-vide
Boiling
Steaming
Root vegetables

Article history:

Received 21.09.2020
Received in revised form 14.11.2020
Accepted 25.03.2021

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DOI:
10.24263/2304-974X-2021-10-1-7

Abstract

Introduction. The aim of the study was to compare the effect of sous-vide treatment, cooking in boiling water, and steaming on changes in the colour, texture, and retention of selected bioactive compounds in root vegetables.

Materials and methods. Carrots and parsley were subjected to sous-vide thermal treatment (SV) at 80 °C (SV 80) and 90 °C (SV 90), cooking in boiling water (B), and steaming (S) for 10, 20, and 30 minutes. Instrumental texture properties were assessed by texture profile analysis (TPA). The colour was measured with a colorimeter, and the content of total phenolic compounds and carotenoids was determined using the spectrophotometric method.

Results and discussion. The hardness, cohesiveness, and chewiness of the analysed vegetables differed significantly depending on the method and duration of culinary processing. The highest hardness, cohesiveness, and chewiness values were demonstrated for the SV 80 variants.

Most of the colour parameters of the vegetables differed statistically significantly depending on the method and duration of culinary processing. The lowest brightness (L*) was demonstrated for parsley B (20-min treatment), and the highest value of the parameter was noted for parsley samples SV 80 (10-min treatment). The highest values of parameter a*, which is the component of orange colour and determine the consumer attractiveness of carrots, were recorded in samples SV 80 and SV 90. Statistically significant differences were found in the b* value between the types of thermal treatment applied. The highest value of yellowness was noted for samples R (raw), whereas samples B (20-min treatment) had the lowest values of this parameter. In the case of carrots, the highest values of parameter b* were recorded in samples SV 90 (10-min treatment), and samples R exhibited the lowest yellowness value.

The highest retention of phenolic compounds was detected in parsley B (20-min treatment) and carrots SV 90 (20-min treatment). The highest value of carotenoid retention was reported for parsley SV 90 (10-min treatment) and carrot SV 80 (10-min treatment).

Conclusions. Compared to the boiled and steamed samples, sous-vide vegetables have higher hardness, cohesiveness, chewiness, and consumer-attractive colour. Carrots processed with this technique exhibit higher retention of carotenoids.

Introduction

Vegetables are a rich source of nutrients and bioactive compounds. They are often subjected to heat treatment before consumption to increase their digestibility and improve their flavour. These treatments are accompanied by physical and chemical processes that result in changes in the texture and chemical composition (Saikai et al, 2013). The appearance and texture of vegetables determine the perception of their freshness by consumers (Saba et al., 2018) and influence the consumer willingness to eat this type of food (Torres de Castro et al., 2020). An adequate choice and a proper course of the culinary processing method yield products with high health-enhancing and sensory quality (Saikai et al, 2013).

The sous-vide technology is an increasingly popular method for thermal treatment. It is based on the use of low process temperature (below 100 °C) and vacuum packing of raw materials. Sealing raw foods in plastic bags prevents loss of nutrients, as is the case when cooking in boiling water (Michalak-Majewska et al., 2018). Vegetables cooked with the sous-vide technique are characterised by higher antioxidant potential than those subjected to other culinary processing methods (Kosewski et al., 2018). The sous-vide cooking method contributes to reduction of losses of chlorophyll, carotenoids, phenolic compounds (Guillén et al., 2017), anthocyanins (Iborra-Bernad et al., 2014), and volatile aroma substances during the thermal treatment of vegetables (Rinaldi et al., 2013). There are promising results of research on the retention of minerals in raw materials of plant origin (Rondanelli et al., 2017). Sous-vide products are perceived by consumers as the highest quality food with a number of advantages. Nevertheless, a dose of scepticism is raised by the need to pack the raw material in plastic bags and the fear of chemical compounds permeating from the packaging to the raw material or reactions of food ingredients with the packaging (Roascio-Albistur et al., 2018).

The aim of the study was to compare the effect of the sous-vide thermal treatment, cooking in boiling water, and steaming on the colour, texture, and content of some bioactive compounds in root vegetables.

Materials and methods

Vegetable materials and sample preparation

Fresh carrots (*Daucus carota* var. 'Aneta F1') and root parsley (*Petroselinum crispum* ssp. *Tuberosum* var. 'Sonata') were obtained from crops grown on the Felin Experimental Farm of the University of Life Sciences in Lublin (Poland; 51°22'N, 22°64'E). The vegetables were harvested a week before the experiments and stored at 4 ºC until use.

All vegetables were washed, cleaned, peeled, and sliced into 10 mm±1 mm thick cylinders using a stainless steel knife. For the sous-vide treatment, the raw vegetables discs were vacuum sealed in plastic cooking vacuum bags (Stalgast, Poland). Air was removed from the bags, which were then sealed using a vacuum packaging machine (Lerica, model: Levac 3, Italy). For boiling and steaming, the vegetables were placed in water without vacuum packaging.

After cooking, all samples were immersed in an ice-water bath for rapid cooling. Boiled and steamed vegetables were sealed (no vacuum was applied) in plastic bags to prevent contact with water. The SV samples were cooled in the same bags.

The texture and colour measurements were performed after cooling the samples. Vegetables intended for chemical analysis were cooled, frozen, and lyophilised in a laboratory freeze drier (Alpha 1-2 LD plus; Martin Christ, Osterode am Harz, Germany). The freeze-dried material was fragmented in a mill and subjected to a further procedure.

Cooking conditions

Three cooking methods were applied: boiling, steaming, and sous-vide cooking.

Boiling (B) was carried out in a stainless steel pot and a cooking top (Stalgast IP 23, Poland) for 10 min, 20 min, or 30 min. The raw vegetable discs were placed in the pot when the water reached its boiling point (100 ºC at atmospheric pressure) with a constant product weight:water volume ratio of 1:4.

For the steaming treatment (S), the samples were placed in a single layer on a steamer floor (Zelmer, model: 37Z010, Poland) with an appropriate distance, following the instruction manual. The raw material was steamed for 10 min, 20 min, or 30 min.

Cooking with the sous-vide method (SV) was performed using a sous-vide water bath (Hendi, model: 225448, Netherlands). The bags were immersed in hot water, and covered with a special limiter to prevent floating. The SV cooking conditions were 80 ºC (SV 80) for 10 min, 20 min, or 30 min and 90 ºC (SV 90) for 10 min, 20 min, or 30 min.

Colour measurement

Colour was measured using a Minolta CR-310 colorimeter (Osaka, Japan). The instrument was calibrated using standard white and black ceramic standards before use. The results were given in the CIELab colour system for illuminant D65. Six replicates were carried out for each vegetable and cooking condition. Parameters L* (brightness), a* (greenness-redness), and b* (blueness-yellowness) were registered. These parameters were used to calculate the total colour difference using the following equation:

$$\Delta E = [(L^* - L^*_0)^2 + (a^* - a^*_0)^2 + (b^* - b^*_0)^2]^{1/2}$$

where L^*, a^*, and b^* are the colour values of SV (SV 80 and SV 90), B, and S samples. L^*_0, a^*_0, and b^*_0 are the colour values of the raw vegetables (R).

Textural analysis

The Textural Profile Analysis (TPA) test was carried out using a TA-XTi texture analyser (Stable Micro Systems, Surrey, UK) with the Texture Expert program (version 1.22). To obtain a sample with the same tissue orientation and dimension, a 40-mm-diameter cylindrical sample was drilled out of the vegetable slices with a cork borer and compressed to 30% of the original height with a 75-mm cylindrical aluminium probe. The compression was carried out with 5 seconds waiting time between the first and the second compression. The test speed was kept at 1 mm/s. Each group was analysed in six replications. The raw vegetable discs (R) were not analysed due to their high hardness. The textural parameters, i.e. hardness [N], springiness, cohesiveness, and chewiness, were determined from the curves obtained from TPA. Six replicates were carried out for each vegetable and cooking condition.

Solvent extraction

For the preparation of ethanolic extracts, powdered samples of the vegetables (1 g) were extracted for 0.5 h with 30 ml of 80% (*v*/*v*) ethanol and centrifuged (4500× *g* for 15 min). The supernatants were used for further analysis. All extractions and chemical analyses were conducted in triplicate.

Total phenolic content

Total polyphenol content (TPC) was determined according to the method proposed by Singleton and Rossi (Singleton, 1965) with some changes (Radzki et al., 2014). The amount of TPC was expressed as gallic acid equivalents (GAE) in mg per 100 g of dry matter (d.m.).

Carotenoid determination

Carotenoids were determined according to the Polish standard (Przetworów Owocowych i Warzywnych, 1990) and expressed in mg per 100 g of d.m.

Statistical analysis

Statistical analysis was performed using Statistica 13.1 program (Statsoft, Cracow, Poland), applying Tukey's HSD test in the analysis of variance (ANOVA) to estimate the significance of the differences between the mean values at $p < 0.05$.

Results and discussion

Colour

The results of the colour of parsley are presented in Table 1.

Table 1

CIELab coordinates in root parsley under different conditions

Cooking conditions	Cooking time	CIELab coordinates			
		L*	a*	b*	ΔE
Raw (R)	N.A.	74.82 ± 0.94^{g}	-2.47 ± 0.08^{b}	6.95 ± 0.66^{d}	N.A.
Sous-vide 80 ºC (SV 80)	10 min	61.04 ± 0.69^{f}	-3.75 ± 0.20^{a}	6.25 ± 0.26^{d}	13.86 ± 0.69^{a}
	20 min	55.24 ± 2.01^{cde}	-3.67 ± 0.38^{a}	4.03 ± 0.18^{c}	19.84 ± 1.94^{bcd}
	30 min	57.35 ± 1.11^{ef}	-3.61 ± 0.39^{a}	3.92 ± 0.24^{c}	17.78 ± 1.09^{ab}
Sous-vide 90 ºC (SV 90)	10 min	55.88 ± 1.40^{def}	-3.55 ± 0.14^{a}	3.63 ± 0.25^{c}	19.26 ± 1.4^{abc}
	20 min	53.33 ± 1.31^{cde}	-3.64 ± 0.22^{a}	2.64 ± 0.22^{b}	21.95 ± 1.26^{bcd}
	30 min	52.87 ± 3.08^{cde}	-3.56 ± 0.38^{a}	2.33 ± 0.31^{b}	22.47 ± 2.97^{bcd}
Steaming (S)	10 min	50.99 ± 1.91^{bcd}	-3.54 ± 0.3^{a}	1.84 ± 0.17^{b}	24.40 ± 1.91^{cde}
	20 min	49.85 ± 0.34^{abc}	-3.56 ± 0.37^{a}	1.80 ± 0.17^{b}	25.52 ± 0.33^{def}
	30 min	50.91 ± 2.10^{abcd}	-3.53 ± 0.24^{a}	2.08 ± 0.32^{b}	24.42 ± 2.09^{cde}
Boiling (B)	10 min	50.76 ± 1.57^{abcd}	-3.37 ± 0.46^{a}	4.33 ± 0.33^{c}	24.23 ± 1.58^{cde}
	20 min	45.19 ± 4.02^{a}	-3.32 ± 0.25^{ab}	0.65 ± 0.22^{a}	30.32 ± 3.91^{f}
	30 min	45.59 ± 1.66^{ab}	-3.40 ± 0.13^{a}	4.07 ± 0.11^{c}	29.39 ± 1.66^{ef}

N.A.: not available. $^{a–f}$ Values in the same column are significantly different ($p \leq 0.05$).

Parameter L* expressing the brightness of the samples ranged from 45.19 to 74.82. The brightness of the thermally treated samples differed significantly from samples R. The lowest value of the parameter was demonstrated for parsley samples B (20-min treatment), whereas samples SV 80 (10-min treatment) were characterised by the highest brightness. Samples SV exhibited higher brightness than samples B and S; hence, they can be more attractive to consumers. The brightness of the colour of fresh-cut vegetables influences the perception of their freshness by consumers (Barret et al., 2010). In the case of the carrots, samples SV 80 (10-min treatment) had the highest brightness, while the lowest value of the parameter was shown for carrot samples B (10-min treatment). However, the value of brightness was not statistically significantly different from most of the samples (Table 2).

Table 2

CIELab coordinates in carrot under different conditions

Cooking conditions	Cooking time	CIELab coordinates			
		L*	a*	b*	ΔE
Raw (R)	N.A.	43.42 ± 1.03^{ab}	4.76 ± 0.37^{a}	14.68 ± 0.51^{a}	N.A.
Sous-vide 80ºC (SV 80)	10 min	45.16 ± 2.02^{b}	12.51 ± 0.56^{cd}	32.54 ± 1.42^{f}	19.63 ± 1.33^{e}
	20 min	43.73 ± 3.51^{ab}	11.30 ± 0.23^{bcd}	27.62 ± 0.82^{de}	14.79 ± 0.74^{d}
	30 min	43.65 ± 1.44^{ab}	11.76 ± 0.27^{bcd}	27.40 ± 1.05^{de}	14.58 ± 0.79^{cd}
Sous-vide 90 ºC (SV 90)	10 min	42.82 ± 1.48^{ab}	12.80 ± 1.10^{d}	27.48 ± 1.43^{de}	15.20 ± 1.56^{d}
	20 min	43.68 ± 1.42^{ab}	12.73 ± 0.40^{d}	27.32 ± 1.29^{cde}	15.02 ± 0.98^{d}
	30 min	42.46 ± 1.07^{ab}	11.75 ± 1.08^{bcd}	25.23 ± 1.05^{bcd}	12.73 ± 1.34^{bcd}
Steaming (S)	10 min	41.76 ± 2.20^{ab}	10.58 ± 0.6^{bcd}	24.42 ± 1.07^{bcd}	11.64 ± 0.69^{abc}
	20 min	41.64 ± 1.56^{ab}	10.36 ± 1.36^{bc}	24.12 ± 0.9^{bc}	11.25 ± 0.88^{ab}
	30 min	40.38 ± 1.11^{ab}	9.53 ± 0.59^{b}	23.55 ± 1.07^{b}	10.59 ± 0.78^{ab}
Boiling (B)	10 min	39.64 ± 2.04^{a}	5.83 ± 1.08^{a}	22.39 ± 1.29^{b}	8.78 ± 1.94^{a}
	20 min	41.40 ± 1.11^{ab}	9.75 ± 0.68^{b}	29.11 ± 1.00^{e}	15.44 ± 0.88^{d}
	30 min	41.67 ± 0.51^{ab}	6.91 ± 0.55^{a}	25.47 ± 0.82^{bcd}	11.16 ± 0.84^{ab}

N.A.: not available. $^{a–f}$ Values in the same column are significantly different ($p \leq 0.05$).

As demonstrated by Mazzeo et al. (Mazzeo et al., 2011), steaming (S) significantly reduced the brightness of carrots, whereas treatment B did not change this parameter significantly. The authors emphasise, however, that the stability of the colour in their experiment may have been influenced by the fact that the carrots were blanched prior to the

treatment. It has also been reported that sous-vide (SV) cooking does not significantly alter the brightness of thermally processed cruciferous vegetables, compared to thermally unprocessed vegetables (Lafarga et al., 2018). The present study showed that parsley that was in direct contact with water (B and S) was characterised by a greater total difference in the colour and lower brightness than samples SV. Similar observations were reported by Tomaszewska et al. (Tomaszewska et al., 2012), who showed that the direct contact of the heating agent with the raw material (steaming or boiling in water) had a significant impact on the differentiation of carrot brightness. In turn, Guillen et al. (Guillén et al., 2007) observed no significant differences in the brightness of vegetables subjected to the sous-vide treatment, cooked in boiling water, and cooked in water at <100 °C.

The values of parameter a* differed significantly between parsley samples R and the thermally treated samples. There were no statistically significant differences in parameter a* of the samples between the type of the thermal treatment. In the case of the carrot samples, significant differences were found in the values of parameter a* depending on the thermal treatment type. The significant differences in the values of this parameter may result from the fact that carotenoids, responsible for the yellow and red colour, are present mainly in chromoplasts surrounded by the cell wall and membrane (Zielińska et al., 2011). Thermal treatment destroys the cell structure and promotes the penetration of crystalline chromoplasts into the intercellular space (Marx et al., 2003). This is probably the reason why the untreated carrots in the present study were significantly less yellow and red than the thermally treated carrots. These results indicate that the SV 90 carrot samples (10-min treatment) had the highest value of redness, while samples R were the least red. In the case of carrot samples that were in direct contact with the thermal agent (B and S), the red colour was less saturated than in samples R, SV 80, and SV 90. A decline in the intensity of the red colour of carrots during boiling and steaming was reported by other authors as well (Mazzeo et al., 2011). It can be observed that the intensity of the red colour decreased with the duration of the thermal treatment in the case of all the methods employed, with the exception of sample B. Similar observations were reported by Biller and Wierzbicka (Biller et al., 2015), where the mean values of parameter a* declined successively after each heating stage.

The values of parameter b* differed significantly between the untreated and thermally treated parsley samples. Similarly, statistically significant differences were found in the b* value between the types of thermal treatment applied. The highest value of yellowness was noted for samples R, whereas samples B (20-min treatment) had the lowest values of this parameter. In the case of carrots, the highest values of parameter b* were recorded in samples SV 90 (10-min treatment), and samples R exhibited the lowest yellowness value. It was evident that the intensity of the yellow colour declined with the duration of the thermal process in all processing methods except for samples B. Schifferstein et al. (Schifferstein et al., 2019) have demonstrated that consumers most often choose orange carrots for consumption. At low saturation of the orange colour, carrots are perceived as unattractive and less fresh. Therefore, high values of parameters a* and b* (components of the orange and yellow colour respectively) are particularly important in consumer assessment of the attractiveness of these vegetables. High values were noted for samples SV 80 and SV 90, which may suggest that sous-vide carrots will be particularly attractive to consumers.

Taking parsley R as the standard, the smallest total difference in the colour (ΔE) was demonstrated for SV 80 (10-min treatment), while the highest value of the parameter was recorded for parsley samples B (20-min treatment). As reported by Marić et al. (2020), the highest ΔE was noted in parsley subjected to convection drying at a temperature of 70 ºC, and the lowest value of the parameter was recorded in the same type of convection drying at a temperature of 50 ºC. In the present study, the high temperature of the thermal treatment

(B) was associated with highest ΔE value, and the heat treatment at low temperature (SV 80) induced the lowest values of the parameter. In the case of carrots, the lowest and the highest values of the total colour difference (ΔE) were demonstrated for samples SV 80 (10-min treatment) and samples B (20-min treatment), respectively. Torres de Castro et al. (Torres de Castro et al., 2020) showed greater changes in the colour of carrots induced by treatment S *vs*. B. The present study demonstrated larger colour differences between these two processing methods only in the 20-min treatment variant.

Texture

The hardness of parsley ranged from 24.11 to 218.53 N, with significant differences between the treatment methods and duration of processing (Table 3). The lowest hardness was exhibited by parsley samples S (30-min treatment). In turn, parsley SV 80 was the hardest (10-min treatment). A comparison of the samples revealed that the hardness parameter in each time variant decreased in the following order: SV 80> SV 90> B> S. The hardness of the carrots also differed significantly depending on the variant and duration of the thermal treatment (Table 4). A comparison of the samples showed the following order of the decline in the hardness parameter in each time variant (except the 10-min B and S treatments): SV80> SV 90> S> B. The lowest value of hardness was recorded for samples B and S (30-min treatment), whereas the highest values of this parameter were determined for samples SV 80. In their investigations of the hardness of sous-vide cooked carrots, (Koç et al., 2017) showed a decline in this parameter along the processing time and with a rise in the process temperature. The hardness of carrots cooked in boiling water for 30 min was comparable to that in samples subjected to sous-vide cooking at 75 °C for 120 min. Similar results were obtained in the present study, i.e. the SV vegetables were even 3 times harder than the B and S vegetables at the same duration of the treatments. The results reported by Xu et al. (Xu et al., 2015) show that the hardness parameter in carrots is more potently influenced by high temperature than by the duration of the thermal process. Similar observations were reported by other authors as well (Vu et al., 2004). High temperatures directly damage the cell walls, thereby changing the texture. The non-enzymatic degradation of cell walls in food of plant origin occurs most effectively at a temperature > 80 °C and contributes to depolymerisation of pectins (Sila et al., 2008). This is a probable explanation of the higher hardness value in samples SV 80 than SV 90 in each time variant. The analysis of the present results indicates that the significant differences in hardness may also be associated with the absence of direct contact with the heating medium (hot water) in the case of samples SV *vs*. samples B and S. This may contribute to inhibition of pectin dissolution. The absence of direct contact with hot water in the case of SV reduces heat transfer to the heated product, which may also influence texture changes. Similar observations of the impact of the absence of direct contact of the heated product with water on the texture of vegetables were reported by other authors as well (Xie,2000; Iborra-Bernad et al., 2015)

No statistically significant differences were found in the case of springiness of the analysed vegetables (Tables 3 and 4).

Cohesiveness is described as a tendency of food to lose its structural form after the first compression test in texture analysis. Potato chips and mashed potatoes are products with low cohesiveness (Wee et al., 2018). The cohesiveness of the analysed vegetable samples differed statistically significantly (Tables 3 and 4). The highest value of this parameter was recorded for parsley samples B (20-min treatment) and carrots SV 80 (20-min treatment). The SV technique yielded vegetables with the highest cohesiveness, compared with the other methods. The lowest values of this parameter were found for parsley samples S (30-min treatment) and carrot samples S (30-min treatment).

Table 3

Results of the TPA measurement of root parsley

Cooking conditions	Cooking time	Texture diameter			
		Hardness (N)	**Springiness**	**Cohesiveness**	**Chewiness**
Sous-vide 80 ºC (SV 80)	10 min	218.53 ± 35.18^{f}	0.78 ± 0.01^{a}	0.50 ± 0.02^{de}	8638.01 ± 1529.28^{d}
	20 min	178.44 ± 50.36^{ef}	0.75 ± 0.02^{a}	0.50 ± 0.02^{de}	6825.53 ± 1587.74^{cd}
	30 min	170.13 ± 3.25^{ef}	0.78 ± 0.02^{a}	0.47 ± 0.02^{cde}	6454.49 ± 522.45^{cd}
Sous-vide 90 ºC (SV 90)	10 min	135.59 ± 20.77^{de}	0.77 ± 0.06^{a}	0.49 ± 0.04^{cde}	3960.82 ± 2336.18^{bc}
	20 min	104.81 ± 28.46^{cd}	0.78 ± 0.08^{a}	0.38 ± 0.02^{abc}	3801.18 ± 1565.85^{abc}
	30 min	46.61 ± 9.32^{abc}	0.78 ± 0.06^{a}	0.34 ± 0.06^{ab}	970.49 ± 966.34^{ab}
Steaming (S)	10 min	63.22 ± 10.54^{abc}	0.82 ± 0.05^{a}	0.36 ± 0.05^{ab}	1878.91 ± 404.92^{ab}
	20 min	38.95 ± 9.01^{ab}	0.82 ± 0.01^{a}	0.35 ± 0.05^{ab}	1122.08 ± 245.30^{ab}
	30 min	24.11 ± 4.20^{a}	0.74 ± 0.02^{a}	0.28 ± 0.02^{a}	509.08 ± 118.04^{a}
Boiling (B)	10 min	91.77 ± 12.35^{bcd}	0.87 ± 0.01^{a}	0.45 ± 0.03^{bcde}	4196.23 ± 611.44^{bc}
	20 min	46.86 ± 20.11^{abc}	0.88 ± 0.01^{a}	0.52 ± 0.06^{e}	2210.79 ± 1166.75^{ab}
	30 min	44.83 ± 3.52^{abc}	0.80 ± 0.11^{a}	0.40 ± 0.03^{bcd}	1470.73 ± 267.73^{ab}

$^{a–f}$ Values in the same column are significantly different ($p \leq 0.05$).

The comparison of the processing methods demonstrated the following order of the decline in the chewiness parameter in each time variant: SV 80> SV 90> B> S. Significant differences were found between the treatment methods and time variants. The highest chewiness parameter was recorded for root parsley samples SV 80 (10-min treatment) and carrot SV 80 (20-min treatment). In turn, root parsley samples S (30-min treatment) and carrot B (30-min treatment) exhibited the lowest chewiness.

Table 4

The results of the TPA measurement of carrot

Cooking conditions	Cooking time	Texture diameter			
		Hardness (N)	**Springiness**	**Cohesiveness**	**Chewiness**
Sous-vide 80 ºC (SV 80)	10 min	265.79 ± 15.99^{d}	0.71 ± 0.03^{ab}	0.49 ± 0.01^{c}	9344.98 ± 613.74^{bc}
	20 min	274.03 ± 32.69^{d}	0.75 ± 0.06^{ab}	0.55 ± 0.06^{c}	11579.84 ± 3243.77^{c}
	30 min	265.51 ± 2.67^{d}	0.74 ± 0.01^{ab}	0.48 ± 0.02^{c}	9517.82 ± 479.64^{bc}
Sous-vide 90 ºC (SV 90)	10 min	244.03 ± 5.14^{d}	0.74 ± 0.02^{ab}	0.48 ± 0.01^{c}	8726.07 ± 329.30^{bc}
	20 min	255.84 ± 19.84^{d}	0.66 ± 0.07^{a}	0.47 ± 0.01^{c}	8127.94 ± 1078.16^{b}
	30 min	163.75 ± 6.89^{c}	0.71 ± 0.02^{ab}	0.32 ± 0.01^{ab}	3781.48 ± 159.52^{a}
Steaming (S)	10 min	118.64 ± 19.02^{bc}	0.78 ± 0.02^{b}	0.30 ± 0.03^{ab}	2998.06 ± 689.80^{a}
	20 min	145.60 ± 5.02^{bc}	0.73 ± 0.07^{ab}	0.31 ± 0.06^{ab}	3255.01 ± 270.22^{a}
	30 min	103.61 ± 0.29^{ab}	0.75 ± 0.02^{ab}	0.26 ± 0.03^{a}	2042.05 ± 306.50^{a}
Boiling (B)	10 min	136.05 ± 36.46^{bc}	0.79 ± 0.01^{b}	0.37 ± 0.03^{b}	4088.03 ± 1309.73^{a}
	20 min	112.35 ± 14.63^{bc}	0.68 ± 0.02^{ab}	0.37 ± 0.04^{b}	2871.98 ± 94.64^{a}
	30 min	60.41 ± 17.29^{a}	0.76 ± 0.05^{ab}	0.32 ± 0.02^{ab}	1483.86 ± 382.36^{a}

$^{a–d}$ Values in the same column are significantly different ($p \leq 0.05$).

Bioactive compounds content

The content of total phenolic compounds in the vegetables differed depending on the method and duration of the thermal treatment (Tables 5 and 6). The lowest content of these compounds was detected in parsley samples SV 90 (30-min treatment) and carrot samples R. The highest phenolic compound levels were determined in samples parsley B (20-min treatment) and carrot samples SV 90 (20-min treatment). The R samples of parsley cv. Sonata analysed in the present study had lower content of phenolic compounds (181 mg/100 g d.m.) than other cultivars available on the Polish market (303 mg/100 g d.m.) (Cieślik et al., 2006). The content of phenolic compounds was significantly higher in the vacuum non-packed samples (B and S) subjected to the higher temperature treatment than in samples SV 90. However, some authors indicate that vacuum packing increases the content of phenolic acids in vegetables (Ravichandran et al., 2012). The present study also demonstrated that the content of phenolic compounds in samples R was lower than that in the thermally treated samples. This may have been related to the more severe thermally induced damage to the cell walls and thus more efficient release of phenolic compounds. Similar observations were reported by other authors as well (Ismail et al., 2004; Chumyam et al., 2013; Juániz et al., 2016).

Table 5

Carotenoids (mg/100 g dry matter) and total phenolics content (mg GAE/100 g dry matter) in root parsley cooked under different conditions

Cooking conditions	Cooking time	Carotenoids	Total phenolics
Raw (R)	N.A.	0.135 ± 0.00^{gh}	180.79 ± 2.07^{cd}
Sous-vide 80 ºC (SV 80)	10 min	0.110 ± 0.00^{ab}	176.39 ± 7.66^{c}
	20 min	0.127 ± 0.00^{ef}	190.43 ± 2.13^{cde}
	30 min	0.126 ± 0.00^{de}	141.94 ± 5.22^{ab}
Sous-vide 90 ºC (SV 90)	10 min	0.142 ± 0.00^{h}	176.98 ± 2.51^{c}
	20 min	0.120 ± 0.00^{cd}	154.04 ± 3.15^{b}
	30 min	0.104 ± 0.00^{a}	136.39 ± 5.86^{a}
Steaming (S)	10 min	0.123 ± 0.00^{cde}	189.61 ± 6.88^{cde}
	20 min	0.127 ± 0.00^{de}	196.89 ± 9.41^{de}
	30 min	0.134 ± 0.00^{fg}	185.96 ± 4.54^{cde}
Boiling (B)	10 min	0.137 ± 0.00^{gh}	178.08 ± 7.44^{c}
	20 min	0.123 ± 0.00^{cde}	203.51 ± 9.19^{e}
	30 min	0.117 ± 0.00^{bc}	185.44 ± 3.68^{cd}

N.A.: not available. $^{a–f}$ Values in the same column are significantly different ($p \leq 0.05$).

Table 6

Carotenoids (mg/100 g dry matter) and total phenolics content (mg GAE/100 g dry matter) in carrot cooked under different conditions

Cooking conditions	Cooking time	Carotenoids	Total phenolics
Raw (R)	N.A.	26.76 ± 0.51^{f}	94.74 ± 2.37^{a}
Sous-vide 80 ºC (SV 80)	10 min	32.70 ± 0.33^{g}	113.69 ± 3.44^{cdef}
	20 min	31.04 ± 0.36^{g}	119.44 ± 3.84^{ef}
	30 min	32.10 ± 0.09^{g}	113.92 ± 5.45^{cdef}
Sous-vide 90 ºC (SV 90)	10 min	26.25 ± 0.84^{f}	121.92 ± 3.79^{f}
	20 min	27.32 ± 0.71^{f}	123.54 ± 2.32^{f}
	30 min	14.06 ± 0.29^{c}	104.16 ± 2.68^{abc}
Steaming (S)	10 min	22.92 ± 0.99^{e}	106.10 ± 5.11^{abcd}
	20 min	6.21 ± 0.10^{a}	110.26 ± 1.35^{bcde}
	30 min	22.73 ± 0.78^{e}	116.86 ± 4.19^{def}
Boiling (B)	10 min	25.73 ± 0.47^{f}	121.44 ± 7.26^{ef}
	20 min	19.68 ± 0.80^{d}	113.92 ± 2.94^{cdef}
	30 min	11.75 ± 0.32^{b}	101.21 ± 0.79^{ab}

N.A.: not available. $^{a–f}$ Values in the same column are significantly different ($p \leq 0.05$).

The content of carotenoids in the parsley was negligible (0.104-0.142 mg/100 g d.m.). The level of these compounds in the carrots ranged from 6.21 mg/100 g d.m. in samples S (20-min treatment) to 32.70 mg/100 g d.m. in samples SV 80 (10-min treatment). The content of carotenoids may increase with the duration of thermal treatment of some vegetables. After exceeding certain treatment duration, this value was found to decline again (Kao et al., 2014). As suggested by Cuéllar-Villarreal et al. (Cuéllar-Villarreal et al., 2016), the degradation of carotenoids in carrots is associated with the activity of oxidative enzymes, and their

inactivation promotes the retention of these compounds. The present results confirm these observations. The vacuum packing and simultaneous reduction of the oxygen content in the package inhibited the activity of oxidative enzymes. This resulted in the highest content of carotenoids in the analysed carrots in samples SV 80. Carrot samples SV 90 had a lower carotenoid level than samples SV 80; therefore, temperature may be an additional determinant of the retention of these compounds.

Conclusion

The present results indicate that the different heat treatment types have a variable impact on the colour, texture, and retention of some bioactive compounds in root vegetables. In comparison with boiled or steamed vegetables, sous-vide vegetables are characterised by higher hardness, cohesiveness, chewiness, and a desirable colour as well as higher retention of carotenoids (in the case of carrots). Thermally treated carrots have significantly higher content of phenolic compounds than untreated carrots. The content of phenolic compounds in boiled and steamed parsley is significantly higher than in samples of this vegetable subjected to sous-vide treatments at 90 °C.

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Technological properties of potato starch treated by Heat-Moisture Treatment with addition of organic acids

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Keywords:

Starch
Modification
HMT
Lactic acid
Citric acid

Article history:

Received 20.09.2020
Received in revised form 10.12.2020
Accepted 25.03.2021

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DOI: 10.24263/2304-974X-2021-10-1-8

Abstract

Introduction. Starch has been modified to overcome industrial limitations present in its native form. Heat-moisture treatment (HMT) is essentially a physical and thermal method applied to modify starch. It can be combined with some chemicals to provide unique technological characteristics to these biopolymers.

Materials and methods. In this study, the potato starch was modified by conventional HMT using water and with 0.2 mol L^{-1} solutions of the organic acids lactic and citric up to 22% ratio. The influence on pasting and thermal properties of the potato starch was investigated. With the action of HMT, with water and acid, it was possible to observe structural changes in the starch.

Results and discussion. The commercial potato starch shown BC pattern type (B type with characteristics of C type). The treated starches have a C pattern type, with main diffraction peaks 2θ at 5.6º, 15.3º, 17.3º and 23.5º, respectively. A decrease in intensity peaks at 5.6º according each treatment was observed. The relative crystallinity (RC) of each starch was calculated being 27.2% from commercial potato starch which decrease to 19.9% to the treated with citric acid. Through pasting properties analysis (RVA) it was possible to observe that the viscosity peak presented a notable reduction from 7,824.00 mPa/s (native), to 90.00 mPa/s (HMT + citric acid). The Differential Scanning Calorimetry (DSC) was used to determine the values of onset (T_o), peak (T_p) and endset (T_c) temperatures of gelatinization as well as the enthalpy (ΔH_{gel}). The native (commercial) starch + HMT and the one treated with 22% water + HMT shown enthalpy 14.5 and 9,9 J g^{-1}, respectively. The others, treated with 22% solution of lactic acid + HMT and 22% solution of citric acid + HMT showed an enlargement between T_o and T_c with drastic reduction of viscosity.

Conclusions. The main application of this modified starch is in foods that require low viscosity, such baby food, soups and as an ingredient for baked products like cookies.

Introduction

Starch is a biodegradable polysaccharide, from a renewable source. It is composed mainly by amylose and amylopectin molecules. The amylose in which each unit of glucose is linked by α- (1 → 4) glycosidic bonds, and the amylopectin, which molecule is highly branched by glycosidic portions of both α- (1 → 4) and α- (1 → 6) glycosidic bond (Yassaroh et al., 2019). This biopolymer is the main storage carbohydrate produced by plants. It is widely processed and consumed by human as an energy source (Leonel et al., 2011). The source of starch can be very broad. The most used sources for human consumption regards to starches from cereals and tubers such as corn, wheat, potato and cassava starch (Zaman et al., 2016). The World Health Organization (WHO) recommends that more than 55% of the energy ingested by humans should be from carbohydrates, which means that studies on starches and their health benefits are highlighted (Pereira,2017). As ingredient starch is responsible for technological properties that characterize most products, contributing with texture, thickeness, colloidal stabilization, gelling, volume, adhesiveness, water retention, and many others (Jiranuntakul et al., 2011).

Potato (*Solanum tuberosum* L.) is the third most important food crop on the planet; it is the first non-grain commodity (dos Santos et al., 2016). This tuber has a significant importance when we talk about starch, because although most of its production is destined for fresh consumption. Potato starch, has been gaining market with thermal and thickening application in the food industry (Noda et al., 2006). Potato starch is composed of approximately 80% amylopectin and 20% amylose. In comparison with cereal starches, potato starch, gel forms with high viscosity, good consistency and clarity. Due to the presence of phosphate, gelatinized material shows high transparency (Noda et al., 2006; Shin et al., 2007). All these characteristics make it interesting to be used as thickeners for dehydrated soups and sauces, binding agents in sausages, puddings and desserts (dos Santos et al., 2016).

Despite having value in the industry, use of native or untreated starch present several problems such as handling, tendency to retrogradate, low freeze–thaw stability, and poor tolerance to many technological processes such as poor thermal resistance and low shear resistance (Barreti et al., 2020). To improve this limitation, starch granules can be modified by several methods, such as chemical, physical, biological and even combined processes (Schafranski et al., 2021). Among the most used starch modification methods in the industry, the most well-known are heat-moisture, dry heat, annealing, pre-gelatinization, high pressure, radiation, ultrasound, cross-linking, substitution, acid hydrolysis and oxidation treatments (Noda et al., 2006). The starch modification extent is affected by botanical source, composition, proportion of amylose to amylopectin, as well as by the disposition of the chains within the amorphous and crystalline regions of the starch granules (Schafranski et al., 2021).

HMT is essentially a physical modification comprises of heat-treating starch with low moisture content (10–30%), and high temperatures above starch gelatinisation (90–120 °C). This type of method modifies the physicochemical properties of starch without destroying its granular structure. The first research to use HMT to modify a cereal starch was reported in 1944, and since then several studies have been carried out to investigate the effects of this method applied to different botanical sources. Besides other modification methods like chemical and biological, HMT brings high effectiveness, simplicity, low costs and mainly the non-generation of hazardous chemicals residues. Thus, HMT can be an attractive technique for industrial food applications (Zavareze et al., 2011). Recently, some published studies have shown that the addition of organic acids solutions instead or combined with water (moisture) during HMT process is able to modify starch, due to partial hydrolysis and promoting structural alterations. These researches have been carried out using lactic, citric, and acetic acids at lower concentrations with the advantage of being food grade ingredients. Furthermore, they can be

used without limitations when inorganic acids are added during food applications (Barreti et al., 2020). Thus, the granules have been modified to be suitable for further applications. Modified starch is responsible for the main technological properties that configure many processed products.

Technological aspects and impacts provided by starch modifications can be observed by several analytical methods such as differential scanning calorimetry (DSC), powder x-ray diffractometry (PXRD), rapid visco analyser (RVA), among many others. DSC provides information regarding starch gelatinization, which is a regular event during an industrial process. DSC can provide the difference in enthalpy between a sample and a reference (normally an empty pan) as a function of temperature or time under controlled heating and cooling program. Furthermore, the equipment helps to obtain the event beginning or onset temperatures (To), endothermic peak (Tp), conclusion temperature (Tc), and gelatinisation enthalpy difference (ΔH) (Schafranski et al., 2021). RVA is a rotational viscometer that can measure the shear resistance or pasting properties of a sample. In addition, the equipment can reproduce thermal processing conditions when a sample is heated and cooled. During RVA analysis it is possible to obtain some important information such as maximum viscosity reached during the heating period and tendency to retrograde during cooling process (Barreti et al., 2020). PRXD is a useful tool to evaluate the impacts on the characteristics of starch crystalline structures. It is possible to observe the behaviour of peaks intensities, the pattern characteristic and the relative crystallinity (Zavareze et al., 2011).

Potato starch has been modified to overcome industrial limitations present in its native form, generating more suitable industrial products (Colussi et al., 2020). The modification technique, such as HMT (Heat-moisture treatment) combined with the addition of others, can bring more expressive and interesting results from the functional point of view, results that have been proven in several studies (dos Santos et al., 2016; Hung et al., 2016). Thus the present research aimed to investigate the impact of treatment by HMT using water (moisture) and solutions of lactic acid and citric acid on the structure and the pasting and thermal properties of the potato starch.

Materials and methods

Samples and reagents

The potato starch was purchased at a local supermarket in the city of Ponta Grossa (25° 05′ 42” S, 5° 09′ 43” W) Paraná, Brazil. The reagents for carrying out the analysis were at least analytical grade.

Methods

Heat-moisture-treatment (HMT) and Acid HMT

30 g of potato starch (dry base) was suspended in deionized water and in different solutions of citric acid and lactic acid each at 0.2 mol L^{-1}. Then, the samples treated in an acid medium were neutralized with sodium hydroxide 0.1 mol L^{-1} and then washed with deionized water. After, the slurry was filtered and dried, obtaining the adjusted level of final humidity at 22% and placed in hermetically sealed bottles. The samples were so maintained for 24 h at room temperature and then submitted to 110 °C in an oven (Tecnal, TE 394/1, Piracicaba, SP, Brazil) for 8 h. Until the analyzes were carried out, the samples were stored in a desiccator with anhydrous calcium chloride (Hung et al., 2016). The treated samples were identified as: native (commercial sample), HMT H_2O (heat-moisture treatment with 22% deionized water); HMT LA (heat-moisture treatment with 22% lactic acid) and HMT CA (heat-moisture treatment with 22% citric acid).

Powder X-Ray diffractometry (PXRD)

Diffractograms of samples were obtained using Ultima IV X-ray diffractometer (Rigaku, Japan) under following conditions: CuKα radiation (λ = 1.541 Å), voltage of 40 kV and current of 20 mA. The observed interval was from 3 to 40° to 2 (θ), step of 0.02° with scanning speed of 2° min^{-1} (Kubiaki, 2018). The main diffraction peaks were observed and Equation (1) was used to calculate the relative crystallinity (Hung et al., 2016; Shaikh et al., 2019):

$$X_c = A_p / (A_p + A_b)\ 100 \quad (1)$$

where X_c refers to relative crystallinity; $A_{p|}$ refers to the area of the X-ray diffractogram; and A_b refers to the amorphous area of the diffractogram.

Pasting properties (rapid visco analyser – RVA)

The pasting properties of the samples were determined with RVA-4 instrument (Newport Scientific, Australia). A dispersion in water of 8% (m / m) of starch on a dry basis in 28 g of total mass, was subjected to a cycle of heating and cooling under constant agitation, where they were kept at 50 °C for 2 min, heated by 50 to 95 °C to 6 °C min^{-1} and maintained at 95 ° C for 5 min; cooled to 50 ° C to 6 °C min^{-1} and held at 50 °C for 2 min (Maior at al., 2020).

Differential Scanning Calorimetry (DSC)

The DSC curves were obtained in a DSC 60 equipment (Shimadzu, Japan). The mass of the starch samples (2.5 mg) were weighed aluminum crucibles and it was added water up to a ratio 1:4 starch water. The crucibles were sealed, maintained by 60 min and so performed the DSC analysis. The DSC instrument conditions were: heating rate of 5 °C min^{-1}, heating from 30 to 100 °C under air atmosphere with flow of 50 mL min^{-1}. The instrument was calibrated according to the manufacturer's specifications and verified with a Indium standard purity index of 99.999%, m.p. = 156.6 °C, ΔH = 28.71 J $g.^{-1}$ (Lacerda et al., 2014; Andrade et al., 2014).

Statistical analysis

The results were expressed as mean ± standard deviation and were analyzed using the Action Stat 3.3 software (Estatcamp, São Paulo, Brazil). To determine the behavior of the samples, unilateral analysis of variance – ANOVA was used. To determine the differences between the means, the Tukey test was used, with a 95% confidence level ($p < 0.05$) (Barretti, 2020).

Results and discussion

Powder X-Ray diffractometry (PXRD)

Potato starch presented pattern characteristics of BC type (B with characteristics of C) whereas the other three have a C pattern with 2θ at approximately 5.6°, 15.3°; 17.3° and 23.5°. Similar behavior were found in literature (Hung et al., 2016). It is possible to observe the decrease in peaks at 5.6° according the HMT modified starches. In Figure 1, are depicted the X-ray diffractograms. They were used to analyze the main peaks and calculate the relative degree of crystallinity (%) of each sample.

Native starch showed the highest relative crystallinity (27.2%). Starch modified only with HMT + H_2O and starch modified with HMT + lactic acid shows similar crystallinity (25.6%), the lowest relative crystallinity was observed for a sample modified with HMT +

citric acid (19.9%). These decreases can be explained due to a lamellar destabilization promoted by the treatment by HMT (Maior et al., 2020; Liu et al., 2019).

According to authors, the effect of heat treatment on starch crystallinity depends on the source of starch used and the conditions of moisture and heating used in the treatment. The decrease in crystallinity caused by the treatment of HMT combined with citric acid, which was 19.9%, is much smaller when compared to the other values obtained, this can be explained by a possible decomposition of the crystalline structure of starch by citric acid, this phenomenon causes the substitution of citrate groups in the starch chains, forming a starch with limited mobility (Barretti et al., 2020; Andrade et al., 2014). Xia et al. (2016) also raises a reduction in relative crystallinity when studying sweet potato starches treated by HMT and citric acid.

On the other hand, other sudies showed an increasing in relative cristallinity values. It was observed that HMT, is able to transform the fraction of amorphous amylose to the crystalline state. It is possible that during the treatment the double helical chains help to form a crystalline matrix which presents a structure more ordered than an untreated starch (Zavareze et al., 2011)

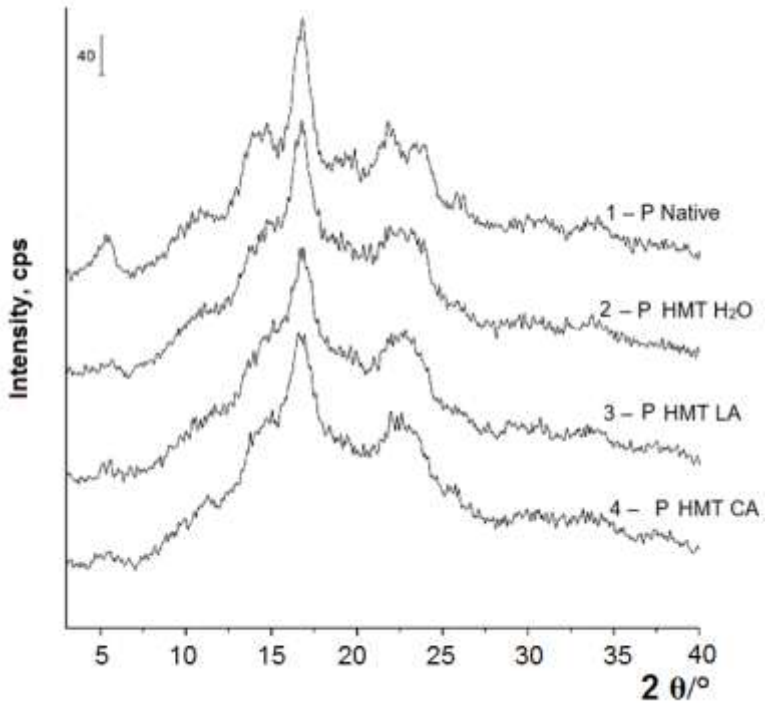

Figure 1. Diffractograms and relative crystallinity (XC%) of native and modified potato starches

Pasting properties (rapid viscoanalyser– RVA)

Data relating to viscoamylographic analyzes of native and treated potato starch are provided in Table 1 and the viscosity profiles are illustrated in Figure 2.

Table 1

Results obtained from the RVA curves of native and modified potato starches

Samples	Treatments	P °C s	V mPa·s s	B mPa·s e	S mPa·s t	Final V mPa·s
Native	P Native	65,50[b] ± 0,07	7824,00[a] ±1,41	5868,5[a] ± 1,41	847,00[b] ± 2,12	2802,50[b] ± 2,33
HMT + H_2O	P HMT H_2O	81,60[a] ±0,57	1732,00[b] ±2,82	-	1592,00[a] ± 1,41	3329,00[a] ± 1,41
HMT + LA	P HMT LA	-	94,50[c] ±1,41	41,00[b] ± 0,05	30,00[c] ±1,41	77,00[c] ±2,12
HMT + CA	P HMT CA	-	90,00[d] ± 1,41	45,00[c] ± 0,02	31,50[c] ± 2,12	76,50[c] ± 0,71

mPa·s – millipascal-second, *s* – second.Values presented as mean values ± standard deviation. Values followed by the same letter in the same column are not significantly different by Tukey's test ($p < 0.05$)

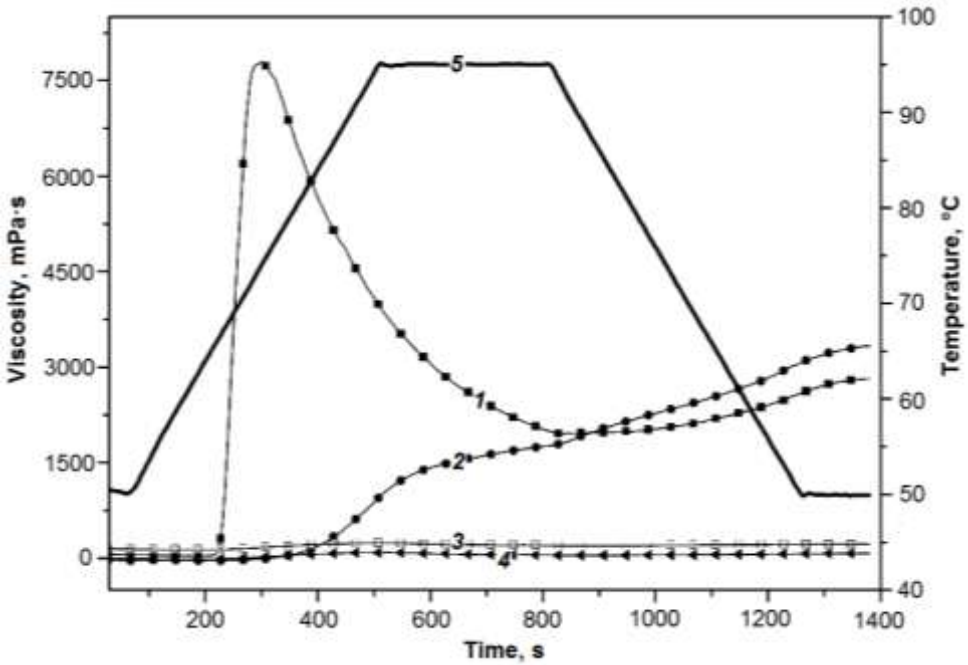

Figure 2. Pasting properties (RVA) curves of native and modified potato starches (mPa·s)

1 – P (Potato starch);
2 – HMT H_2O (Heat-moisture treatment with deionized water);
3 – HMT LA(heat-moisture treatment with lactic acid);
4 – HMT CA (Heat-moisture treatment with citric acid).
5 – temperature.

With the results obtained, it is possible to observe that the temperature for mass formation increases for H_2O modified starch from 65.5 to 81 °C, in relation to native starch. The transforms modified with the organic acids did not form masses, showing that the treatment of the starch by HMT with a gel formation.

It is also possible to notice an increase in the final viscosity for H_2O modified starch, which may be related to intra-granular curtains, making the starch more resistant to deformation, in addition to the reorganization of the starch chains. For how modified with citric and lactic acid, it hears a decrease in the final viscosity, suggesting the rupture of the granular structure (Barreti et al., 2020; Maior et al., 2020; Shaikh et al., 2019).

Viscosity reduction can occur by altering the amorphous fraction of the starch. In acidic conditions this amorphous fraction can be hydrolyzed and the starch starts to move more freely which can result in the closure of its chain and consequently starts to absorb less water which results in reduced viscosity (Maior et al., 2020).

In the sample of starch modified with citric and lactic acid, it is possible to observe that lower as lower final viscosities and peak viscosity, reduced breakage and less tendency to retrograde, this is due to the fact that they do not form paste. One of the major causes of texture deterioration in bakery products is retrogradation. Pastes with low viscosity values are desirable in these products since they have a lower tendency for this phenomenon to run (Hornung et al., 2015; Bet et al., 2020).

Differential scanning calorimetry (DSC)

In the Figure 3 it is possible to observe the DSC curves and in the Table 2 are the obtained values of T_o, T_p, T_c as well as the gelatinization enthalpy ΔH_{gel} for the samples submitted to this analysis.

It can be observed that the gelatinization event occurred in native starch, with a single well-defined peak in the endothermic profile, which is due to the fact that they have low levels of lipids. In the other three samples, the absence or almost absence of the gelatinization curve shows that the changes made to the starch showed the expected decrease in the gelatinization characteristic. This is due to a rupture of the double helices or partial gelatinization of the amylose and amylopectin content during heating. It may also be due to the formation of new starch crystals that can present different thermal stability in the face of physical modification, leading to a high energy need for the dissociation of the crystals to occur and thus the gelatinization phenomenon to happen (Andrade et al., 2014).

Only two of the samples had the gelatinization event, being that of native starch and starch modified with H_2O. The native starch presented a higher ΔH_{gel} value when compared to the H_2O modified starch, showing that the H_2O modification already reduces the gelatinization power of the starch. This change in the thermal characteristic of starch can be explained by the reduction of the destabilizing effect of the amorphous region in the fusion of the lens and by the dissociation of the double helices present in this region (Hung et al., 2017).

Regarding the values of T_o, T_p and T_c can see that they are similar between the two samples. In studies already carried out, the ΔH_{gel} values of native potato starch revolve around similar values, as it is verified by (Andrade et al., 2014), where the value presented is ΔH_{gel} (14.72 J g^{-1}) being very close to the value ΔH_{gel} (14.5 J g^{-1}) found in the present study. The starches modified with organic acids do not show gelatinization (It is observed an enlargenment between T_o and T_c with drastic reduction of viscosity and impossible to stablish values). This behavior can be explained by the occurrence of an esterification reaction caused by these acids (Barreti et al., 2020).

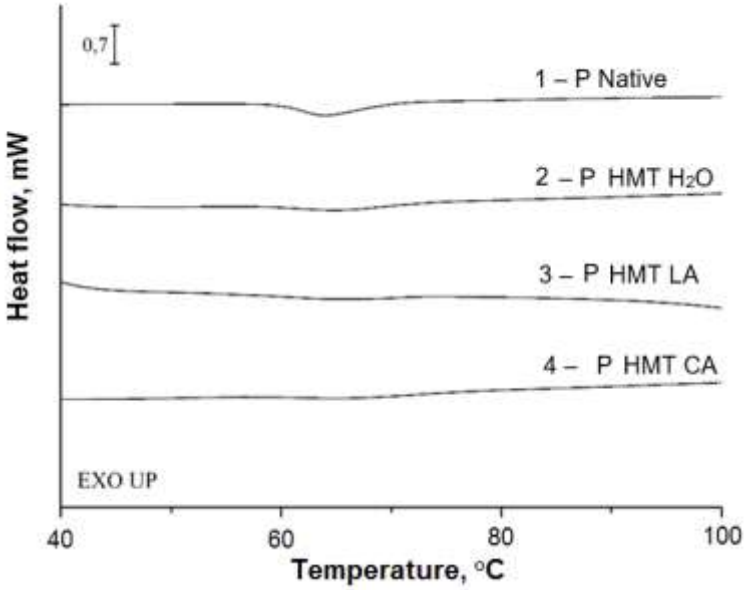

Figure 3. DSC curves of native and modified potato starches
1 – P (Potato starch);
2 – HMT H_2O (Heat-moisture treatment with deionized water);
3 – HMT LA(heat-moisture treatment with lactic acid);
4 – HMT CA (Heat-moisture treatment with citric acid).

Table 2. Results of DSC curves for native and modified potato starches

Samples	T_o, °C	T_P, °C	T_c, °C	ΔH_{gel} J g^{-1}
Native	$59,8^{a}\pm0,06$	$64,0^{a}\pm0,01$	$70,6^{a}\pm0,09$	$14,5^{a}\pm0,08$
HMT + H_2O	$58,5^{b}\pm0,16$	$64,9^{a}\pm0,01$	$72,9^{b}\pm0,14$	$9,9^{b}\pm0,29$
HMT + LA	-	-	-	-
HMT + CA	-	-	-	-

T_o – onset temperature, T_p – peak temperature, T_c – conclusion temperature, ΔH_{gel} – gelatinisation enthalpy. Values presented as mean values ± standard deviation. Values followed by the same letter in the same column are not significantly different by Tukey's test ($p < 0.05$)

According to (Maior et al., 2020) corn starch showed a higher ΔH_{gel} when compared to starches that underwent HMT modification, and that the modification combined with organic acids promoted an even greater reduction in ΔH_{gel}. Regarding the values of T_o, T_p, T_c, the work reports that after the modification the values obtained were higher, being in accordance with the shown results. Other studies have also shown similarities in the behavior of starches when subjected to HMT combined with organic acids (Barreti et al., 2020; Hung et al., 2016; Hung et al., 2017; Xia et al., 2016).

Conclusions

Starch modified by HMT presents with appropriate and different characteristics comparing to the native form. It was possible to observe changes on gelatinization, cristallinity, and pasting properties. The treatment of potato starch using HMT + H_2O, showed some properties alterations, however the modifications were more evident for the treatments with HMT + organic acids, which presented important results and altered technological properties such as the viscosity profile of the samples. The DSC and RVA analysis showed that the characteristic thickener of the starch can be lost after the modifications combined with the organic acids. PXRD analysis showed a decrease in the relative crystallinity after the modifications. The heat-moisture treatment with lactic and citric acids can be considered safe and ecologically correct treatments with organic acids of food grade. Processed modified samples proved to be suitable for application in foods that require low viscosity, such baby food and soups for example. On the other hand, treated granular starch can be an interesting alternative to be used as ingredient for baked products like cookies. It is possible that they have altered the digestibility of this polymer in the human body.

Acknowledgements. The authors would like to thank CNPq – Brazilian Council for Scientific and Technological Development (Proc. nº.310534/2020-8 and 308515/2018-8) and CAPES – Brazilian Coordination for the improvement of Personell in High Level.

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Mass transfer during osmotic dehydration of quince using different osmosis solutions

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Keywords:
Cydonia oblonga
Quince
Colour
Polyphenols
Ascorbic acid

Article history:

Received 21.09.2020
Received in revised form 16.01.2020
Accepted 25.03.2021

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DOI:10.24263/2304-974X-2021-10-1-9

Abstract

Introduction. Fructose and sucrose osmotic solutions, were tested in order to evaluate their effects on the characteristics of the osmosed quince. The effect of slice thickness, solution concentration and immersion time on the color and chemical characteristics of dehydrated quince (*Cydonia oblonga*) were studied.

Materials and methods. Weight reduction (WR), CIE color parameters, total phenolic (TPC) and ascorbic acid (AA) content were investigated using fructose and sucrose osmotic solutions for 3 hours immersion time. The content of the total polyphenols (TPC) was determined by the Folin-Ciocalteu reagent at 765 nm using spectrophotometer. Ascorbic acid (AA) content was separated, identified and dosed in a HPLC SHMADZU system coupled with UV–VIS detector (DAD).

Results and discussion. Weight reduction (WR, %) of osmosed quince showed significant differences depending on the type and concentration of the osmotic agent and process time. Significantly higher moisture loss of fructose (monosaccharides) as an osmotic agent is a considerable advantage compared to sucrose (disaccharide). The higher values of WR were obtained when quince samples were dehydrated with fructose solution of 80% concentration. It was observed that after 180 min of osmotic dehydration with fructose solution 40%, thinner slices (10 mm) have a higher WR value compared to thicker slices (20 mm). The total polyphenols content increased during the osmotic dehydration treatments with 80% osmotic solution. The ascorbic acid content increased during the treatments with fructose solution from 18.66 mg/100 g (in fresh quince samples) to 30.9 mg/100 g (in quince samples after osmotic dehydration with fructose solution 80%). The samples treated with 80% fructose had a lower L^* coordinate, showing an enzymatic browning. The value of a^* was minimal in the case of samples treated with 80% fructose, indicating that the hydrated quince showed a darker color compared to the fresh samples.

Conclusions. Osmotic dehydration for quince with the two osmotic solutions only slightly affected the compositional properties, such as total polyphenols and ascorbic acid content, and weight gain of osmotically dehydrated quince.

Introduction

By air drying the fruits biological substances mainly vitamins, provitamins, polyphenols and other bioactive compounds sensitive to heat, light and oxygen are degraded (Zielinska et al., 2017). In order to reduce nutrient losses, pretreatment is often applied to improve the quality of dry products (Turkiewicz et al., 2020). Osmotic dehydration leads to prolongation of the shelf life, small loss of colour in apple samples osmosed with glucose or sucrose solutions 30%, 45% (w/w) at different times, and in glucose osmosed samples a greater texture hardening rate was observed (Mandala et al, 2005).This process consumes less energy than the air drying or vacuum drying process, osmotic dehydration of apple cubes at room temperature acts as a pre-treatment with a beneficial effect on the firmness of the rehydrated samples that had been air-dried at 50°C (Prothon et al., 2001).

Osmotic dehydration (OD) is the process in which pieces of fruit or vegetables are immersed for different periods of time in a hypertonic solution, so that some of the water and natural sugar in the fruit are replaced with sugar from the syrup (Fernandes et al., 2009).The cellular structure of food acts as a semi-permeable membrane, which is not completely selective and which results in two counter-currents of mass transfer: the diffusion of water from food into solution and the diffusion of osmotic solution into fruit (Yadav et al., 2014). Osmotic dehydration with different osmosis solutions is used to preserve the fruit, because the enzymatic browning (initiated by the enzyme polyphenol oxidase) is reduced, the retention of volatile substances is increased, the color is saved and the sweet taste is preserved (McEvily et al., 1992). The selection of a particular osmotic agent depends on its cost, its molecular weight, and the sensory characteristics of the product to be dehydrated (Ahmed et al., 2016). The most common osmotic agents are (Ahmed et al., 2016): sucrose, corn syrup solution, sodium chloride, maltose, honey, glucose, fructose, lactose, glycerol and ethanol. In general, osmotic dehydration is considered as a pretreatment used in the conventional drying of many agricultural products (da Silva et al., 2013).

Quinces (*Cydonia oblonga Miller*), are a species belonging to the *Rosaceae* family and represents one of the most important sources of vitamins, calcareous salts, bioactive components such as flavonoids and dietary fiber, compounds with health-promoting properties (Dehghannya et al., 2018). Traditional Quince fruits uses are in the preparation of jams, marmalades, purees and jellies, but can be added in ice cream, yogurt or confectionery products (Turkiewicz et al., 2019).

Although, quince fruits are less suitable for direct consumption due to their astringent and strong flesh, the most important advantage of quince over other fruits is its high content of vitamin C. Thus, for example, in some varieties at the ripening stage of consumption, the vitamin C content was over 70 mg/100 g fresh weight (FW) (Rop et al., 2011).

Quince is rich in polysaccharides, which is 11% (dry weight) in the flesh (Qin et al., 2020), pectin (0.53 to 1.83 g pectin/ 100 g fresh quince) (Borazan et al., 2017), contain organic acids such as oxalic, citric, ascorbic, malic, quinic, shikimic, and fumaric acid (Silva et al., 2004) and phenolic compounds (16), which have health promotion roles and medicinal properties (Wojdyło et al., 2014).

Although, osmotic dehydration of quince is little studied, there are some papers available in the literature. For example, the effects of pretreatment on the osmotic dehydration of cubic pieces of quince fruit with sucrose osmotic solution (control, 10, 30, 50, and 70% (w/w)) using intermittent microwave (IM) – dry hot air (HA) at a low temperature (40 °C) were studied by Dehghannyaet et al. (2018). Thus, the increase in the concentration of the osmotic solution, the power and the pulse ratio led to significant decreases in contraction (Dehghannyaet et al., 2018). Recently, Turkiewicz et al. (2020) studied the effect of osmotic

dehydration (DO) using fruit concentrates (apple, pear, pineapple, sour cherry, blackcurrant and hot pepper), as well as the antioxidant, antidiabetic and anticholinergic activity of dried Japanese quince before and after osmotic dehydration. After the completion of osmotic dehydration (in water baths at 45 °C for 1.5 h), Japanese quince fruits had an increased sugar content (up to 20 times more) and a significant reduction in organic acid content (even a 77% reduction compared to non-OD fruits) (Turkiewicz et al., 2020)).

Therefore, the purpose of this article is to present the effect of osmotic dehydration pretreatment (fructose and sucrose 40, 60 and 80%) on the mass transfer color and chemical characteristics of quince fruits.

Materials and methods

Materials

Fresh Quinces (*Cydonia oblonga Miller*), were purchased from a local producerin Falticeni (Romania). For each experiment, the healthy fruits were carefully sorted and washed, cleaned by hand, and after removing the seeds were cut into 2 cm thick slice. All chemicals used for total phenolic content analysis, Folin's reagent and gallic acid, acid ascorbic content, were procured from Sigma Aldrich, Germany. Deionizer water was used.

Osmotic pre-treatment

In this report, the influence of fructose and sucrose solutions concentration and immersion time on mass transfer during osmotic dehydration was studied. Osmotic dehydration was performed at room temperature and three concentrations of fructose and sucrose solution (40%, 60% and 80%) were used. Osmotic solutions were prepared by blending with distilled water on a weight-to-weight basis. The high concentration of osmotic solution was based on a comparative study in the literature (İspir et al., 2018). A ratio of 2:1 (200 ml osmotic solution: 100 g quince) was chosen to monitor changes in hypertonic solution concentration.

The fruits were placed in glasses and after the addition of the osmosis agent it was stirred with an orbital stirrer. The mass transfer between the sample and the fructose solution during osmotic dehydration was measured within 30, 60, 90, 120, 150 and 180 min, the two dimensions of the cubes Quince and showed a different behavior during osmotic dehydration. The samples were removed from the osmotic solution then wiped gently with filter paper to remove adherent water and then weighed.

Each treatment was performed three times and each time with other fruits. Weight reduction (WR) is used to characterize osmotic dehydration, and was calculated according to the following equation (Leahu et al., 2020):

$$\text{WR } \% = \frac{W_i - W_t}{W_i} * 100$$

where W_i is the initial weight of sample cubes (g) and W_t the weight of sample cubes after osmotic dehydration for at each sampling times t (g).

Water activity

Water activity (a_w) was measured using a water activity meter (AQUALAB).

Total phenolic content (TPC)

The degree of the methanolic extract of fruits was expressed as equivalent gallic acid (GA) (mg of gallic acid 100 g^{-1}fw fresh weight) using a standard curve prepared at different concentrations of GA, and were assayed using the Folin-Ciocalteau reagent (Stankov et al., 2020). 1 ml of freshly prepared Folin-Ciocalteau's reagent (1:10, v/v, with water) and 0.8 ml sodium carbonate (7.5%) were added in 0.2 ml of aqueous extract to quince fruit sample. Then, the mixture was incubated at room temperature for 2 hours, and spectrophotometric absorbance was measured at 765 nm using a spectrophotometer T70 UV-VIS PG Instruments Ltd.

Determination of the ascorbic acid

The ascorbic acid (AA – expressed in mg/100 g FW of fruits) content, was separated, identified and dosed in a HPLC SHMADZU system coupled with UV–VIS detector (DAD), A ZORBAX – C18 column (5µm, 250x4.6) (Leahu, 2019).

Colour measurement

Colour was measured on the surface of the fruit in terms of '*L*' (lightness), '*a*' (+a is red, -a is green) and '*b*' (+b is yellow, -b is blue) values (Leahu et al., 2018). Variations in the values of L^*,' a^* and b^* were measured due to different osmotic pretreatments using a Minolta Chroma counter (Model CR 400/410) (Leahu et al., 2018).

Moisture content

The content of water from the quince fruits was determined after drying to constant weight at the standard temperature of 105 ± 2°C (Rop et al., 2011). The samples used in the research constituted 5 g of the sample that has been dried in an oven at 103°Cat atmospheric pressure until constant weight was obtained. Moisture (g water/100 g of sample) % was calculated by using the following equation:

$$M\ \% = \frac{M_i - M_s}{M_i} * 100$$

where M_i is the initial weight of sample cubes (g) and M_s is the mass of sample's dry solids (g). The sample mass was determined using a digital balance.

Statistical analysis

Data were expressed as means ± standard deviations for triplicate determination. Statistical significance of differences between the individual treatments was evaluated by using one-way ANOVA (Minitab 17 software). The null hypothesis was "*All means are equal*", while the alternative hypothesis was "*At least one mean is different*". The significance level considered was $\alpha = 0.05$.

Results and discussion

Physico-chemical parameters of fresh and osmotic dehydrated quince

Osmotic dehydration is a simple process that facilitates the processing of fruits and vegetables, such as bananas, figs, pineapples, mangoes, apples, grapes, carrots, pumpkins, etc. with preservation of the initial characteristics of the fruit. Quince samples were treated with different osmotic solutions concentrations, 40, 60 and 80%, to evaluate the effect of immersion time on moisture content and biochemical characteristics. Table 1 shows the moisture content data during osmotic dehydration versus quince sampling immersion time.

Table 1

Physico-chemical parameters of fresh and osmotic dehydrated quince samples

Parameters	M %	Dry matter %	a_w	AA[a] mg/100 g	TP[a] mg GAE/100g
Fresh quince	83.8±0.554	16.36±0.554	0.63±0.11	18.66±0.425	80.3±0.929
FOD_{40}	76.6±0.79	23.4±0.79	0.56±0.15	29.17±0.23	81.47±0.40
FOD_{60}	72.66±0.44	27.09±0.44	0.54±0.14	29.36±0.49	91.13±0.85
FOD_{80}	68.09±0.34	31.91±0.49	0.50±0.16	30.9±0.26	94.83±0.504
SOD_{40}	78.9±0.19	21.1±0.34	0.56±0.12	21.06±0.31	84.9±0.58
SOD_{60}	75.20±0.21	25.79±0.48	0.56±0.13	21.43±0.27	91.13±0.85
SOD_{80}	71.92±0.38	31.40±0.75	0.52±0.11	22.06±0.24	97.06±0.81

[a]Values are referred to mg/100 g fresh weight vegetable;
Values (means ± SE) (n = 3);
M=Moisture (g water/100 g of sample) %;
AA =Ascorbic acid mg/100 g;
TP = content of Total Phenols (mg GAE/100 g);
FOD= Osmotic dehydration with fructose solution 40%; 60% and 80%;
SOD= Osmotic dehydration with fructose solution 40%; 60% and 80%.

Time of osmotic dehydration had a significant effect on moisture content. Thus m*oisture* content of fresh quince samples ranged from 83.8% in fresh quince to 68.09% in sample treated with 80% fructose solution. This decrease in moisture content is in agreement with previous research (Derossi et al., 2008). As can be seen, the use of the osmotic dehydration (OD) process resulted in an increase in the dry matter content.

Water is the environment for the development of chemical processes that take place in food during processing, but water also participates as reactant in hydrolytic processes. Food stability is a characteristic related to the variation of the water content in them. The value of water activity for fresh quince is high, which may explain the higher L*. A high-water content may cause an increase of the reflected light. The effect of osmotic dehydration on quince was achieved by varying the concentration of the sucrose solution (40–60°Brix) and the immersion time (60–120 min). At a constant water activity, the decrease in the value for the equilibrium moisture content with the increase of air temperature was observed for quince (Noshad et al., 2012).

The total phenolic content recorded in fresh quince pulp was 80.3 mg GAE/100 g, it can be seen that osmotic dehydration treatments with 80% osmotic solution increase the content of total polyphenols (18.09% for fructose solution and 14.32% for sucrose solution).The

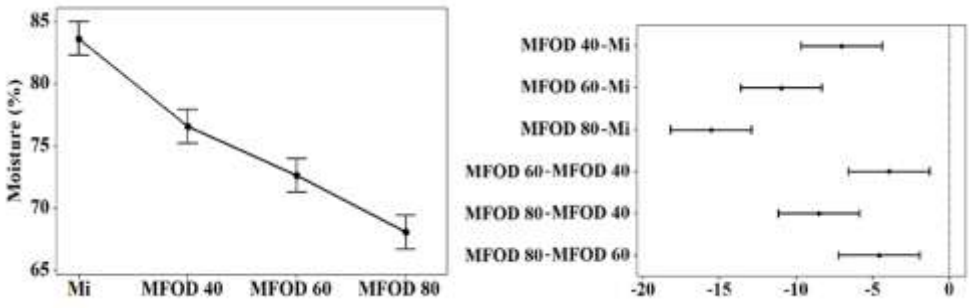
results of the phenolic content obtained in this research are almost similar to the findings published by Rasheed et al. (2018), they reported a total phenolic content in quince fruit pulp from 65.73 mg GAE/100 g to 68.13 mg GAE/100 g.

During the osmotic dehydration of quince fruit, some differences can be observed in the ascorbic acid content. The ascorbic acid content increased in a non-linear manner over time, at all concentrations of fructose and sucrose during osmotic dehydration of quince. The ascorbic acid values found in the present study agreed well with previously reported data. Studying the chemical characteristics of 22 quince genotypes and cultivars (*Cydonia oblonga Mill*.), the content of vitamin C was the highest in the Muškatová variety containing up to 79.31±2.01 g/100 g FW (Rop et al., 2011).

Riva et al. (Riva et al., 2005) studied the relationship between shrinkage and colour stability during osmodehydration and air dehydration, and chemical characteristics of apricot cubes. The ascorbic acid content increased with the pre-treatment time and was similar in the treatment with sorbitol, while the values were higher in the osmodehydration of sucrose (Riva et al., 2005).

Effect of osmotic agent's types and its concentration on weight loss

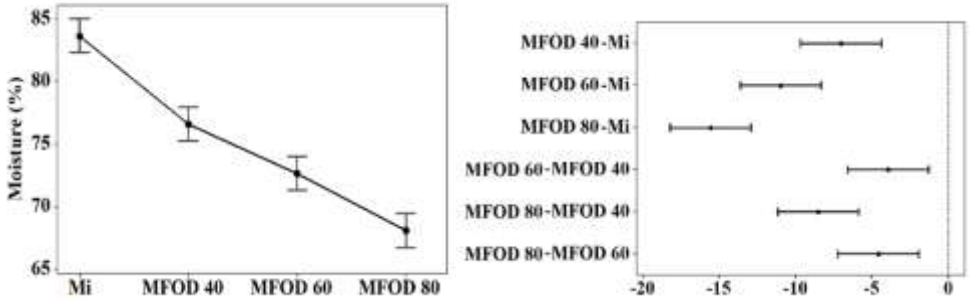
Results obtained by applying the statistical one-way analysis of variance (ANOVA) method showed that the moisture of quince samples after osmotic dehydration with fructose solution 80% had the lowest mean (Figure 1a), comparative with the fresh quince samples which had obvious, the highest mean regarding the moisture content. As shown in Figure 1b, the moisture of quince samples after osmotic dehydration, considering all three concentrations (40, 60 and 80%) of fructose solution, cannot be grouped in the same category and the means are significantly different. Based on the value obtained for R^2 (95.37%) it can be said that the model fits well with our data and the factor explains 95.37% of the variation of the response. It can be established that the differences between some of the means are statistically significant due to the *p-value* obtained (0.000), in the case of quince samples moisture when the osmotic dehydration is performed with fructose solution.



a ***b***

Figure 1.
***a* – Interval plot for quince moisture (moisture of fresh quince samples – Mi, moisture after osmotic dehydration with fructose solution – MFOD (40%, 60% and 80%));**
***b* – comparison of data by using Tukey method**

As well, in the case of quince samples moisture after osmotic dehydration with sucrose solution, it was obtained $p = 0.000$. These means that the differences between some of the means are statistically significant. Results illustrated in Figure 2a indicate that quince samples after osmotic dehydration with sucrose solution 80% has lowest mean, similar to osmotic dehydration with fructose solution. Moisture loss through dehydration of quince samples is higher when osmotic dehydration is performed with fructose solution compared to sucrose solution. In Figure 2b it can be observed that the means are significantly different, since the intervals do not contain zero. The value obtained for R^2 was 96.36%, which means that the factor explains 96.36% of the variation in the response.



a ***b***

Figure 2.
***a* – Interval plot for quince moisture (moisture of fresh quince samples – Mi, moisture after osmotic dehydration with fructose solution – MFOD (40%, 60% and 80%));**
***b* – comparison of data by using Tukey method**

Weight reduction (WR, %) of quince samples (10 mm), during the osmotic dehydration, increased in time according to the results presented in Figure 3. It can be observed that the higher values of WR were obtained when quince samples were dehydrated with fructose solution of 80% concentration (Figure 3a), and with sucrose solution of 80% concentration (Figure 3b). Results showed that weight reduction increases with increasing concentration of solutions, and this was also stated by (Leahu et al., 2020; Kayak-Ertekin et al., 2000). The WR values are also influenced by the quince slices size (Figure 4), it was determined that thinner slices (10 mm) have a higher WR value compared to thicker slices (20 mm) after 180 min of osmotic dehydration with fructose solution (40%), otherwise WR had higher values for 20 mm slices.

The results showed that the values of weight loss of quince slices of 10 mm, dehydrated in 40% sucrose solution are higher after 30 min, 120 min, 150 min and 180 min of dehydration, compared to the values obtained for 20 mm slices. Also, it can be seen that WR values are higher when the fructose solution is used (Figure 4).

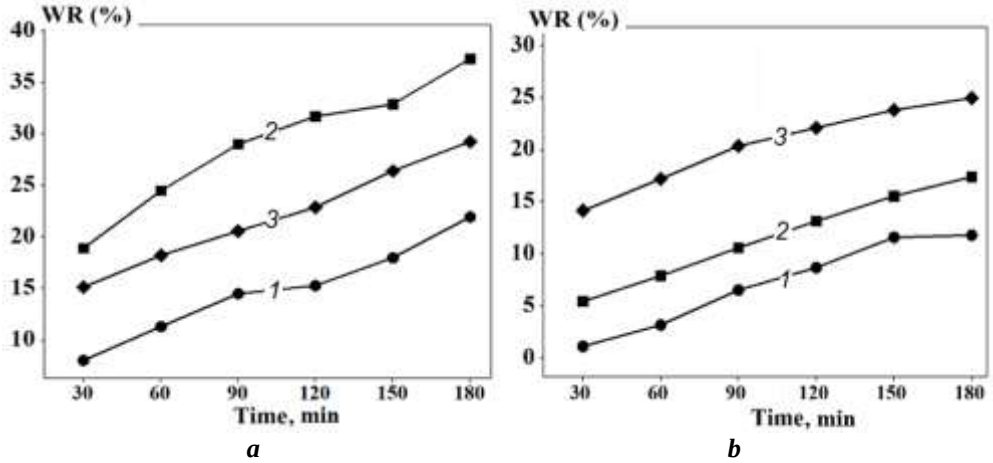


Figure 3. Variation of weight reduction (WR, %) in time of quince samples (10 mm) during osmotic dehydration with different concentration of *a* – fructose solution; *b* – sucrose solution:
Variable:
1 – WR_FS 40%;
2 – WR_FS 60%;
2 – WR_FS 80%.

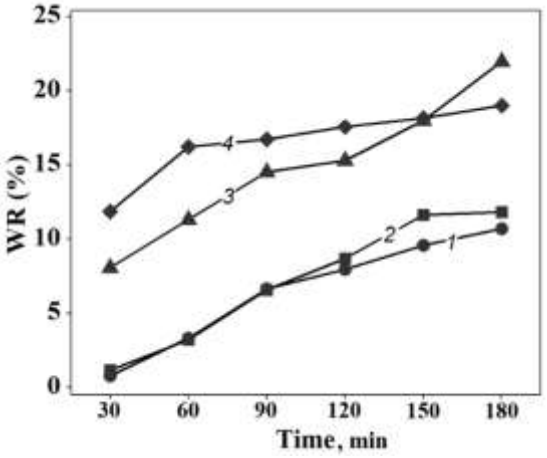


Figure 4. Variation of WR (%) in time of quince samples (10 and 20 mm) during osmotic dehydration with sucrose solution (SS) and fructose solution (FS), both with a concentration of 40%.
Variable:
1 – quince (20mm) in SS
2 – quince (10mm) in SS
3 – quince (20mm) in FS
4 – quince (10mm) in FS

The *p-values* obtained were lower than *α – value*, for both types of solution used for osmotic dehydration (*p=0.002* in case of WR obtained for dehydration of quince slices with fructose solution and *p=0.000* when dehydration is done with sucrose solution) and these mean that the differences between some of the means are statistically significant. From Figures 5a and 5c it can be seen that the WR of quince samples after osmotic dehydration with fructose solution 80% and sucrose solution 80% have the highest mean. The WR of quince samples after osmotic dehydration with fructose solution (FS) 40% and 80% can be grouped in the same category, as well as WR FS 60% and 80% (Figure 5b). In the case of osmotic dehydration with sucrose solution (SS), the WR of quince samples can be grouped in the same category only for WR SS 40% and 60% (Figure 5d). Their means are not significantly different, when they can be grouped in the same category. The factor explains 37.25% of the variation in the response (R^2 = 37.25%) in the case of samples dehydrated with fructose solution and 50.70% (R^2 = 50.70%) for samples dehydrated with sucrose solution, respectively.

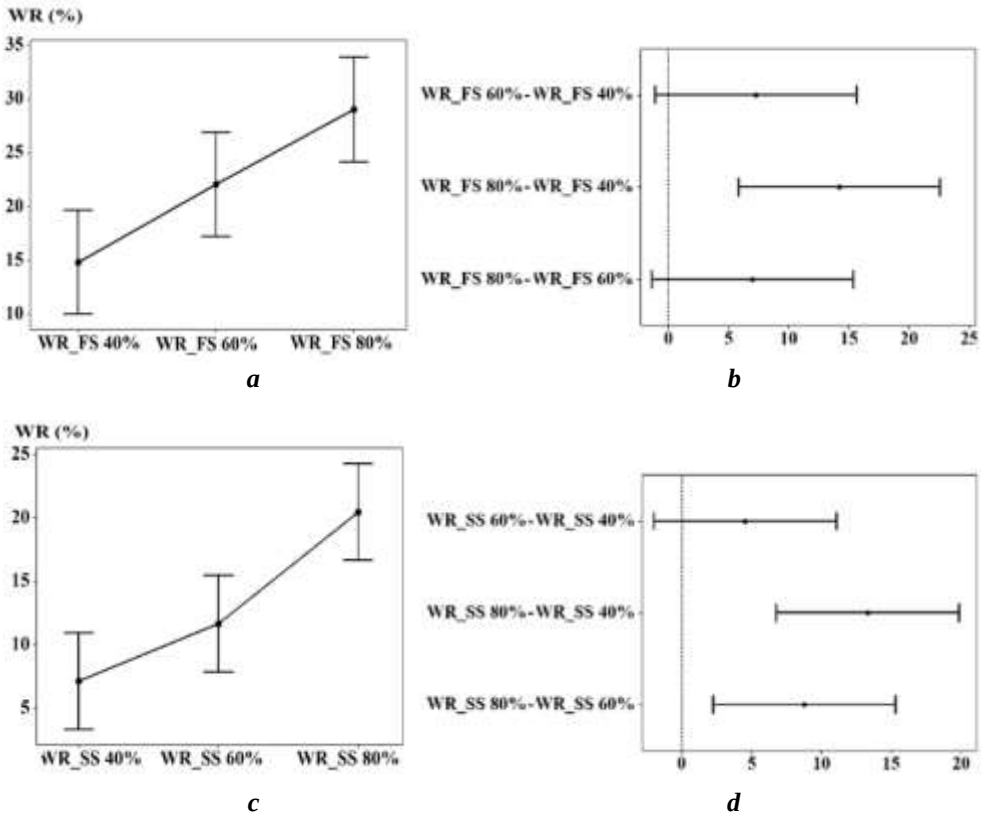


Figure 5. Interval plot for WR of quince samples (10 mm) (WR (%) after osmotic dehydration (OD) with: a – fructose solution (FS) and c – sucrose solution (SS); WR comparison of data by using Tukey method b – OD with FS and d – OD with SS

CIE colour coordinates

CIE L^*, a^*, and b^* values of quince slices after osmotic dehydration using different osmotic solutions are presented in Table 2.

Table 2

Colour CIELAB parameters for samples during the osmotic dehydration

Samples	L^*	a*	b*	Hue angle	Chroma
Quince initial	41.8	1	3.33	66.43	3.73
Quince after treatment with fructose 40 % solution	36.06	4.53	-1.26	33.93	4.76
Quince after treatment with fructose 60 % solution	33.73	-1.8	1.26	28.05	4.13
Quince after treatment with fructose 80 % solution	29.25	-2.31	1.01	26.41	4.86
Quince after treatmentwith sucrose 40 %solution	35.73	4.1	-1.76	33.06	4.53
Quince after treatmentwith sucrose 60 %solution	32.06	-1.55	1.11	27.63	4.38
Quince after treatmentwith sucrose 80 %solution	28.18	-2.12	0.90	25.73	4.95

The use of different osmotic agents affected the L^*, a^*, and b^*values of the final product during the osmotic process. Thus, for fresh quince the values were L^*=41.8, a^*=1.00, b^*=3.33, and for OD fruits with fructose solution these values ranged as follows: L from 36.06 to 29.25, a^* from 4.53 to -2.33, and b^* from -1.26 to 1.01. It can be observed that coordinate *L** presents the lowest values 28.18 after treatment with sucrose 80 %solution indicating that quince dehydrated presented a darker colour compared to the fresh samples. Chauhan et al. (Chauhan et al., 2011) reported that the color changes of the fruits (darkening) due to the enzymatic browning are correlated with the L^* and a^* values. Changes in the * (green – red) coordinate for dehydrated samples with sugar solution show a change in color from greenish yellow to fresh samples (1) in reddish yellow (-2.12).

The results of the current study were quite in agreement with those of El-Aouar et al. (El-Aoua et al., 2006); these authors reported that the concentration of the osmotic solution was the most important effect on weight loss of osmotically dehydrated slices of papaya (*Carica papaya L.)* with sucrose and corn syrup (El-Aoua et al., 2006).

Conclusion

1. Processing of quince by osmotic dehydration using three concentrations of fructose and sucrose solution (40%, 60% and 80%) osmotic solutions significantly increased the total phenolic and acid ascorbic content in comparison to control sample.
2. Weight reduction (WR) were higher for fructose osmosis samples compared to sucrose osmosis samples at the same concentration of the solution. Both weight reduction (WR) increased with increasing osmotic agent concentration. This can be attributed to the fact

that fructose is a low molecular weight monosaccharide and therefore has a more pronounced effect on water loss compared to sucrose which is a polysaccharide.

3. The studied factors of osmotic dehydration (process duration, type of agent and sugar solution concentration) showed a significant influence on weight reduction.

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Nutritional value of fish soup from cultured brook trout (*Salvelinus fontinalis*, Mitchill, 1814)

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Keywords:

Brook trout
Fish
Soup
Fatty acid
Mineral
Nutritional value

Article history:

Received 20.08.2020
Received in revised form 28.10.2020
Accepted 25.03.2021

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DOI: 10.24263/2304-974X-2021-10-1-10

Abstract

Introduction. The aim of this study is to estimate the nutritional value of fish soup from cultured brook trout in terms of a healthy diet for human consumption.

Materials and methods. Fish soup was prepared from 23.65% previously cooked trout mince and 18.76% vegetables, and cooked for 35 mins. The final product was analyzed for the proximate composition, fatty acids and, mineral contents as well as carotenoids and vitamins B1, B2 and B6. Inductively coupled plasma Mass Spectrometer (ICP-MS) was used for mineral content after decomposition of lyophilized samples. Fatty acids methyl esters were separated by gas chromatography by flame ionization detector (FID). High performance liquid chromatography (HPLC) was used for the estimation the contents of vitamins and carotenoids.

Results and discussion. The proximate contents of soup were represented by 87.79% moisture, 8.18% protein, 2.89% crude fat, 1.17% dietary fibre, 0.62% ash and 0.03% carbohydrate. The energy value was calculated as 58.82 kcal/100g. The value of total polyunsaturated fatty acids (∑PUFA) was higher than the values of total monounsaturated (∑MUFA) and saturated fatty acids (∑SFA) and accounted as 43.89, 34.93 and 19.83%, respectively. The main PUFA corresponded to linoleic acid as 27.14% followed by docosahexaenoic acid (DHA) as 7.92%. Total eicosapentaenoic acid+docosahexaenoic acid (∑EPA+DHA) was observed as 9.21% which was accounted as 239.04 mg/100g soup. The results of this study demonstrated that a portion of trout soup (about 200g) would well cover daily recommended n-3 PUFA intake while slightly higher amount is required for daily EPA+DHA intake. Mineral contents were varied in the range of 1.77–31.52 mg/g (dwb), while the results obtained for vitamin B1, B2 and B6 and for carotenoids were comparable with the data given for different types of soups in literature.

Conclusion. This study indicates that a nutritious fish soup can be produced from brook trout for human consumption suitable for a healthy diet.

Introduction

Brook trout (*Salvelinus fontinalis*, Mitchill, 1814) belongs to *Salmonidae* family and is distributed to North America. It is native to most of eastern Canada from Newfoundland and Labrador to western side of Hudson Bay; south in Atlantic, Great Lakes, and Mississippi River basins to Minnesota and northern Georgia, USA. It is widely introduced in North America and temperate regions of other continents of the world (FISHBASE, 2020). Brook trout is not a native species of Turkey. It has been introduced from Europe for aquaculture purposes. Today, the species is reared in some rainbow trout farms in eastern Black Sea region (Atasaral Şahin et al., 2011). Although the world production, particularly aquaculturing of brook trout has been increasing, the world production was reported lower than Rainbow trout as 1546.5 tonnes in 2018 (FAO, 2020). However, most countries include this species within the other trout species in their statistical reports, such as Turkey. Therefore, the actual production value is unclear.

Soup is defined as a liquid food usually made by stewing ingredients such as meat, vegetables, and fish often in a stock and with seasoning or a chemical mixture (Kiin-Kabari et al., 2017). Due to health benefits of seafood, proximate composition and nutritional properties of trout have been investigated in the past research (Guillou et al., 1995; Atasaral Şahin et al., 2011; Öz and Dikel, 2015). Therefore, consumption of fish soup has also been advised by some authorities (CFS, 2020).

Despite nutrient database reports of various organisations provide information on nutritional values of various foods including few fish soup products, the actual processing and ingredients of the reported processed foods are not clear. Although limited studies reported the proximate composition and nutritional value of edible muscle for brook trout (Atasaral Şahin et al., 2011), no study exists on the fish soup originated from trout including brook trout.

Therefore, our aim of this study is to estimate proximate composition and nutritional value of fish soup prepared from brook trout in terms of a healthy diet for human consumption.

Materials and methods

Production of fish soup

The type and amount of ingredients used in fish soup were given in Table 1. The weight and the sizes of fish were 17.5–32.7 cm and 162.7–410.5 g, respectively. In total around 17 kg brook trout was used. The other ingredients used were obtained daily from a supermarket situated in central Trabzon, Turkey. They were transferred to the laboratory within an hour and kept in cool conditions until use.

Preparations of ingredients

All fish were first measured and weighed before using. After prewashing, they were cut into 2-4 pieces depending on the sizes and then placed into chilled water containing 5% salt for 30 mins. Then, they were washed again and transferred in a sieve (stainless Steel 18/8, Large Colander: 250 x 90 mm) and enclosed to keep ingredients.

The vegetables were cleaned from their skins and other unwanted parts (e.g. stem/stalk of green pepper and parsley, and spoiled parts), then washed and drained. They were chopped and weighed before using. The lemons were freshly squeezed to obtain fresh juices just before cooking. The wheat flour was kept in oven at 200–250 °C until it started browning.

Preparation of fish soups

The ingredients were separated into two groups, namely fish (containing fish heads, skins, flesh and bones) and vegetables (garlics, onions, fresh carrots, fresh tomatoes and fresh green peppers) are placed in enclosed stainless-steel sieves separately (Table 1). Then, they were transferred into a boiler (Stainless Steel 18/10, 28×18 cm) containing tap water. Then, the boiler containing fish and vegetables were placed on an industrial type heater (340×550×740 mm, LPG, 3.8Kw). Bay leaves and lemon pieces were added into water to provide some aroma into the soup and they were taken out after the first cooking step.

Table 1

Ingredients used in the preparation of fish soup from of brook trout

Ingredients	%
Fish mince (Boiled and separated from bones and skin)	23.65
Carrot	5.91
Tomatoes	3.65
Onion	3.68
Green Pepper	3.50
Parsley	0.71
Garlic	0.71
White flour (roasted)	0.60
Salt	0.69
Lemon juice	1.18
Water	55.72
TOTAL	~100 (Water loss due to evaporation was not taken into account)

Bay leaves and lemon pieces separated into two or three pieces were added to the product during the first cooking phase and then discarded.
The ratio of these amounts is not considered.

The cooking was carried out in three different steps. First cooking was completed in 20 mins at about 93±3 °C and stopped. The fish was taken out onto a container (Stainless Steel 18/10, 80x40cm) which was previously cleaned and sterilized in an oven at 250 °C for 1 hour. The flesh of fish was separated from the bones and skin in hygienic conditions, then weighed into an aluminium foil before adding back to the boiler. The bay leaves and lemon pieces were removed from the boiler while the vegetables were freed into the water in boiler. The cooking was continued further for about 5 mins and stopped. All the vegetables were blended into small pieces using a blender (Arçelik K8525, Turkey) to get an even mixture. The fish mince obtained from previously cooked fish was added into the broth of homogenized vegetables. Later, chopped fresh parsley, lemon juice and salt were added into the mixture. The roasted wheat flour was dissolved in cold water was also fortified into the soup mixture. The second cooking step took place for 10 mins at 93±3 °C. All the ingredients in the soup was homogenized using the same blender for 1–3 mins.

Analytical methods

Proximate composition and energy value

The AOAC method numbers of 985 (AOAC, 1995a), 7.009 and 2.507 (AOAC, 1980) were used to analyse moisture, ash and protein contents, respectively. The dietary fibre of the fish soup was analysed using AOAC Official Method 991.43 (AOAC, 1995b). Crude fat content was analysed by using solvent extractor Velp SER 148/6 with petroleum ether (130°C) (Tufan et al., 2016). The Atwater method was applied to calculate total energy value of the soup (Merrill and Watt, 1973).

Metal (Mineral) analysis

The samples were transferred to the laboratory in frozen form. The fish soup samples were lyophilized using a lyophilizer (ALPHA 1-4, CHRIST, Germany). Then, the samples were decomposed by microwave method and analysed for metals using inductively coupled plasma Mass Spectrometer (ICP-MS). About 0.5 g of lyophilized sample was weighed into a TFM vessel (Microwave oven ETHIOS E – touch control) and treated with 6vmL of HNO_3 (65% Fluka-Sigma, Germany) and 2mL of hydrogen peroxide (30%, Belinka) was added. The vessels were closed and transferred into the microwave oven. The microwave digestion program was carried out under two steps as 1st step included 10 mins ramp time at 180ºC with 1000-Watt microwave power, then 2nd step with 10 mins hold time at 180ºC with 1000-Watt microwave power. Then the vessels were cooled, the digested solutions were transferred into the 25 mL volumetric flasks and made up to volume with MQ water (for chromatography, > 18,2 MΩ/cm, Aldrich, Germany). Dilution according to the desired concentration range was made before measurements. Multi element standard solution (Merck KGaA, Germany) for calibration was used in metal analysis. A blank digest is carried out in the same way. All minerals were determined using an ICP-MS (Agilent Technologies, Model 7900) against aqueous standards. The mineral concentration is expressed as mg mineral/kg fish dry weight.

Analysis of fatty acids

A modified method of Park and Goins (1994) was used to analyse fatty acids of fish soup. Before esterifying of fatty acids and analysis by GC-FID, the soup samples were lyophilized to remove the water. Esterification of fatty acids was performed according to the procedure developed by Park and Goins (1994). The method consists of *in situ* transesterification without prior extraction of fat from the sample. About 0.2 g of each anhydrous sample was weighed into a test tube with a stopper and added 300 µL dichloromethane (CH_2Cl_2) and 3 mL of freshly prepared 0.5 M sodium hydroxide (NaOH) in methanol. Tubes were purged with nitrogen to prevent the oxidation from the air and closed by stoppers. Well mixed content was heated for 10 mins at 90°C in a thermal block. In that time the hydrolysis of fats expired. After heating, tubes were rapidly cooled under running water. Then, 3 mL of 12 % boron trifluoride-methanol solution ($BF_3.CH_3OH$) was added to each tube, re-purged with nitrogen, and heated for 10 mins in thermal block at the temperature of 90°C. In that time the transesterification expired. Then, 3 mL of deionized water and 1.5 mL of hexane were added in cooled tubes. Methyl esters were extracted in a non-polar solvent by shaking for 1 min. The reaction mixture was centrifuged for 10 mins at 2000 rpm. The hexane layer was separated from the aqueous phase and was transferred to the bottle

with a Pasteur pipette, which was further purged with nitrogen. Until analysis it was stored at -20°C.

Methyl esters of fatty acids were separated by gas chromatograph Agilent 6890 and detected by flame ionization detector (FID). Capillary chromatographic column Omegawax 320 was used. The column was silica with stationary phase of polyethylene glycol (30 m x 0.32 mm x 0.25 μL). Injection volume was 10 μL. Split injection mode with ratio 30:1 was used. For instrument calibration mix standard NuCheck 85 was used. Argon was used as a carrier gas with flow rate 1.5 mL/min. Flow rate of make-up gas (nitrogen) and fuel gas (hydrogen) was 30 mL/min. The injector temperature was 250°C and the detector temperature was 290°C. Temperature program of separation had set up the initial temperature of the column at 170°C, which was heated with a rate of 1°C/min till 215°C. After 9 min at the maximum temperature there was cooling with a rate of 5°C/min till 170°C.

The amount of mg fatty acid in edible portion of trout soup was calculated by Greenfield and Southgate (2003) using the following formulae:

FA content (mg FA per 100g edible portion of fish soup) = [FAME% x FACF x lipid content% (g lipid / 100g food)]/100;

Where FAME is fatty acid methyl esters, FACF: the lipid conversion factor (fatty acid conversion factor, g FA g^{-1} lipid). FACF is reported as 0:933 for fish finfish (Weihrauch et al., 1977).

Vitamin Analysis

For vitamin B_1 and B_2, the samples were extracted according to the method used by Esteve et al. (2001), for vitamin B_6, the method used according to Kall (2003). About 5-30 g samples were transferred into an Erlenmeyer with the size of 100 mL. Then, 60 mL of 0.1N hydrochloric acid (HCl) was added. The mixture was autoclaved at 121 °C for 30 min. Then it was cooled at room temperature before adjusting the pH at 4.5 using 2.5 M sodium acetate solution. After addition of 100 mg taka-diastase and 5 mg acid phosphatase enzyme onto samples, they were incubated at a shaking water bath at 45°C'de 3 hours for the extraction of vitamin B_1 and B_2 and for 18 hours for the extraction of vitamin B_6. Then, the samples were cooled down to room temperature. The extract was made up to 100 ml using 0.1N HCl and filtered using a qualitative filter paper. The solution was derivatised for the analysis of vitamin B_1. Twenty-five ml of filtered extract was transferred into polyethylene tubes and then 1.5 mL potassium ferricyanide solution was added. The pH was adjusted to 7.0-7.1 using Orto-phosphoric acid, then the solution was filtered through 0.45 μm filtrate before injection to HPLC.

Fluorescent detector was used for the analysis. HPLC conditions used for vitamin B_1 was as follows; Wavelength: Excitation: 366 nm and Emission: 445 nm, injection volume: 20-50 μl, the flow rate: 1mL/mins and the total analysis time was 25 mins. HPLC conditions used for the analysis of vitamin B_2 was as follow; mobile phase: water: methanol (75:25 v:v), wavelength: Excitation: 445 nm and Emission: 525 nm, injection volume: 20-50 μL, flow rate:1 mL/mins, total analysis time was 20 mins. The HPLC conditions of the vitamin B_6 was as follows; Wavelength: Excitation: 290 nm and Emission: 395 nm, extraction volume: 50 μL, flow rate: 1 mL/mins, column temperature: 25°C, and total analytical time was 40 mins.

Analysis of Carotenoids

The method of Konings and Roomans (1997) was used to analyze carotenoids. Five gram of homogenized fish soup was taken into a mortar and crushed (mushed). Then 50 ml

of extraction solution (methanol/tetrahydrofuran (1:1) was added and mixed. The solution was transferred into 100 ml volumetric measuring flask, then the rest of the sample in the mortar was cleaned with the extraction solution and added to previous solution. Later, it was adjusted to 100 ml with the extracted solution. The sample was filtered using a standard filter paper, the filtrate was then filtered again using 0.45 μm filter prior to injection to HPLC. The HPLC conditions were as follows; Mobile Phase: Methanol: Tetrahydrofuran (95:5), Detector: UV, Wavelength: 450 nm, Injection volume: 20 μl, flow rate: 0.8 ml/min, Analytical time: 20 mins.

Results and discussion

Table 2 shows the results for proximate composition and energy value of fish soup prepared from cultured brook trout.

Table 2

Proximate composition and energy value of trout soup

Analytical parameters	Value (Wet weight bases)
Moisture (%)	87.79±0.81
Protein (%)	8.18±0.31
Crude Fat (%)	2.89±0.18
Carbohydrate (%)	0.03±0.88
Dietary Fibre (%)	1.17 ± 0.00
Ash (%)	0.62±0.13
Energy (kcal/100g)	58.82±0.88
	Values (Dry Weight bases)
Dry Matter (%)	12.21±0.81
Protein (%)	65.96±0.69
Crude fat (%)	22.24±1.34

± *SD*

Past studies demonstrated that the moisture contents of soups highly depend on their consistency and affect the ratios of other components such as protein and ash contents (Chan et al., 1994; Obiakor-Okeke et al., 2014; Zhang et al., 2018; USDA, 2020). Moisture contents of dried soups were reported as below 10% (Venugopalan and James, 1969; Rahman et al., 2012; Priscilla and Vigasini, 2017) although the values were over 90% after their dilution prior to use. Moisture contents of ready to eat soups were usually between 80 and 89%, while lower values were reported for condensed soups (Obiakor-Okeke et al., 2014). The moisture content obtained in this study was within the values obtained in the literature indicating the thickness of it was similar to commercial soup products. Although Tolasa et al. (2012) reported similar amount of moisture content for fish soup prepared from smoked Rainbow trout trimmings, they obtained higher protein content and lower lipid value in comparison to the current study. Lower moisture contents were reported by different studies for different types of fish soups (Lied and Julshamn, 1986; Chan et al., 1994). Zhang et al. (2018) reported very high levels (around 98%) for the fish soups prepared from Crucian carp and snakehead fish. USDA food database (2020) reported two different home-prepared/made fish soups contained 84.0 and 96.6% water.

Chan et al (1994) reported higher protein content as 10.4% protein for bouillabaisse soup. However, lower values were obtained by different studies (Lied and Julshamn, 1986). USDA food database (2020) reported as 2.26 and 7.4% protein content for two different home-prepared/made fish soups. Tümerkan (2015) incorporated fish into dried soups and showed that addition of fish meat can increase protein contents of the soups.

The fat content of fish soups not only depend on the fish species used in the soup, the ingredients, particularly the addition of oil or butter during cooking can also change the fat composition of final product. The fat content of brook trout soup was obtained in the current work was higher than the values reported by USDA food database (2020) for two different home-prepared/made fish soups. Lied and Julshamn (1986) found higher values of fat and carbohydrate contents responding to higher energy values as 143.5 kcal/100g for fish soup sold in Norwegian markets. Proximate composition of traditional fish soups served in Nigeria were reported higher than this study since the soup was a condensed soup with a moisture content lower than 60% (Obiakor-Okeke et al., 2014). Similarly, much higher proximate composition can also be expected to find for dried/soup powders (Venugopalan and James, 1969; Jeyakumari et al., 2016; Fasogbon et. al., 2017; Kiin-Kabari and Akusu, 2017). Our results also proved high values of protein and fat contents when calculated as dry weight bases (dwb). Health benefits of fatty acids in fish soup are mostly related to its contents of highly unsaturated fatty acids, particularly, omega-3 fatty acids. The fatty acid values were presented in Table 3 as FAME% and mg/100 g in trout soup.

The results showed that trout soup has good nutritional value in terms of fatty acids since it contains higher amount of total polyunsaturated fatty acids (∑PUFA) in comparison to total saturated fatty acids (ΣSFA). The highest value was represented by oleic acid followed by linoleic acid. Various health benefits of omega-3 fatty acids, particularly, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been reported (Gogus and Smith, 2010).

Total EPA+DHA values were calculated as 9.21% which corresponds to 239.04mg/100g of edible portion of trout soup. Previous studies indicated that fatty acid values of farmed trout are highly affected by its diet (Trbović et al. 2012), and it contains higher n-6 fatty acids compared to n-3 PUFA, particularly linoleic acid (Atasaral Şahin et al., 2011; Yeşilayer and Genç, 2013). Therefore, high levels of omega 6 were also expected in trout soup used in this study as its being farmed origin. The ratios of n3/n6 and n-6/n-3 have been suggested as a useful indicator for comparing relative nutritional values of oils. Varying levels were suggested for n-3/n-6 as low as 0.2 and up to 1, while the UK Department of Health recommends an ideal ratio of n6/n3 of 4.0 at maximum (HMSO, 1994; Gogus and Smith, 2010). Both values were found within the suggested levels indicating the benefits of trout soup for healthy diet. Different health institutions have recommended daily intake of n-3 PUFA and EPA+DHA in varying rates within the range of 0.2–0.45g and 0.5–1.0g, respectively (Gogus and Smith, 2010). The results of this study demonstrated that a portion of trout soup (about 200g) would well cover daily recommended n-3 PUFA intake while slightly higher amount is required for daily EPA+DHA intake. Chan et al (1994) and USDA (2020) reported percentages of PUFA levels were lower than SFA and MUFA values in different fish soups.

Table 3

Fatty acid composition of brook trout soup

Fatty Acids	FAME (%)	mg/100g of soup
C14:0 (Myristic acid)	1.59±0.02	41.25±3.14
C15:0 (Pentadecanoic acid)	1.59±0.02	5.72±0.35
C16:0 (Palmitic acid)	13.39±0.04	347.84±20.75
C17:0 Heptadecanoic acid)	0.21±0.00	5.46±0.34
C18:0 (Stearic acid)	4.22±0.04	109.68±5.80
C20:0 (Eicosanoic acid)	0.20±0.00	5.20±0.32
∑SFA (Saturated Fatty Acids)	**19.83±0.05**	**515.13±30.71**
C14:1 (Myristoleic acid)	0.20±0.01	5.21±0.56
C16:1 (Palmitoleic acid)	2.82±0.04	73.39±5.50
C18:1n-9 (Oleic acid)	31.54±0.17	819.59±55.02
C22:1n-11 (Cetoleic acid)	0.37±0.00	9.61±0.60
∑MUFA (Monounsaturated Fatty Acids)	**34.93±0.21**	**907.80±61.67**
C18:2n-6 cis (Linoleic acid)	27.14±0.14	705.16±47.18
C18:3n-6 (Gama-linoleic acid)	0.66±0.01	17.07±1.20
C18:3n-3 (Linolenic acid)	1.92±0.01	49.79±3.02
C18:4n-3 (Stearidonic acid)	0.45±0.01	11.61±0.87
C20:2n-6 (Eicosadienoic acid)	1.29±0.01	33.59±1.94
C20:3n-6 (Homo- γ -Linolenic acid)	0.92±0.01	23.80±1.19
C20:3n-3 (Eicosatrienoic acid)	0.76±0.23	19.74±5.89
C20:4n-6 (Arachidonic acid)	0.76±0.03	19.72±0.58
C20:4n-3 (Eicosatetraenoic acid)	0.28±0.00	7.27±0.45
C20:5n-3 (Eicosapentaenoic acid)	1.30±0.02	33.67±1.58
C22:5n-3 (Clupanodonic acid)	0.51±0.01	13.15±0.53
C22:6n-3 (Docosahexaenoic acid)	7.92±0.28	205.37±5.74
∑PUFA (Polyunsaturated Fatty Acids)	**43.89±0.39**	**1139.94±63.61**
∑EPA+DHA	9.21±0.30	239.04±7.32
∑n-3 (Omega-3)	13.13±0.47	340.60±12.97
∑n-6 (Omega-6)	30.11±0.09	782.27±50.88
∑n3/∑n6 (Omega-3/Omega-6)	0.44±0.02	
∑n6/∑n3 (Omega-6/Omega-3)	2.30±0.09	
Unidentified	1.35	

± *SD*

Jeyakumari et al. (2016) reported that the highest fatty acid profile of carrageenan and fish soup were corresponded to SFA followed by MUFA. The results of Zhang et al. (2018) also showed similar trend by obtaining high values of ∑SFA and ∑MUFA in comparison to low values of ∑PUFA in two different fish soups. Udari et al. (2015) obtained slightly higher EPA and lower DHA values for fish soup powder from *Sardinella longiceps*.

Table 4 shows mineral contents of the fish soup prepared from cultured brook trout. Minerals are reported to play a key role in biological processes and metabolism. Therefore, they are considered as nutrient minerals related to specific health benefits (Zhang et al., 2018).

Table 4

Mineral contents of trout soup (dry weight bases)

Minerals	Values
Sodium (Na) (mg/g)	31.52±2.45
Potassium (K) (mg/g)	11.93±0.85
Magnesium (Mg) (mg/g)	0.91±0.014
Calcium (Ca) (mg/g)	1.38±0.147
Iron (Fe) (μg/g)	12.12±1.13
Copper (Cu) (μg/g)	1.77±0.16
Zinc (Zn) (μg/g)	15.30±0.43
Barium (Ba) (μg/g)	3.77±2.09

n=3, SD: standard deviation.
The values of minerals named Li, Be, B, Al, V, Mn, Cr, Co, Ni, Cu, Ga, Rb, Sr, Mo, Ag, Cd, Te, Tl and U are reported to be below the detection limit (1ng/g(ppb).

In this study, the highest value was obtained for sodium (Na) followed by potassium (K), calcium (Ca) and magnesium (Mg). Trace amounts were found for copper (Cu), Zn, Fe and barium (Ba). High amount of Na level was related to the addition of salt to the trout soup during cooking. Food Standards Agency (2002) has reported twice higher amounts of Na level in instant soup powder. High Na intake is not recommended for health risk arise from high salt intake. Therefore, 1500 mg Na intake was advised by the health authorities for adults and lower amounts were suggested for children (National Academy of Sciences, 2019). The Na content obtained in this study was well below the upper limit given by this mineral since the values in the current study represents dry weight bases. Mineral contents obtained in this study were higher than several types of fish soups obtained by different researchers with some exceptions (Chan et al., 1994; Obiakor-Okeke et al., 2014; Zhang et al., 2018; USDA, 2020) while higher Ca, Fe and Mg contents were reported for some dried soup containing fish (Fasogbon et al., 2017; Priscilla and Vigasini, 2017).

Table 5 represents the values of carotenoids and vitamins of trout soup.

Table 5

Values of carotenoids and vitamins of trout soup

Vitamins	Values (mg/100g)	Carotenoids	Values (μg/100g)
B1 (Thiamine)	0.0175±0.001	**Lutein**	109.5± 4.9
B2 (Riboflavin)	0.0245±0.001	**Beta carotene**	342.5± 27.6
B6	0.0745±0.002	**Lycopene**	82.5± 4.9

± *SD*

Vitamins are also important nutrient elements in foods relating to different health functions (Lee et al., 2000; National Institute of Health, 2017). Thiamine and riboflavin are important for the growth, development, and function of the cells in the body while vitamin B_6 is need for more enzyme reactions involved in metabolism. Vitamin B_6 is also involved

in brain development during pregnancy and infancy as well as immune function. Average daily recommended amounts were reported as in the range of 0.2–1.4 mg thiamine, 0.3–1.6 mg for riboflavin, and 0.1–2.0 mg for vitamin B_6 depending on the age of humans (National Institute of Health, 2020). Although the amounts for the relating vitamins were determined the below the recommended values, the results obtained for thiamine and riboflavin in this study closely supported the values obtained for various types of soups including chicken, fish and different vegetables soups as reported by Venugopalan and James (1969), Chan et al. (1994) and USDA (2020). Food Standards Agency (2002) reported higher value for Vitamin B_6 and beta carotene with some exceptions. Therefore, this study suggests that trout soup has good contribution for the mentioned vitamins to the human diet. Lied and Julshamn (1986) determined higher riboflavin and thiamine contents in fish soup sold in Norwegian fish market.

Carotenoids are compounds of great dietary importance not only as precursors of Vitamin A, but also as molecules that take part in cell protection and consumer attraction due to the visual colour they provide to food. Epidemiological studies that strongly suggest that consumption of carotenoid-rich foods reduces the incidence of cancers, cardiovascular diseases, age-related macular degeneration, cataracts, diseases related to low immune function and other degenerative diseases (Perera and Yen, 2007; Pal and Bhattacharjee, 2019). National Institute of Health (2020) recommended 700 μg of retinol activity equivalents (RAE) for adult woman and 900 μg of RAE for adult man. Perera and Yen (2007) pointed out that the retinol equivalency for β-carotene and other provitamin A carotenoids differ much and they reported 12 μg of dietary beta-carotene is equivalent to 1 μg of retinol (12:1 RAE). Therefore, our results suggest high contribution to the daily amount of RAE for human consumption.

USDA (2020) has not observed any beta carotene, lycopene and lutein values in various fish soups, they found higher lycopene values in Mexican style fish and vegetable soup, and found lower values of beta carotene and lutein in comparison to the present study. Higher carotene values were also found compared to the data reported by different food database (Chan et al., 1994; Food Standards Agency, 2002). However, National Institute of Health (2020) reported higher contents of beta carotene in various vegetable soups containing, particularly carrot. Therefore, higher values are likely to arise from high percentages of vegetable ingredients in the relating soup products.

Conclusion

The values obtained in this study indicate that soup prepared from cultured brook trout has good nutritional value and therefore, it is suggested for a healthy diet for human consumption.

Acknowledgements. This work was supported by the Scientific and Technological Research Council of Turkey (TÜBİTAK) and Slovenia Research Agency (ARRS) under a bilateral project Grant Numbers: TOVAG 213O112 and BI-SLO-TR-14-16/08).

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Extraction, rheological and textural analyses and grading of pectin from stem pith of banana

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Keywords:

Banana
Pectin
Extraction
Rheology
Texture
Grading

Article history:

Received 20.09.2020
Received in revised form 17.03.2021
Accepted 25.03.2021

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DOI: 10.24263/2304-974X-2021-10-1-11

Abstract

Introduction. Purpose of the work – to identify suitable conditions for the extraction of pectin from stem pith of banana and to investigate how it affects rheological and textural properties of fruit jams.

Materials and methods. Stem pith of local banana cultivar. Pectin extracted using hot water acidified with sulfuric acid. Yield was studied and expressed as percentage of dried raw material used. Degree of methylation was studied using titration method, monomeric composition was studied using high performance anion exchange chromatography with pulsed amperometric detector. Effect of banana pectin upon flow behavior and texture profile of the pineapple jam prepared with it was studied using rheometer. The pectin was graded using modified line spread method.

Results and discussion. 27.91% of yield was obtained. All independent variables were found to affect the yield to varying degrees. Temperature and pH were found to be the most important conditions affecting yield, while time of heating and SLR (time of heating and solid to liquid ratio) were also found to be affecting the yield, but to a lesser extent. The highest of the yield was at a SLR of 50, pH of 1.5, temperature of 82℃ and a time of heating of 52.5 minutes.

Degree of methoxylation was found to be 62% with a composition similar to that of pectin from other sources reported by other workers.

Banana pectin was found to be affecting the rheological and textural properties of pineapple jam. Yield stress of pineapple prepared using banana pectin was found to be 113 Pa, compared with 96 Pa for control. At the same time highest shear modulus for the test was 94 Pa, against a value of 60 Pa for the control. While banana pectin was found to be affecting the strength of pineapple jam, more of it was required to achieve it compared with citrus pectin. More force was required to make the jam flow, indicating a higher yield stress compared with citrus pectin.

Textural properties of the jam were affected by the addition of banana pectin. Hardness was reduced from 6.18 for the control to 2.3 for the test while cohesiveness was reduced from 5.3 for control to 1.96 for test. At the time springiness was increased from 6.24 to 7.52. Grade banana pectin was found to be 90.9.

Conclusions. Stem pith of banana is a potential alternate raw material for the extraction of pectin and the pectin so extracted is suitable to use as a gelling agent in food materials.

Introduction

Pectin is used as a thickening and gelling agent in food and other industries for a long period of time. Traditionally, it is obtained as a value-added product from agro-waste materials. As new pectin-containing food (and non-food) products are developed continuously, the demand for pectin increases throughout the world, making it necessary to identify new sources for its feasible large-scale extraction. (Arachchige et al., 2020), (Dimopoulou et al., 2019). The newly identified sources should be abundant and locally available at low-cost, should yield pectin with minimum or no damage to the environment, should give a reasonable yield per unit weight of the raw material and the extracted pectin should contribute significantly towards the flow behaviour and textural properties of food materials. The conditions affecting the yield need to be identified, in order to device a financially viable strategy for its large-scale extraction. Moreover, source – structure-usability relationship need to be established (Ciriminna et al., 2019). This is especially important because the structure, and therefore the applications, of pectin greatly depend upon the sources from which it is extracted.

Banana is the most abundantly cultivated fruit in the world. After using the fruits of banana, most of the remaining parts of the cultivated plant are thrown away as waste. Natural calamities and pest infestation also lead to the loss of a large quantity of banana plants before harvesting. These wasted components in banana cultivation have been explored as a source of pectin recently. The authors of this article have shown that peel (N. S. Rajendran and B.S. Harikumaran Thampi, 2019) and underground stem (Rajendran and Harikumaran Thampi, 2021) of banana can be used as a viable source of pectin.

Stem pith of banana – the white part, enclosed by leaf sheaths, rising above the soil, terminating with flower and fruit bunch- has rarely been explored as a source of pectin, and there is no publication in this regard.

Therefore, in this work, we aim to substantiate the feasibility of using stem pith of banana as a source of pectin, and also to study the usefulness of the extracted pectin as a gelling agent in food industries.

Materials and methods

Studied products

Stem pith of *Poovan* (AAB) cultivar (local name) was collected from a local farm in Palakkad district, Kerala state of India. The plant used as raw material in this study is being described in "Cytotaxonomical Studies on Banana Cultivars", by P.K. Valsalakumari (Valsalakumari, 1984), Kerala Agricultural University, Kerala, India in 1984, as picture plate no. 16, page no. 87-88).

Extraction of pectin

Fresh stem pith was washed with tap water, cut and pulverized, dried under sun to constant weight, powdered and packed until use. Measured quantities were transferred to particular volume of sulfuric acid-water mixture (Bouhenni1 et al., 2019) of particular pH, heated at pre-defined temperature for definite time period, cooled to room temperature, dust removed by centrifugation, pH raised to 6 using barium carbonate, pectin precipitated with double volume of isopropyl alcohol, vacuum dried and powdered.

Estimation of Degree of methyl esterification – The method provided in Food Chemicals Codex was used (Birch, 2003).

Analysis of monomeric composition

Powdered pectin was hydrolysed with trifluoroacetic acid at 105℃ for 5 hours, acid removed by evaporation, 10 µg was injected in to high performance anion exchange chromatography (HPAEC) CarboPacPA-1 column (4mm x 250mm) with 100 mM sodium hydroxide and sodium acetate gradient, equipped with pulsed amperometric detector (PAD). Dionex ICS-3000 was used for monomer analysis.

Rheological analysis

Preparation of pineapple jam: Ripe pineapple was purchased from local market, washed, chopped in to small pieces, pulverised and filtered through a 2mm mesh to get the juice. 52g of this juice was measured in to a vessel, 25g sucrose was added, boiled to a certain extent, powdered mixture of 1g sucrose and 0.25g of pectin was added, boiled again until a brix of 60 % was reached, citric acid was added to bring the pH to 3.6 cooled to room temperature and packed (Hlaing, 2019).

Estimation of Yield stress of pineapple jam: Amplitude sweep curves of the above jams were obtained at 25℃, 11Hz frequency, shear strain range 0.1 to 100, with a Rheometer (Aanton Paar) model MCR 52, plate-plate method, with a 1mm thickness of sample in between.

Texture profile analysis

The above pineapple jam was placed on the lower plate of Universal Testing Machine, Lloyd, LR-5K model, cylindrical probe – 80mm, load cell of 50N, speed of 50mm/min, at 25℃ and the values were processed with NEXYGENPlus data Analysis Software provided by the manufacturer. Gumminess was calculated as the product of cohesiveness and hardness while chewiness was calculated as the product of springiness and gumminess (Hussain et al., 2020).

Grading

The consistency of a gel prepared using pectin, sucrose and water was measured and compared with that of a gel made using 150 grade, standard citrus pectin. pH of the gel was adjusted using citric acid and sodium citrate. The process was repeated by adjusting the concentration of pectin, until a gel with consistency matching with that of gel made with citrus pectin was obtained. The ratio of grams of sucrose to that of banana pectin used for making this gel was expressed as the grade of pectin.

Preparation of gel: Measured amounts of sucrose and water were boiled, pectin was powdered with sucrose and added, 1ml 25% citric acid and 2ml 12.5% sodium citrate were added with continuous boiling, removed from flame, 4 ml of 25% citric acid and 1 ml of 12.5% sodium citrate were added, cooled to room temperature overnight, then consistency was compared with a gel made in the same manner using standard citrus pectin (Ranganna S., 1986).

Measurement of consistency of pectin-sucrose gel using modified Line-Spread method: 3.0 ml of the gel was poured in to a horizontal glass plate at room temperature, and allowed to spread, length of the gel was measured at the widest part using a scale after three minutes (Kim et al., 2018). This process was repeated until similar values were obtained for gels prepared with banana pectin and standard pectin. Grade of banana pectin is determined using its concentration in this gel.

Statistical analysis

Data were analysed using t-test, two sample, assuming unequal variance, (MS Excel 2017), $p \leq 0.05$. Results were used to assess the impact of changing conditions of extraction upon yield of pectin. The same test was used to analyse the effect of banana pectin upon rheological and textural properties of the pineapple jam.

Results and discussion

Yield

The highest yield was 27.9 %. Temperature and pH were found to be the most important conditions affecting yield while time of heating and SLR were also found to be affecting the yield, but to a lesser extent. These findings are in agreement with data published by other workers (Wang et al., 2016).

Effect of temperature on yield

Table 1 shows the effect of increase in temperature upon yield.

Table 1

Effect of temperature of extraction upon yield of pectin from stem pith of *Poovan* cultivar of banana

Pairs	SET	SLR	pH	Time, minutes	Temperature, ℃	Yield, (%)
Pair 1	A	30	1.5	52.5	54	1.29±0.24
	B	30	1.5	52.5	82	14.20 ± 1.16^{A}
Pair 2	A	30	1.5	97.5	54	4.55±0.64
	B	30	1.5	97.5	82	26.35 ± 2.50^{A}
Pair 3	A	30	2.5	52.5	54	2.51±0.13
	B	30	2.5	52.5	82	3.98 ± 0.52^{A}
Pair 4	A	30	2.5	97.5	54	1.93±0.23
	B	30	2.5	97.5	82	2.78±0.34
Pair 5	A	40	2.0	75.0	68	8.34±0.73
	B	40	2.0	75.0	96	18.02 ± 1.87^{A}
Pair 6	A	50	1.5	52.5	54	2.44±0.49
	B	50	1.5	52.5	82	27.91 ± 3.22^{A}
Pair 7	A	50	1.5	97.5	54	15.17±2.06
	B	50	1.5	97.5	82	26.52 ± 2.88^{A}
Pair 8	A	50	2.5	97.5	54	1.42±0.23
	B	50	2.5	97.5	82	5.54 ± 0.84^{A}
Pair 9	A	50	2.5	52.5	54	1.81±0.18
	B	50	2.5	52.5	82	3.95 ± 0.38^{A}

*Values of yield are average of six independent analyses ±SEM.
Set A in each pair is at a lower temperature, while set B is at a higher temperature.
Superscript A against the value of yield indicates that the particular value of yield is significantly different from that of set A of the same pair.

Temperature was found to be an important factor in deciding yield (Chen et al., 2021). It is found that in most of the cases, yield significantly increased upon increase in temperature, probably due to the increased penetration of the cell wall material by the extractant liquid at the higher temperature (Wang et al., 2016).

Effect of pH upon yield

Table 2 shows the effect of increasing pH upon yield of pectin. It is evident that, in most of the cases, increase in pH resulted in decreased yield. Thus, it may be concluded that, as a general rule, increased concentration of acid favors penetration of the extractant mineral acid solution in to the cell wall matrix of the raw material, releasing more pectin (Hlaing, 2019).

Table 2

Effect of pH of extraction upon yield of pectin from stem pith of *Poovan* cultivar of banana

Pairs	SET	SLR	Time, minutes	Temperature, ℃	pH	Yield, (%)
Pair 1	A	30	52.5	54	1.5	1.29±0.24
	B	30	52.5	54	2.5	2.51±0.13^{A}
Pair 2	A	30	52.5	82	1.5	14.20±1.16
	B	30	52.5	82	2.5	3.98±0.52^{A}
Pair 3	A	30	97.5	54	1.5	4.55±0.64
	B	30	97.5	54	2.5	1.93±0.23^{A}
Pair 4	A	30	97.5	82	1.5	26.35±2.50
	B	30	97.5	82	2.5	2.78±0.34^{A}
Pair 5	A	40	75.0	68	2	8.34±0.73
	B	40	75.0	68	3	3.35±0.47^{A}
Pair 6	A	50	52.5	54	1.5	2.44±0.49
	B	50	52.5	54	2.5	1.81±0.18
Pair 7	A	50	52.5	82	1.5	27.91±3.22
	B	50	52.5	82	2.5	3.95±0.38^{A}
Pair 8	A	50	97.5	54	1.5	15.17±2.06
	B	50	97.5	54	2.5	1.42±0.23^{A}
Pair 9	A	50	97.5	82	1.5	26.52±2.88
	B	50	97.5	82	2.5	5.54±0.84^{A}

* Values of yield are average of six independent analyses ±SEM.
Set A in each pair is at a lower pH, while set B is at a higher pH.
Superscript A against the value of yield indicates that the particular value of yield is significantly different from that of set A of the same pair.

Effect of time period of heating upon yield

A quick look at the Table 3 indicates that more pectin was released from the raw material when it was heated for longer duration as was demonstrated by other workers (Fakayode and Abobi, 2018). This might be due to the more effective penetration of the

extractant solution in to the deeper layers of the cell wall, leading to breakdown of chemical bonds both within pectin and also between pectin and other molecules in it (Li et al., 2019). Generally, in most of the cases, change in yield upon increased time of heating was statistically not significant, under the experimental conditions used for this study.

Table 3

Effect of time of heating upon yield of pectin from stem pith of *Poovan* cultivar of banana

Pairs	SET	SLR	pH	Time, minutes	Temperature, ℃	Yield, (%)
Pair 1	A	30	1.5	54	52.5	1.29±0.24
	B	30	1.5	54	97.5	4.55 ± 0.64^{A}
Pair 2	A	30	1.5	82	52.5	14.20±1.16
	B	30	1.5	82	97.5	26.35 ± 2.50^{A}
Pair 3	A	30	2.5	54	52.5	2.51±0.13
	B	30	2.5	54	97.5	1.93±0.23
Pair 4	A	30	2.5	82	52.5	3.98±0.52
	B	30	2.5	82	97.5	2.78±0.34
Pair 5	A	40	2.0	68	75	8.34±0.73
	B	40	2.0	68	120	8.61±0.64
Pair 6	A	50	1.5	54	52.5	2.44±0.49
	B	50	1.5	54	97.5	15.17 ± 2.06^{A}
Pair 7	A	50	1.5	82	52.5	27.91±3.22
	B	50	1.5	82	97.5	26.52±2.88
Pair 8	A	50	2.5	54	52.5	1.81±0.18
	B	50	2.5	54	97.5	1.42±0.23
Pair 9	A	50	2.5	82	52.5	3.95±0.38
	B	50	2.5	82	97.5	5.54±0.84

*Values of yield are average of six independent analyses ±SEM.
Set A in each pair is at a lower time period, compared with set B.
Superscript A against the value of yield indicates that the particular value of yield is significantly different from that of set A of the same pair.

Effect of solid to liquid ratio (SLR) upon yield of pectin

Results are given in Table 4. It is evident that in half of the experiments increase in SLR resulted in a statistically significant change in yield. In many of the cases, increased SLR resulted in increased yield, because:

a. Increased dilution of the solution stabilised the released pectin, thereby helping releasing of more bound pectin from cell wall materials;

b. Of the increased penetration of extractant liquid in to the biomass to release more pectin.

Table 4

Effect of SLR upon yield of pectin from stem pith of *Poovan* cultivar of banana

Pairs	SET	pH	Time, minutes	Temperature, ℃	SLR	Yield, (%)
Pair 1	A	1.5	52.5	54	30	1.29±0.24
	B	1.5	52.5	54	50	2.44±0.49
Pair 2	A	1.5	52.5	82	30	14.20±1.16
	B	1.5	52.5	82	50	27.91 ± 3.22^{A}
Pair 3	A	1.5	97.5	54	30	4.55±0.64
	B	1.5	97.5	54	50	15.17 ± 2.06^{A}
Pair 4	A	1.5	97.5	82	30	26.35±2.50
	B	1.5	97.5	82	50	26.52±2.88
Pair 5	A	2	75.0	68	40	8.34±0.73
	B	2	75.0	68	60	3.87 ± 0.56^{A}
Pair 6	A	2.5	52.5	54	30	2.51±0.13
	B	2.5	52.5	54	50	1.81 ± 0.18^{A}
Pair 7	A	2.5	52.5	82	30	3.98±0.52
	B	2.5	52.5	82	50	3.95±0.38
Pair 8	A	2.5	97.5	54	30	1.93±0.23
	B	2.5	97.5	54	50	1.42±0.23
Pair 9	A	2.5	97.5	82	30	2.78±0.34
	B	2.5	97.5	82	50	5.54 ± 0.84^{A}

*Values of yield are average of six independent analyses ±SEM.
Set A in each pair is at a lower SLR, compared with set B.
Superscript A against the value of yield indicates that the particular value of yield is significantly different from that of set A of the same pair

Degree of esterification (D.E.)

The D.E. was found to be 62%, indicating that banana pectin was a high methyl pectin. This is comparable with pectin from other sources (Li et al., 2019).

Monomeric composition

The following monomers (Table 5) were identified from the pectin extracted from stem pith of *Poovan* cultivar. It is similar to the results published by other workers from other raw materials (Sabater et al., 2020).

Table 5

Types and quantity of monosaccharides present in 10 µg of hydrolysed pectin obtained from stem pith of *Poovan* cultivar of banana

Sl. No.	**Name of monomer**	**Quantity (µg)**
1	Fucose	0.024
2	Rhamnose	0.136
3	Arabinose	0.128
4	Glucosamine	0.004
5	Galactose	0.266
6	Glucose	0.428
7	Mannose	0.23
8	Xylose	0.056
9	Galacturonic acid	0.075
10	Glucuronic acid	0.064

Rheological analysis

Comparison of gelling ability of different types of pectin is shown in Table 6. The pectin samples extracted were non-Newtonian and exhibited shear thinning behavior and is consistent with data from other workers (Li et al., 2019). Shear modulus, a measure of the strength of the material, was found to be increased upon addition of pectin and is comparable with pectin from other sources (Koubala et al., 2009). However, more of the banana pectin was required to bring up the shear modulus up to that of citrus pectin. At the same time, yield stress, the stress beyond which the material begins to flow, was much more for banana pectin than for citrus pectin. Thus more force need to applied to induce flow, under the given set of conditions (Dinkgreve et al., 2016). These findings indicate that flow behavior of food gels may be modified by careful use of banana pectin, as was reported earlier by workers using pectin from other sources (Norziah et al., 2000).

Table 6

Comparison effect of pectin from different sources upon flow behaviour of pineapple jam

Experiment	Yield stress	Highest shear modulus
	Average	Average
A – Control	96.48±2.29	60.31±1.43
B – Std.	63.62±1.51	81.91±1.94
C – Test	113.21±2.68^{A,B}	93.97±2.23^{A,B}

*The values are averages of six independent analyses ± SEM. $p \leq 0.05$.

Superscripts A and B against the values of test indicate that the values are statistically significant compared with control (A) and Std (B).

Control contained no pectin, Std. contained 0.25 g of citrus pectin while Test contained 0.25 g of banana pectin.

Texture profile analysis

Textural properties of the pineapple jam prepared using pectin from stem pith of *Poovan* cultivar of banana are shown and compared with standard citrus pectin in Table 7.

Table 7

Texture profile analysis of pineapple jam prepared using pectin

Hardness1 (N)	A – Control	$6.18 \times 10^{-1} \pm 1.21 \times 10^{-2}$
	B – Std	$11.71 \pm 2.23 \times 10^{-1}$
	C – Test	$2.3 \pm 4.46 \times 10^{-2A, B}$
Hardness2 (N)	A – Control	$5.31 \times 10^{-1} \pm 1.03 \times 10^{-2}$
	B – Std	$9.2 \pm 1.78 \times 10^{-1}$
	C – Test	$1.96 \pm 3.8 \times 10^{-2A, B}$
Cohesiveness	A – Control	$7.68 \times 10^{-1} \pm 1.49 \times 10^{-2}$
	B – Std	$5.0 \times 10^{-1} \pm 9.7 \times 10^{-3}$
	C – Test	$7.23 \times 10^{-1} \pm 1.4 \times 10^{-2B}$
Springiness (mm)	A – Control	$6.24 \pm 1.22 \times 10^{-1}$
	B – Std	$7.25 \pm 1.42 \times 10^{-1}$
	C – Test	$7.52 \pm 1.45 \times 10^{-1A}$
Gumminess (kgf)	A – Control	$4.84 \times 10^{-2} \pm 9.42 \times 10^{-4}$
	B – Std	$5.97 \times 10^{-1} \pm 1.15 \times 10^{-2}$
	C – Test	$1.7 \times 10^{-1} \pm 3.27 \times 10^{-3A, B}$
Chewiness (kgf.mm)	A – Control	$3.02 \times 10^{-1} \pm 5.77 \times 10^{-3}$
	B – Std	$4.33 \pm 8.39 \times 10^{-2}$
	C – Test	$1.28 \pm 2.49 \times 10^{-2A, B}$
Adhesiveness (kgf.mm)	A – Control	$2.4 \times 10^{-3} \pm 4.61 \times 10^{-5}$
	B – Std	$6.31 \times 10^{-2} \pm 1.22 \times 10^{-3}$
	C – Test	$1.41 \times 10^{-2} \pm 2.76 \times 10^{-4A, B}$

Values are averages of six independent analyses ±SEM. $p \leq 0.05$.
Superscripts A and B against the value of test indicate that the particular value is statistically significant compared with control and standard respectively.
"Control" contains no added pectin, 'Std' contains standard citrus pectin while "Test" contains pectin extracted from stem pith of *Poovan* cultivar of banana.

Analysis of the textural profile indicates that all textural properties were influenced by banana pectin. Therefore, a different set of textural properties may be achieved for the food material, by replacing citrus pectin with banana pectin (Cruz et al., 2019). Hardness is the most important textural property of food materials, having a direct role in consumer satisfaction and marketability (Monalisa et al., 2020). Hardness of jam increased upon the addition of banana pectin, but the magnitude of increase was less than that of citrus pectin. It indicates that more force is required to compress the jam containing banana pectin compared with the control, but less force compared with standard (Di Monaco et al., 2008). This is an important finding as it helps to prepare food for people with difficulty in mastication and deglutition (Nishinari et al., 2019). By adjusting the concentration of banana pectin, food materials of required hardness and other textural properties may be prepared (Anuar and

Salleh, 2019). Cohesiveness, a measure of the strength of chemical bonds in the jam under study, was brought down upon the addition of banana pectin but not as much as citrus pectin. This means that the energy required for successive rounds of chewing decreases more than that for the control and this concept is helpful while formulating food for the elderly (Nishinari et al., 2016). A chewiness – product of springiness, cohesiveness and hardness – more than control, indicates that more energy is required to chew the jam prepared with banana pectin, until it can be swallowed.

Grading

The grade of banana pectin was found to be 90.9, while many authors have reported 150 grade pectin from other sources (Maskey et al., 2018). This means that more of the banana pectin is required to bring about the same consistency of the gel, compared with citrus pectin. However, this cannot be observed as a demerit of banana pectin, because more of it can be obtained from the waste produced from one banana plant and also because, of the huge quantity of banana plants being cultivated globally (FAO, 2019).

Conclusion

1. Pectin obtained from stem pith of *Poovan* cultivar of banana is an effective alternative plant-derived gelling agent useful in food industries.
2. Temperature and pH of extraction were found to determine the yield predominantly, even though time of heating and SLR also were important parameters.
3. Banana pectin has a monomeric composition similar to that of pectin from other sources.
4. Its rheological and textural properties also were suitable to enable its use for preparation of fruit jams.
5. Grade of the pectin was found to be less than that of citrus pectin.

Acknowledgements. The authors would like to extend their gratitude to CSIR – CFTRI, Mysore, India (Rheology and Textural Profile Analysis) and Biomedical Research Facility, California University, San Diego, USA (HPAEC – PAD) for providing their laboratory facilities for this work.

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Stability of selenium and iodine in the functional meat products prepared with seaweeds under different cooking procedures

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Keywords:

Functional
Food
Seaweeds
Selenium
Iodine

Article history:

Received 21.05.2020
Received in revised form 03.09.2020
Accepted 25.03.2021

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DOI: 10.24263/2304-974X-2021-10-1-12

Abstract

Introduction. The aim of this study was determination of the stability of selenium and iodine in the functional meat products prepared with seaweeds under different cooking procedures.

Materials and methods. Three edible seaweeds *Fucus*, *Cystoseira*, and *Laminaria* were used in the study. Different meat products with addition of seaweeds were prepared. Selenium concentration was measured using diaminonaphtalene method. Content of iodine was determined by inverse voltammetry.

Results and discussion. Meat-based food products prepared with seaweeds to enrich them with iodine and selenium were proposed. Seaweed *Laminaria* had too high iodine and selenium contents to be used for preparation of dietary products. *Cystoseira* was the better source of iodine than *Fucus.* All ready-to-eat products with *Cystoseira* had higher iodine content than ones with *Fucus.* Iodine losses were lower and the contents of iodine in ready-to-eat products were higher in the products prepared at lower temperature 100–110 °C (steamed cutlets) in comparison with 170 °C (fried cutlets). Altogether, method used for product preparation had a great influence on iodine losses during cooking. The biggest losses were observed for products prepared in liquid: 50% iodine losses in meat balls braised in sauce and 61% in quenelles cooked in soup. Loss of iodine in dumpling was lower, 38%, maybe due to protection of meat with the dough cover. The lowest loss of iodine, around 17%, was in grilled sausages due to relatively low temperature of cooking and absence of liquid environment.

Cystoseira was a lit bit better source of selenium than *Fucus.* All ready-to-eat products with *Cystoseira* had slightly higher selenium content than ones with *Fucus.* Influence of temperature and method for food preparation was not so evident, selenium losses varied from 19 to 27% for steam cutlets, meat balls and even for quenelles and dumpling prepared with *Laminaria.* The lowest loss of selenium, around 7%, was in the grilled sausages.

Conclusion. Fried and steamed minced-meat cutlets, meat balls, and grilled sausages prepared with addition of 2% (w/w) of seaweeds *Cystoseira* or *Fucus* can be recommended to be used as the functional food supplying needed daily quantity of iodine and selenium.

Introduction

Meat products are essential part of a daily ration supplying human with valuable nutrients. To improve meat products quality these products can be designed as the functional food by direct incorporation of different ingredients during meat processing (Bhat and Bhat, 2011; Zhang et al., 2010). Among ingredients used for the preparation of functional food, edible seaweeds are considered as a good source of antioxidants, dietary fibers, essential amino acids, vitamins, unsaturated fatty acids, carotenoids and abundant minerals that can be incorporated in meat, fish, bakery and others products (Bocanegra et al., 2009; Circuncisao et al., 2018).

It was shown that the addition of seaweeds or seaweeds extracts can improve the health value, shelf-life and quality of food (Roohinejad et al., 2017). There are some attempts to use seaweeds in the preparation of meat-based functional food products. Seaweeds, for example "wakame" (*Undaria pinnatifida*), "nori" (*Porphyra umbilicalis*), and "sea spaghetti" (*Himanthalia elongata*) were used as the sources of bioactive substances to improve fatty acid content of some modified meat products such as frankfurters, patties and restructured steaks (Cofrades et al., 2017). There were also attempts to prepare bread using seaweeds *Lemna minor* or *Ulva rigida* to extend its shelf-life (Kılınc et al., 2013).

Edible seaweed (macroalgae) is a rich source of essential minerals and trace elements needed for human nutrition, particular selenium (Se) and iodine (I) (Circuncisao et al., 2018; Cherry et al., 2019), adequate intakes of which are required for optimal thyroid function (Schomburg and Kohrle, 2008).

The aim of this study was determination of the stability of selenium and iodine in the functional meat products prepared with seaweeds under different cooking procedures (Figure 1).

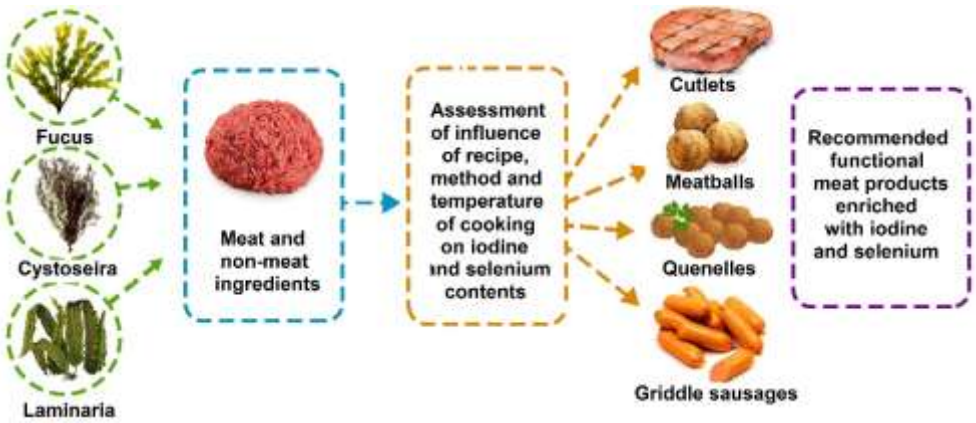

Figure 1. Research scheme

Materials and methods

Seaweeds

Three edible seaweeds from the brown algae family were chosen for this study: (1) *Fucus*, "sea oak", which has high contents of iodine, calcium, magnesium, iron and other minerals (Pereira, 2011); (2) Black sea brown alga *Cystoseira*, which is a source of iodine and selenium and is used as a food additive (Pereira, 2016), and *Laminaria*, which traditionally has been used as a food additive being a source of biological active substances including iodine in natural form and it is considered that it may be useful for preventing lifestyle-related diseases (Kılınc, 2013; Shirosaki and Koyama, 2011). Seaweeds have been dried in the conventional oven at 100 °C for 2–3 hours for the moisture content 12–13 %, and then milled to a fine powder. The fine powder of seaweeds before addition to the minced meat was mixed with water with temperature 20ºC in the following ratio: mass of *Cystoseira* to water 1:3, mass of *Fucus* or *Laminaria* to water 1:4, and exposed for hydration or 6–12 hours. Moisture content of seaweeds was determined after drying at 105ºC to a constant weight.

Trace elements determination

Selenium concentration was measured by minifluorometer (mode TD-360, Turner Design, Sunnyvale, CA, USA) using diaminonaphtalene method (Watkinson, 1966). The samples of biomass were preliminary digested using HNO_3, $HClO_4$ and HCl. The determination of selenium was made in quartz cuvette 3 ml. The excitation wavelength was 369 nm, the fluorescence emission wavelength was 525 nm.

Content of iodine was determined by inverse voltammetry using voltametric analyser ABA2 (Scientific Industrial Enterprise, Saint Petersburg, Russia) (Korzun and Palamarek, 2014).

Thermal treatment of meat products

Meat products were treated at different temperatures: meat-ball were cooked by braising in sauce at 100 °C, quenelle (meat ball cooked in broth) were cooked at 100 °C, boiling of dumplings was done at 100ºC, preparation of steamed minced cutlets was done at 100–110ºC, frying of minced cutlets was done at 150–170ºC, and cooking of sausages on the grill was done at 110ºC.

Technological operations

Fried and steamed minced-meat cutlets. Ingredients for cutlets included chicken meat, beef, semi-fat pork, minced fish, soya mince, and also barley, potato, eggs, onion, carrot, sweet butter, bread, salt and spices. Barley was hydrated with water in ratio 1:2 for 30 minutes. Hydrated seaweeds *Fucus* or *Cystoseira* were added in quantity 2% (by dry weight) to raw stuff and mixed for 7 minutes to obtain indiscrete mass. Eight different recipes were used for cutlets preparation: chicken meat, minced fish, barley with biomass of *Cystoseira* (1) or *Fucus* (2); chicken meat, semi-fat pork, barley with biomass of *Cystoseira* (3) or *Fucus* (4), minced fish, soya mince, with biomass of *Cystoseira* (5) or *Fucus* (6); beef, semi-fat pork, raw potato and biomass of *Cystoseira* (7) or *Fucus* (8). Content of iodine was determined in raw, fried and steamed minced-meat cutlets. Fried minced cutlets were stored at temperature -10 °C for 14 days (freezing) and then content of iodine was checked again.

Meat-ball. Ingredients for meat balls included chicken meat, semi-fat pork, minced fish, taking in different proportions, and also barley, eggs, sweet butter, bread, salt and black pepper. Hydrated seaweeds *Cystoseira* or *Fucus* were added in quantity 2% (by dry weight) to raw stuff and mixed for 7 minutes to obtain indiscrete mass. Meat balls (with weight of 70 g each) were prepared by braising in sauce at temperature 100ºC. Four different recipes were used for meat ball preparation: semi-fat pork, chicken meat, rice grits, barley flour, seaweed *Cystoseira* (1) or *Fucus* (2); minced fish, wheat bread, milk, barley flour, seaweed *Cystoseira* (3) or *Fucus* (4).

Quenelles. Quenelles (small meat balls with weight 15 g each) were cooked in a broth at 100ºC. Hydrated seaweed *Laminaria* was added in quantity 2% (by dry weight) to raw stuff and mixed for 7 minutes to obtain indiscrete mass. Two different recipes were used for quenelles preparation: semi-fat pork, chicken meat, barley flour, egg, carrot, onion, salt, black pepper and seaweed *Laminaria* (1); minced fish hake, white bread, sweet butter, barley flour, egg, carrot, onion, salt, black pepper and seaweed *Laminaria* (2).

Dumplings. Ingredients for dumplings included minced pork, minced beef, fat pork (recipe 1) and fish pike perch (recipe 2), and also onion, chicken eggs, and spices. Hydrated seaweed *Laminaria* was added in quantity 2% (by dry weight).

Grilled sausages. Sausages on the grill were prepared at 110 °C for 60 min. Ingredients for sausage included chicken meat, semi-fat pork, lard, bean, onion, and species. Hydrated seaweeds *Fucus* (recipes 1 and 3) or *Cystoseira* (recipes 2 and 4) were added in quantity 2% (by dry weight).

Results and discussion

Fried minced-meat cutlets. Iodine losses in minced-meat cutlets after frying at 150–170 °C varied from 20 to 37% in products with *Cystoseira* (recipes 1, 3, 5, and 7) and from 20 to 23% in products with *Fucus* (recipes (2, 4, 6, and 8) (Figure 2).

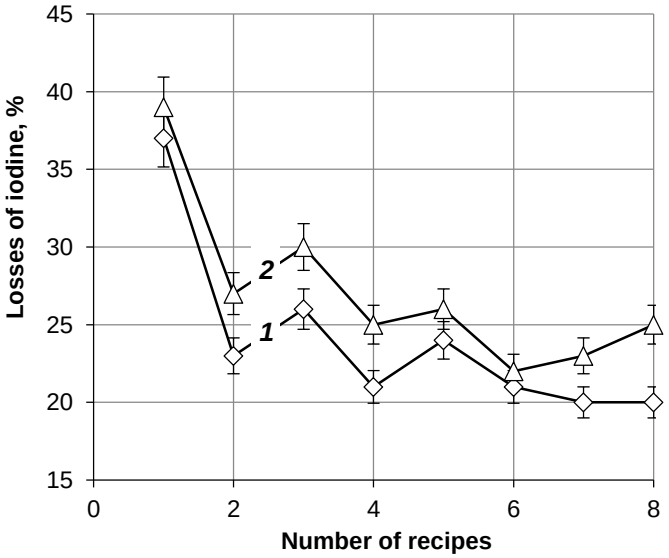

Figure 2. Iodine losses in fried minced-meat cutlets prepared by different recipes
1 – fried; 2 – fried after freezing

Content of iodine in fried minced-meat cutlets with mass 50 g varied from 276 to 315 mcg: 189 mcg (recipe 1); 215 mcg (recipe 2); 276 mcg (recipe 3); 188 mcg (recipe 4); 315 mcg (recipe 5); 216 mcg (recipe 6); 188 mcg (recipe 7); 186 mcg (recipe 8). It was shown that the losses of iodine depend on recipe notwithstanding what kind of seaweeds was used. During preparation of fried cutlets, the higher losses were observed for recipes 3 and 4, which differed only by seaweed added (3 with *Cystoseira*) and (4 with *Fucus*), and the content of iodine in 50 g of fried minced-meat cutlets were 276 and 188 mcg, respectively. Meanwhile, the lowest losses of iodine were observed for recipes 5 and 6, which also differed only by seaweed added (5 with *Cystoseira*) and (6 with *Fucus*), and the content of iodine in 50 g of fried minced-meat cutlets were 315 and 216 mcg, respectively.

Fried minced-meat cutlets were stored at – 10 ºC for 14 days. Losses of iodine during storage under freezing were negligeable, around 2%, and consisted from 23 to 39% in products with *Cystoseira* (recipes 1, 3, 5, and 7) and from 22 to 30% in products with *Fucus* (Figure 2).

Steamed minced-meat cutlets. Losses of iodine in steamed minced-meat cutlets prepared at temperature 100-110ºC were lower than in fried ones and were around 22% in products with *Cystoseira* (recipes 1 and 3) and were around 15% in products with *Fucus* (recipes 2 and 4) (Figure 3).

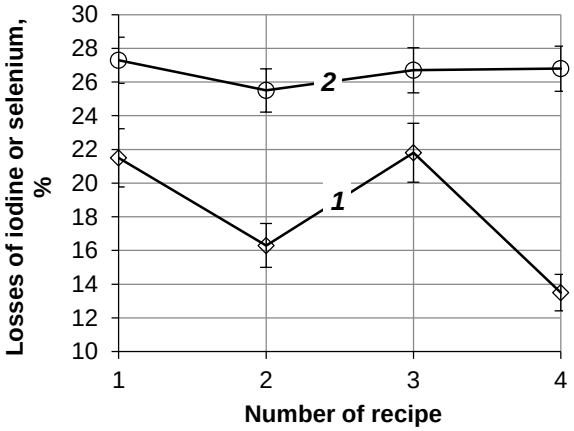

Figure 3. Iodine and selenium losses in steamed minced-meat cutlets prepared by different recipes: 1 – iodine; 2 – selemim

Content of iodine in steamed minced-meat cutlets with mass 50 g varied from 202 to 342 mcg: 342 m cg (recipe 1); 214 mcg (recipe 2); 321 mcg (recipe 3); 202 mcg (recipe 4). Content of iodine in fried minced-meat cutlets prepared without seaweeds (control) with mass 50 g varied from 0.32 mcg to 1.06 mcg.

Losses of selenium in steamed minced-meat cutlets were almost the same for all recipes: around 27% in products with *Cystoseira* (recipes 1 and 3) and around 26% in products with *Fucus* (recipes 2 and 4) (Figure 2). Content of selenium in steamed minced-meat cutlets with mass 50 g were: 40 mcg (recipe 1); 35 mcg (recipe 2); 33 mcg (recipe 3); 30 mcg (recipe 4). Content of selenium in steamed minced-meat cutlets prepared without seaweeds (control) with mass 50 g varied from 20 mcg to 28 mcg.

Meat-balls. Meat-balls and fish-balls were cooked by braising in sauce at 100 °C. Iodine loss in both types of balls with *Cystoseira* (recipes 1 and 3) *Fucus* (recipes 2 and 4) was similar 50.1±1.6%. Contents of iodine in 50 g of balls with *Cystoseira* were 186.2 mcg (recipe 1) and 181.1 mcg (recipe 3); with *Fucus*; 134.9 mcg (recipe 2); 146.3 mcg (recipe 4). Content of iodine in meat balls prepared without seaweeds (control) with mass 50 g varied from 0.01 mcg to 0.04 mcg.

Selenium loss in both types of balls with *Cystoseira* (recipes 1 and 3) or *Fucus* (recipes 2 and 4) was similar 20.1±1.2%. Content of selenium in meat balls with mass 50 g were: 38.9 mcg (recipe 1); 34.1 mcg (recipe 2); 19.2 mcg (recipe 3); 21.6 mcg (recipe 4). Content of selenium in meat balls with mass 50 g prepared without seaweeds (control 1) was 3.6 mcg (control 1 for recipes 1 and 3) and to 6.2 mcg (control 2 for recipes 2 and 4).

Quenelles. Meat quenelles (recipe 1) and fish quenelles (recipe 2) with *Laminaria* with weight 15 g each cooked in the soup at 100ºC. Iodine loss was 60.9% for meat quenelles and 56.1% for fish quenelles. Content of iodine in 50 g of quenelles was 574 mcg (recipe 1) and 693 mcg (recipe 2). Selenium loss was 15.9% for meat quenelles and 20.3% for fish quenelles. Content of selenium in 50 g of quenelles was 136 mcg (recipe 1) and 125 mcg (recipe 2).

Dumplings. Meat dumplings (recipe 1) and fish dumplings (recipe 2) with *Laminaria* were boiled at 100 °C. Iodine loss was 38.2% for meat quenelles and 40.6% for fish quenelles. Content of iodine in 50 g of dumplings was 373 mcg (recipe 1) and 339.0 mcg (recipe 2). Selenium loss was 20.1% for meat dumpling and 21.2% for fish quenelles. Contents of selenium in 50 g of dumplings were 82 mcg (recipe 1) and 80 mcg (recipe 2).

Griddle sausages. Sausages on the grill were prepared at 110 ºC for 60 min. Hydrated seaweeds *Cystoseira* (recipes 1 and 3) or *Fucus* (recipes 2 and 4) were added in quantity 2% (by dry weight). Losses of iodine and selenium were 15.4 and 7.2% (recipe 1), 16.0 and 6.1 % (recipe 2), 15.0 and 8.2% (recipe 3), 20.5 and 7.7 % (recipe 4), respectively. Content of iodine and selenium, mcg/50g, were 270 and 45.4 (recipe 1), 243 and 39.1 (recipe 2), 306 and 40.2 (recipe 3), 218 and 36.0 (recipe 4), respectively.

Compatible analysis of seaweeds application as a source of selenium and iodine to enhance meat products

Average data of all meat products without taking into account influence of recipes are shown in Table 1.

Cystoseira was the better source of iodine than *Fucus.* All ready-to-eat products with *Cystoseira* had higher iodine content than ones with *Fucus.* Influence of temperature is evident, especially due to comparison of iodine content in fried and steamed minced-meat cutlets: iodine losses were lower and the contents of iodine in ready-to-eat products were higher when in the products prepared at lower temperature 100–110 °C (steamed cutlets) in comparison with 170 °C (fried cutlets). Altogether, method used for product preparation had a great influence on iodine losses during cooking. The biggest losses were observed for products prepared in liquid: 50% iodine losses in meat balls braised in sauce and 61% in quenelles cooked in soup. Loss of iodine in dumpling was lower, 38%, maybe due to protection of meat with the dough cover. The lowest loss of iodine, around 17%, was in grilled sausages due to relatively low temperature of cooking and absence of liquid environment.

Table 1

Losses of iodine and selenium in meat products enriched with seaweeds prepared by different cooking methods

Meat products	Temperature, °C	Seaweed	Iodine		Selenium	
			loss, %	mcg/50g	loss, %	mcg/50g
Fried cutlets	150–170	*Cystoseira*	27	242	N/d	N/d
		Fucus	22	201	N/d	N/d
Steamed cutlets	100–110	*Cystoseira*	22	332	27	37
		Fucus	15	208	26	32
Meat ball	100	*Cystoseira*	50	186	21	39
		Fucus	50	135	19	34
Quenelles	100	*Laminaria*	61	574	16	136
Dumpling	100	*Laminaria*	38	373	51	82
Grilled sausages	110	*Cystoseira*	16	305	8	43
		Fucus	18	200	7	38

Recommended Dietary Allowances (RDAs) for iodine is 150 mcg per day for most adults (Trumbo et al., 2001) and 250 mcg per day for pregnant women (WHO, 2007). It looks that *Laminaria* has too high iodine content, so, it is not reasonable to use it for preparation of functional meat product to prevent over dosage of iodine daily intake. However, *Cystoseira* and *Fucus* can be recommended to be used in quantity 2% for preparation of functional meat product to enrich diet with iodine.

Fried and steamed minced-meat cutlets, meat balls, and grilled sausages prepared with addition of seaweeds *Cystoseira* or *Fucus* can be recommended to be used as functional food supplying needed daily quantity of iodine.

Cystoseira was a lit bit better source of selenium than *Fucus.* All ready-to-eat products with *Cystoseira* had slightly higher selenium content than ones with *Fucus.* Influence of temperature and method for food preparation was not so evident, selenium losses varied from 19 to 27 % for steam cutlets, meat balls and even for quenelles and dumpling prepared with *Laminaria.* The lowest loss of selenium, around 7%, was in the grilled sausages.

According to World Health Organization, the recommended daily intake of selenium is from 30 to 40 μg/day (Kieliszek and Blazejak, 2016). Daily consumption of selenium by population in different countries varies from 28 to 150 μg Se per day. Daily recommended allowances of selenium in Ukraine are 70 μg for men and 55 μg for women (Stabnikova et al., 2019). However, a large part of the Ukrainian population has a shortage of selenium in their rations. For example, daily consumption of selenium by people living in town Slavutich, near Chernobyl nuclear power plant accident site, consists only 26% of recommended daily allowance (Spirichev et al., 2006). It looks that *Laminaria* has too high content of selenium to be used for preparation of dietary products as a source of selenium. However, minced meat cutlets, meat balls and grilled sausages prepared with seaweeds *Cystoseira* and *Fucus* can serve as the sources not only iodine but selenium also.

The quality of meat products with seaweeds were estimated by organoleptic properties such as product appearance, view of the cross-section, consistency, color, taste and smell. The volunteers from the National University of Life and Environmental Sciences and the National University of Food Technologies made conclusion that there was no significant difference between meat products prepared with seaweeds and control ones.

Conclusions

Fried and steamed minced-meat cutlets, meat balls, and grilled sausages prepared with addition of seaweeds *Cystoseira* or *Fucus* can be recommended to be used as functional food supplying needed daily quantity of iodine and selenium.

Losses of iodine in meat products prepared with seaweeds depended on recipe, notwithstanding what kind of seaweeds was used. Meanwhile, losses of iodine during storage at – 10ºC for 14 days were negligeable, around 2 %. The method that was used for product cooking as well as temperature of cooking had also a great influence on iodine and selenium losses during food preparation. Lowest losses of both elements were observed for grilled sausage.

Seaweed *Laminaria* has too high iodine and selenium contents to be used for preparation of functional meat products. However, minced meat cutlets, meat balls and grilled sausages prepared with seaweeds *Cystoseira* or *Fucus* can serve as the sources for iodine and selenium in preparation of functional meat products for consumption by Ukrainian population.

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Effect of *Spirulina platensis* and *Kelp* algae on the content of thiamine and riboflavin in wheat bread

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Keywords:
Bread
Spirulina platensis
Kelp
Thiamine
Riboflavin

Article history:

Received 22.07.2020
Received in revised form 01.11.2020
Accepted 25.03.2021

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Abstract

Introduction. The purpose of the present study is to investigate the effect of some edible algae – *Spirulina platensis* and *Kelp* on the content of thiamine and riboflavin in wheat bread.

Materials and methods. Bread is obtained from wheat flour with the addition of *Kelp* and *Spirulina platensis* (powder) in the amount of 2 or 4% by the weight of flour. The vitamin content was evaluated by liquid chromatography with mass spectrometry (LC-MS) method.

Results and discussion. It was found that enrichment with *Kelp* and *Spirulina platensis* (in the amount of 2% and 4% by the weight of flour) leads to an increase in the content of thiamine and riboflavin in wheat bread. The two types of algae have different effects. The use of 2% *Kelp* leads to an increase of 7.35%, and of 4% – by 28.27% of the amount compared to that in the control sample. The most significant increase being observed with the addition of 4% *Spirulina platensis*. The amount of thiamine is 1533.75 μg/kg of bread, which is almost twice as high as in the control sample.

The content of vitamin B_2 in the control bread sample is 310.5 μg/kg. When *Kelp* in the amount of 2% is added, the increase is by 81.7 μg/kg, and at the higher dosage (4%) the increase is by 120 μg/kg compared to the control sample and by 38.3 μg/kg compared to the bread with 2% algae. The highest value was reported for bread enriched with 4% *Spirulina platensis*. The riboflavin content is almost 3 times higher than in the control sample; 2.37 times higher than in the 2% *Kelp* sample and 2.16 times higher than in the 4% *Kelp* sample.

Conclusions. Fortification of wheat bread with some edible algae – *Kelp* and *Spirulina platensis* (especially in the amount of 4% by the weight of the flour) is an effective approach for increasing the content of thiamine and riboflavin. The effect of *Spirulina platensis* on the vitamin content is more pronounced.

Introduction

The importance of vitamins for good health has long been known. Often, to ensure an adequate intake of vitamins, people (especially those on special diets for health reasons, vegetarians and vegans) consume foods fortified with vitamins derived from natural sources. Seaweed can be successfully used for this purpose. Honya et al. (1994) point out that fresh or dried seaweed is a traditional food of the diet in many coastal areas, especially on the Pacific coasts of Asia and South America. On the other hand, Boukid and Castellari (2021) outline a new trend – the use of algae in food formulation is positioning firmly in the food market in Europe. The number of food products containing algae has significantly increased during the period 2015–2019 (13,090 new food products containing algae were globally launched, of which 5720 – in Europe). However, most of the specific nutritional information on edible seaweeds concerns their protein content and favorable amino acid composition, but there are less researches on the vitamin content in micro- and macroalgae.

Fabregas and Herrero (1990) published results on vitamin content of various microalgal species. They noted that microalgae contained high concentration of provitamin A, vitamin E, vitamin B_1 and folic acid, compared to conventional food sources. They point out that *Dunaliella tertiolecta* is able to synthesize vitamin B_{12} (cobalamin), vitamin B_2 (riboflavin), vitamin E (tocopherol) and provitamin A (β-carotene). Pawlak et al. (2014) point out that seaweed containing vitamin B_{12} may be beneficial for people who follow a vegan diet who are at risk of vitamin B_{12} deficiency (as it is found mainly in foods from animal origin). Because certain seaweeds are a valuable source of both fat- and water-soluble vitamins (Norziah and Ching, 2000) their use by the food industry and consumption by the general public is increasing. According to Kennedy (2016) seaweed is a good source of a number of water-soluble vitamins (B_1, B_2, B_{12} etc.). Based on this, algae are of particular interest as food fortifiers due to the high content of biologically active ingredients (including vitamins). According to Kadam and Prabhasankar (2010), bakery products, which are the most widely consumed foods in the world, are best suited to include functional ingredients in the recipe to achieve good health, optimal duration and quality of human life. From this point of view, algae are a promising raw material for the bakery industry. Many authors study the influence of algae on the protein content (Achour et al., 2014), dietary fiber (Raman et al., 2019) and mineral content of wheat bread (Ak et al., 2016). Others pay attention to the effect of algae on the rheological properties of dough and bread (Rosell et al., 2001; Mamat et al., 2014), sensory properties and consumer acceptance of bread (Saharan and Jood, 2017), antioxidant activity (Różyło et al., 2017) and iodine content (Tsyganova et al., 2014). The effect of algae on the vitamin content of bread has been less studied.

Dulinski et al. (2018) conduct a study to design a functional rye bread enriched with algae extracts (*Ascophyllum nodosum, Arthrospira platensis*) and to analyze the content of its selected bioactive ingredients: phenolic acids and vitamins B_1 and B_2. As part of the research, an attempt was also made to estimate the absorbable pool of these compounds by using an in vitro procedure simulating digestion in the human digestive tract. It was found that the addition of algae at the stage of mixing the rye dough significantly contributed to the increase of the available pool of B vitamins in bread, especially riboflavin. The analysis shows that the tendency to increase the vitamin content applies to bread samples enriched with *Arthrospira platensis*. Relatively high in vitro bioavailability of vitamin B_2 in bread was found (45-62%). The authors attribute this to the beneficial effect of hydrocolloids present in its biomass on the absorption of bioactive ingredients. In the case of thiamine, such large increases in the content of this vitamin with the addition of the algae component were not observed, nevertheless the trends noted in the case of riboflavin were confirmed.

There are relatively limited publications on the effects of different types of algae on the content of thiamine and riboflavin in bakery products. Both vitamins are sensitive to the influence of temperature, light and other factors which the cereal grains are subjected to. Technological processes in breadmaking can partially degrade these biocomponents in the range from 25% (thiamine) to even 50% (riboflavin) of their initial level in the raw material (Mihhalevski et al., 2013; Wolak et al., 2017). For this reason, many industrially produced grain products are fortified with B vitamins to restore their original levels. There is a significant gap in our knowledge about the effect of different types of seaweed on thiamine and riboflavin content of wheat bread.

Therefore, the purpose of the present study is to investigate the effect of *Spirulina platensis* and *Kelp* (in the amount of 2 % and 4 % by the weight of flour) on the content of thiamine and riboflavin in wheat bread.

Materials and methods

Materials

For the preparation of the bread samples, the following materials were used:

- Commercial wheat flour type 500 with the following properties: moisture content – 12.8 %; gluten content – 27.07 %; release of gluten – 6 mm; titratable acidity – 2 °H;
- Water – according to ISO 6107-1:2004;
- Commercial yeast (Lesafmaya);
- Salt – according to Codex Standard for Food Grade Salt CX STAN 150-1985;
- *Spirulina platensis* powder (average chemical composition: protein 64 g/100 g; fat 8.2 g/100 g of which saturated 3.42 g; carbohydrates 16.1 g/100 g, of which sugars 0.52 g, fiber 7 g/100 g).
- *Kelp* powder (average chemical composition: protein 5.3 g/100 g; fat 4.2 g/100 g of which saturated 0.9 g; carbohydrates 12.0 g/100 g, of which sugars 0.5 g, fiber 1.25 g/100 g).

Methods

Dough and bread composition

The composition of the bread samples is presented in Table 1.

Table 1

Bread samples composition

Ingredients	Bread samples				
	Control sample	**Sample S2 - with 2 % *Spirulina platensis***	**Sample S4 - with 4 % *Spirulina platensis***	**Sample K2 – with 2 % *Kelp***	**Sample K4 – with 4 % *Kelp***
Wheat flour , g	250	245	240	245	240
Water, cm^3	140	145	155	145	155
Yeast, g	3.37	3.37	3.37	3.37	3.37
Salt, g	3.25	3.25	3.25	3.25	3.25
S. platensis, g	–	5	10	–	–
Kelp, g	–	–	–	5	10

Bread preparation

Bread is obtained from type 500 wheat flour by a two-phase method. Initially, knead the yeast, flour and water dough in a 1:1 ratio in kneading machine (Labomix 1000, Hungary). Pre-mixed *Spirulina platensis* and *Kelp* algae (powder) in the amount of 2 % or 4 % by the weight of flour are added to the mixing water (combinations K2 and K4, for the breads prepared with *Kelp* and combinations S2 and S4, for the breads prepared with *Spirulina platensis*, respectively). The control sample was prepared only with wheat flour. The dough thus prepared matures for 4 hours at 33°C and then mix the dough to obtain a homogeneous mass by adding the remainder of the flour according to the formulation and salt (1.3 kg/100 kg flour). The bread dough divides (440 g) and forms, matures for 55 minutes at 38°C (Tecnopast CRN 45–12, Novacel ROVIMPEX Novaledo, Italy). After the final fermentation, the pieces of dough were put into an electric oven (Salva E-25, Spain) pre-heated to 200–220°C. The baking time is 24 min, until the temperature in the center of the bread crumb reach 96-98°C. After baking, the bread is allowed to cool down for 3 h at room temperature.

Determination of vitamin B_1 and vitamin B_2 content

Sample extraction. 5 g of each sample of bread were weighted into a 50 ml centrifuge tube using an analytical balance. 25 ml of doubly distilled water were added and the samples are dispersed with a Polytron apparatus for 10 minutes. The resulting suspensions were centrifuged at 4000 rpm for 15 minutes. The upper layer was decanted into a centrifuge tube. The precipitate was dispersed twice more with 10 ml of doubly distilled water. After each dispersion, the suspensions were centrifuged and the lower layers were combined, then filtered through a quantitative filter (syringe filter with a pore size of 0.45 μm). About 1 ml was taken from the filtrate and stored at up to 4°C for chromatographic analysis.

Analytical methods. In the samples thus prepared, the content of water-soluble vitamins B_1 (thiamine) and B_2 (riboflavin) was examined on a liquid chromatograph with a high-resolution mass-selective detector (LC-MS). The chromatographic system was equipped with M510 and M45 pumps (Waters Associates), Rheodyne M-7125 injector and scanning fluorescent detector. The mobile phase was methanol/water/acetic acid (31/68.5/0.5) containing 5 mM sodium hexasulfonate. The determination of the chromatographic peaks was carried out by comparing the retention times of the pure substances for chemical analysis (certified reference material for vitamin B_1 and vitamin B_2) with that of the tested samples. The qualitative identification of the analytes was based not only on the retention times of each component, but also on the secondary mass spectrum of the characteristic result ions of the studied vitamins.

Data on the amount of vitamins tested in bread samples were processed by the integrated software of the liquid chromatograph with mass detector used.

Results and discussion

Effect of *Spirulina platensis* and *Kelp* on the content of vitamin B_1 in wheat bread

The human body needs a minimum of 0.33 mg of thiamine for every 1000 kcal it consumes (Osiezagha et al., 2013). According to Harper (2006), many population groups worldwide suffer from vitamin B_1 (thiamine) deficiency and are at risk of severe neurological diseases. Vitamin fortification of daily consumed foods (such as bread) is an effective and

easily achievable measure to improve public health, thanks to which it can overcome their deficiency in all groups of the population.

Some authors (Batifoulier et al., 2006), aim to study by means of HPLC the content of vitamin B_1 in nine varieties of wheat, as well as in the flour and bread obtained from them. It has been found that vitamin B_1 content ranging from 2.60 to 6.13 μg/g dry matter in the different wheat varieties. This reveals that the vitamin content is genetically determined and should be an important factor to consider when selecting different varieties of wheat. After grinding, only 43% of thiamine present into low-ash flour (compared to 80% for wholemeal flour). After baking, the amount of thiamine decreases by 37% in white bread and by 31% into wholemeal bread.

It's well known that bread made from flours, which contain less particles from the peripheral layers of the grain, has a lower vitamin content. This is due to the fact that thiamine is unevenly distributed in the anatomical parts of the wheat grain – the aleurone layer and the germ are much richer in this vitamin than the endosperm. In addition, the high temperature effect during baking leads to the thermal destruction of a significant part of the vitamins. That is why wheat bread from flour type 500 is not a good source of vitamins for the human body. The effect of algae *Spirulina platensis* and *Kelp* on the content of vitamin B_1 in wheat bread is presented in Figure 1.

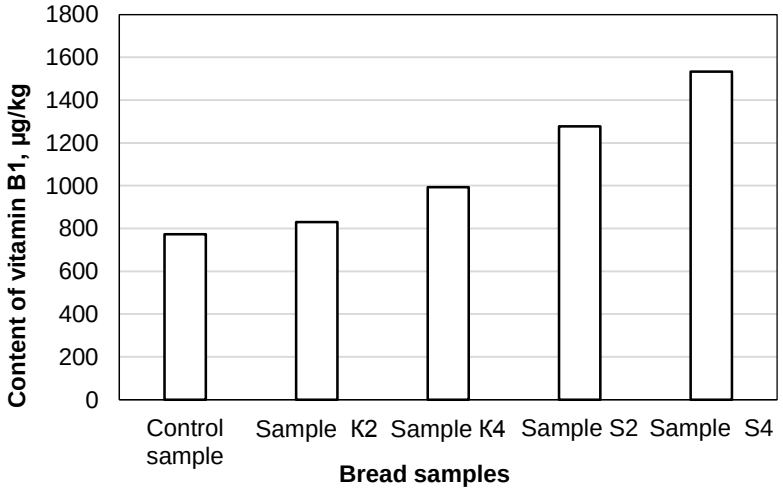

Figure 1. Effect of *Kelp* and *Spirulina platensis* algae on vitamin B_1 content in wheat bread.

The content of vitamin B_1 in wheat bread from flour type 500 is 773.75 μg/kg. Our data on thiamine content are almost identical to those published by Tiong et al. (2015), which found an amount of 1.0 ± 0.028 mg/100 g of product, but expressed the dry matter result (and the data presented by us are of fresh product). Other authors (Umelo et al., 2014) found lower amounts of vitamin B_1 in wheat bread – ranging from 0.034 mg/100 g to 0.044 mg/100 g. In this case, both the genetic and varietal characteristics of the wheat and the specific technological parameters of bread preparation (such as the type and quantity of yeast, the duration of fermentation process) have an influence.

From the data presented in Figure 1, it is clear that the addition of algae in the recipe of wheat bread leads to an increase in the content of vitamin B_1. Both types of aquacultures affect the amount of thiamine to varying degrees. *Kelp* cause an increase in its content to a lesser extent. The use of 2% biomass from algae leads to an increase of 7.35%, and of 4% – by 28.27% of the amount compared to that in the control sample. These results are expected, considering that brown algae contain relatively high amounts of thiamine. According to Sánchez-Machado et al. (2004) vitamin B_1 content vary from 0,14 ± 0,02 to 0,40 ± 0,13 g /g dry matter. Therefore, the replacement of a relatively small portion of wheat flour (2% and 4%) with these brown algae reflects this way on the content of vitamin B_1 in bread.

When *Spirulina platensis* is added to the recipe of wheat bread, the effect on the amount of thiamine is much more pronounced. The addition of 2% caused an increase in the amount of vitamin B_1 1.65 times compared to the control sample. The reported result is 1277.5 μg/kg of bread. This amount is 1.54 times higher than in the sample enriched with 2% *Kelp* algae. When using 4% *Spirulina platensis*, the thiamine content is 256.25 μg/kg higher. The value obtained of 1533.75 μg/kg of bread is almost twice as high as for the control sample; 1.85 times higher than for the 2% *Kelp* sample and 1.55 times higher than for the 4% *Kelp* sample. The high efficiency of enrichment with these algae stems from the fact that they have a very high content of thiamine, which, however, shows strong seasonal variations. Babadzhanov et al. (2004) published the following data on thiamine content in *Spirulina platensis*: 11.6% of dry matter in biomass collected in winter; 15.4% in biomass collected in the spring; 0.8% in biomass collected during the autumn and summer seasons. According to these data the biomass grown in spring-summer had a high vitamin content. This shows a clear seasonal fluctuation in the vitamin content of algae. In order to increase the vitamin content of wheat bread, the biomass of *Spirulina platensis*, intended for enrichment, should be harvested during the spring-summer season. In addition, the amounts to enrich the bread and achieve adequate intake must be calculated according to the amount of thiamine in the biomass.

According to Yusuf et al. (2016) the thiamine content of *Spirulina platensis* ranges from 34 to 50 mg/kg. There is a significant difference between the results published by the two authors. This is due to the fact that the composition of algae is not constant and varies depending on their species, geographical regions and harvesting season (Wells et al., 2017).

To get a better idea of the enrichment effect, a comparison was made between the recommended daily intake of thiamine and the extent to which the bread samples tested satisfies it.

The need to determine adequate vitamin B_1 intake arose decades ago. As early as 1967, the World Health Organization set a recommended intake of thiamine 0.4 mg/1000 kcal. Therefore, an adult male consuming 3200–3300 kcal per day needs a daily intake of 1.3 mg of thiamine, while a woman consuming an average of 2300 kcal needs to take 0.9 mg of thiamine per day. In 1989, the National Council for Research in the United States recommended a daily intake of 0.5 mg/1000 kcal for adults, and the total daily intake not less than 1.0 mg even for those consuming less than 2000 kcal daily (World Health Organization, 1999).

In Bulgaria, Ordinance №1/2018 of the Ministry of Health differentiates the recommended thiamine intake depending on gender and age of the population. For men in the age range of 19 to 60 years, the recommended daily intake of thiamine is 1.2 mg, while for women of the same age – 1.0 mg. The degree to which the daily amount of bread consumed from the tested samples satisfies the recommended daily intake is presented in Figure 2.

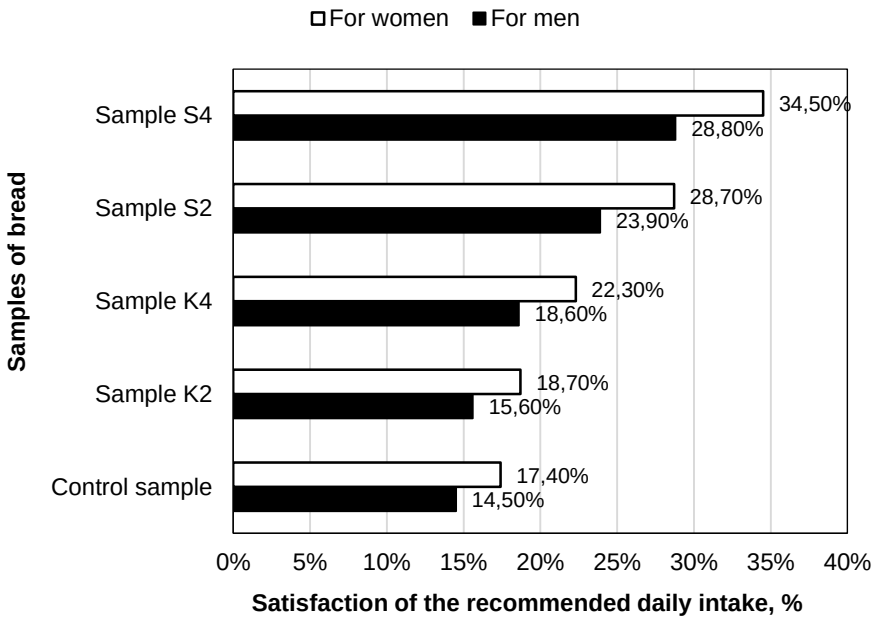

Figure 2. Effect of *Kelp* and *Spirulina platensis* on the degree of satisfaction of the recommended daily intake of vitamin B_1 in men and women aged 19 – 60 years

As can be seen from the figure, the average daily amount of wheat bread consumed in Bulgaria provides less than 15% of the adequate intake of thiamine for men and 17.40% of the adequate intake for women in the specified age range. We must take into account the fact that thiamine may not be properly absorbed by people who have liver problems; there are also some components in food that impair the absorption of thiamine (eg tannins in coffee and tea).

Enriching wheat bread with seaweed leads to a higher degree of satisfaction with adequate thiamine intake. The impact of both the type and the amount of aquaculture input is clear. *Kelp* algae have a less pronounced effect. When 4% of them are added to bread, its daily consumption provides 18.6% of the adequate intake of vitamin B_1 for men and 22.3% of the adequate intake for women.

The impact of the other type of algae is more pronounced. Consumption of bread with 2% *Spirulina platensis* covers almost 24% of the recommended intake for men and almost 29% for women. If the amount of aquaculture is 4%, the highest degree of satisfaction of the needed intake of vitamin B_1 is achieved. Consumption of the usual daily amount of bread provides 28.8% of the required amount for men and over a third (34.5%) of the required amount for women aged 19 to 60 years. Replacing only 4% of wheat flour with *Spirulina platensis* makes bread twice as effective a source of thiamine for the human body (compared to the control sample).

For pregnant women and nursing mothers, a slightly higher adequate daily intake of thiamine was determined – 1.3 mg. Consumption of wheat bread provides 13.38% of this amount, and consumption of bread enriched with 4% *Spirulina platensis* – 26.5%.

Effect of algae *Spirulina platensis* and *Kelp* on the content of vitamin B_2 in wheat bread

Riboflavin deficiency can cause vision problems, particularly cataracts. This disease is the result of abnormal aggregation of proteins in the lens, which causes it to become cloudy. In the elderly, the risk of cataract is increased due to riboflavin deficiency (Skalka and Prchal, 1981). Vitamin B_2 deficiency is found in about 80% of cataract patients. Riboflavin intake of 400 mg/day has a preventive effect against the development of age-related cataracts (Buehler, 2011). People who adhere to a special diet due to health problems (diabetes, peptic ulcer disease, weight loss), as well as smokers, alcoholics and women using certain types of birth control (Škrovánková and Sikorová, 2010) are at increased risk of riboflavin deficiency.

Thus presented literature data show that insufficient intake of vitamin B_2 can affect health status in many different ways. The human body cannot synthesize this vitamin and therefore it must be absorbed through food through absorption in the small intestine. The results on the effect of *Spirulina platensis* and *Kelp* on the content of vitamin B_2 in wheat bread are presented in Figure 3.

As can be seen from the figure, the content of vitamin B_2 in the control bread sample (from wheat flour type 500) is low – 310.5 μg/kg. Similar results were published by Martinez-Villaluenga et al. (2009). According to them the amount of vitamin B_2 in wheat bread is 45.48 ± 0.22 μg/100g dry matter. If this value is recalculated for fresh product, it would mean 272.88 μg/100g. Regarding the content of vitamin B_2 in bread, decisive factors are: its quantitative content in the flour, the duration of fermentation, pH, high temperature exposure, exposure to ultraviolet radiation and others (Ahmad et al., 2004). Whole meal bread is a better source of this vitamin, as it is concentrated mainly in the germ and aleurone layer. The inclusion of *Kelp* seaweed in the bread recipe leads to an increase in the amount of riboflavin, but not very significantly. When the aquaculture is in the amount of 2%, the increase is by 81.7 μg/kg, and at the higher dosage (4%) the increase is by 120 μg/kg compared to the control sample and by 38.3 μg/kg compared to the bread with 2% seaweed.

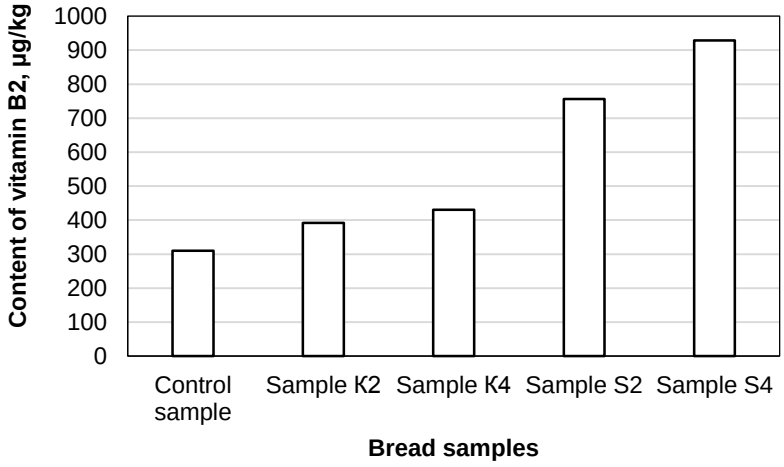

Figure 3. Effect of *Kelp* and *Spirulina platensis* algae on vitamin B_2 content in wheat bread

When *Spirulina platensis* is added to wheat bread, the effect on the amount of riboflavin is much more pronounced. In the sample with the addition of 2% of the algae content was reported 2.44 times higher than in the control sample and 1.93 times higher than in the bread with the same amount of *Kelp*. The highest value was reported for bread enriched with 4% *Spirulina platensis*. The riboflavin content is almost 3 times higher than in the control sample; 2.37 times higher than in the 2% *Kelp* sample; 2.16 times higher than in the 4% Kelp sample. If we compare the result with that for bread containing *2% Spirulina platensis* – the increase is almost 23%. This proves that enriching wheat bread with *Spirulina platensis* is effective and leads to a significant increase in vitamin B_2 content.

Initially, the high protein content and favorable amino acid composition of algae aroused interest among researchers. Subsequently, it was found that they are a good source of other biologically active substances, including – vitamins (Wells et al., 2017). In fact, the literature on analytical methods and the content of riboflavin in biomass (fresh or dried) from *Spirulina* is contradictory. It has been reported that the content of riboflavin in *Spirulina platensis* varies from 37 to 45 μg/g dry matter (Andrade, 2018; Bishop and Zubeck, 2012). Edelmann et al. (2019) examined four varieties (different brands with different countries of origin) of *Spirulina* algae and found results ranging from 33.6 to 40.9 μg/g in the different samples. In the same publication, a comparison was made between the amount of riboflavin in the algae *Spirulina platensis*, *Chlorella* and *N. gaditana* powder. The author points out that of all the aquaculture studied, the best source of riboflavin is the algae *Spirulina*. This reveals the good potential for the use of Spirulina platensis as a supplement to increase the content of vitamin B_2 in wheat bread. A much lower content of riboflavin (2-9 μg/g) in *Spirulina platensis* has been reported by Babazhanov et al. (2004). Probably this is due to the variation in the amount of vitamins depending on the region and the water basin of aquaculture development, the season of their harvest and others.

Škrovánková and Sikorová (2010) point out that the recommended daily intake of riboflavin depends on protein and energy intake and varies from 1.2 to 1.7 mg per day. According to Ordinance №1/2018 of the Ministry of Health in Bulgaria, the recommended intake of riboflavin for men in the age range from 19 to 60 years is 1.3 mg, while for women of the same age is 1.1 mg. The degree to which the daily amount of bread consumed from the various tested samples satisfies the recommended daily intake is presented in Figure 4.

As can be seen from the figure, the average daily amount of bread from the control sample provides a very small portion of the recommended intake – only 5.38% for men and 6.35% for women. If the diet includes other foods rich in riboflavin, this would compensate for its low content in bread. But for people for whom wheat bread has been a staple food for a long time, there is a risk of hypovitaminosis.

When *Kelp* is included in the bread recipe, the average daily amount of bread consumed covers the recommended intake to a higher degree. The impact of aquaculture is stronger when the amount is 4% by the weight of flour (respectively 7.45% of adequate intake for men and 8.80% of adequate intake for women).

The effect of algae *Spirulina platensis* is much more pronounced. If used in an amount of 2%, the daily amount of bread consumed covers 13.10% of the adequate intake of riboflavin in men and 15.50% in women. These values are almost 2.5 times higher than those found in the control sample. To the greatest extent, the amount of thiamine obtained from bread is close to the recommended intake when 4% *Spirulina platensis* is included in the recipe. The daily intake of bread provides 16.1% of the required amount of riboflavin in men and almost one-fifth of the required amount in women. Thus enriched bread is three times more effective than the control sample and 2.16 times more effective than bread containing 4% *Kelp* in terms of meeting the recommended intake of vitamin B_2.

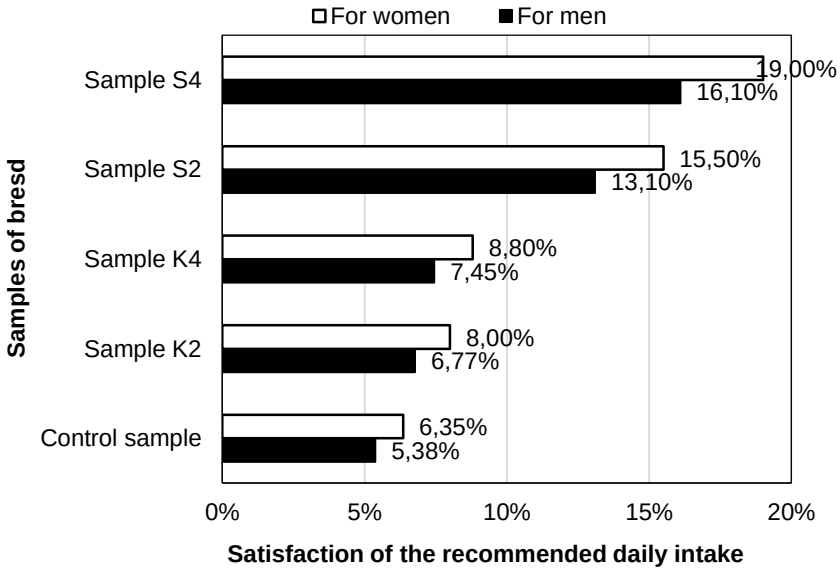

Figure 4. Effect of *Kelp* and *Spirulina platensis* on the degree of satisfaction of the recommended daily intake of vitamin B2 in men and women aged 19 – 60 years

For pregnant women, the recommended daily intake of this vitamin is 1.4 mg. The control sample of bread yields only 5% of this amount, and the bread with 4% *Spirulina platensis* – 14.9%. The highest norms for daily requirement of vitamin B_2 are set for women who are breastfeeding – 1.6 mg. In this case, wheat bread provides only 4.4% of the required amount of riboflavin, while fortified bread – 6% when using *Kelp* and 13% when using *Spirulina platensis* (in the amount of 4%).

Conclusion

1. From the results obtained in the present study, it was found that the fortification of wheat bread with *Kelp* and *Spirulina platensis* algae (in the amount of 2% and 4% by the weight of flour) is a good opportunity to increase the content of thiamine and riboflavin.
2. The effect of *Spirulina platensis* is more pronounced, as the highest values for both vitamins were found when adding 4% of the algae to the raw materials for bread making.
3. By including biomass of algae *Kelp* and *Spirulina platensis* in the bread recipe, a significantly higher degree of satisfaction of the recommended daily intake of thiamine and riboflavin is achieved. This approach would allow prevention of deficiency of these vitamins in some groups of the population and improvement of the health status.

These data show that enriching wheat bread with *Kelp* and *Spirulina platensis* is a successful approach to increasing the content of thiamine (vitamin B_1) and riboflavin (vitamin B_2) in bread. This way a large part of the population could achieve an intake of these vitamins, much closer to the adequate.

Acknowledgements. Authors would like to thank to the Ministry of Education and Science of Bulgaria about the subsidy by Regulation № 3 from 27.11.2015 – in accordance with the Ordinance on the Terms and Procedure for the Evaluation, Planning, Allocation and Expenses of the State Budget Funds for the Financing of the Inherent Research Activities of the State Higher Education Institutions. The acknowledgements are also to the academic management of University of Economics – Varna for the allocations in project NPI-16/2018 "Innovative approaches to increase the quality and biological value of foods".

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Organic acids content, sugars content and physicochemical parameters of Romanian acacia honey

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Keywords:

Honey
Pollen
Acacia
Robinia pseudoacacia

Article history:

Received 26.09.2020
Received in revised form 12.11.2020
Accepted 25.03.2021

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DOI:
10.24263/2304-974X-2021-10-1-14

Abstract

Introduction. Some elements of a honey are added by bees; others occur after the maturation of honey. The physicochemical parameters, pollen content, sugars content and organic acids content were determined for Romanian acacia honey.

Materials and methods. 27 samples of acacia honey from 2020 were examined to determine the physicochemical parameters (moisture, electrical conductivity, pH, free acidity, color, HMF content) and sugars content using the methods proposed by the International Honey Commission. Organic acids composition was determined using the method proposed by Ozcelik.

Results and discussion. The botanical origin of all samples was confirmed by melissopalinological analysis, each sample containing more than 45% *Robinia pseudocacia* pollen granules. According to Codex Alimentarius, moisture content should be lower than 20%, and for the analyzed samples the moisture content ranged from 16,66–20,74%. The pH of the acacia honey samples ranged from 3.61 to 5.33. The free acidity of acacia honey analyzed in this study ranged from 0.32 to 4.14 meq/kg. None of the analyzed samples exceeded the limit imposed by legislation. All honey samples showed similar lightness values (29.62–46.57). The maximum content of HMF in the samples was 23.20 mg/kg thus falling within quality requirements. A value of less than 500 μS/cm indicates a pure floral honey, and in this study the samples of acacia honey had electrical conductivity values between 94.8–405 μS/cm. In the acacia honey samples was identified a percentage of 68.35% monosaccharides, and a small percentage of sucrose (maximum 2.093%). The F/G ratio varied between 1.02 and 1.65 for the studied acacia samples and some samples can crystallize quickly because have high glucose content and the F/G ratio is about 1. In the samples with F/G values above 1.3 the tendency of crystallization is slower. Gluconic acid was the main organic acid in all samples (1.916–2.666 g/kg) followed by propionic and acetic acids. Succinic acid has the lowest concentration in the studied acacia honey samples.

Conclusions. All the investigated honey samples (27 samples) met the quality criteria examined (moisture, pH, free acidity, HMF content, color and electrical conductivity) and the high percentage of pollen grains of *Robinia pseudoacacia* confirmed that the samples analyzed were monofloral acacia honey.

Introduction

Honey is the natural sweet substance produced by bees (Codex Alimentarius, 2001) and most of its elements are derived from plants; while some are added by bees and others occur after the maturation of honey (Teklit and Frehiwot, 2016). The main parameters of honey quality, which also influences its price, derive from its botanical origin (Seisonen et al., 2015). Monofloral honey is a high quality product, which has a unique aroma and taste, but is often adulterated by incorrect labeling and mixing with inferior honey (Trifković et al., 2017; White, J. W., 2000). Monofloral honey is known to have specific therapeutic properties and organoleptic attributes, and for this reason is one of the foods preferred by consumers.

Physicochemical parameters and especially the color influence the price of honey but also the preferences of consumers (Visquert et al., 2014). Specific rules and standards are needed to guarantee the identity and quality of honey so that people can consume honey safely and pay the right price (Danezis et al., 2016). Obtaining data on the physicochemical parameters of honey is important both for its characterization and for ensuring the quality of products on the market.

The identification of the botanical and geographical origins of honey are indicators of its authenticity (Jandrić et al., 2017; Karabagias et al., 2018). Ensuring the authenticity and quality of honey is of real interest in the international honey market for both regulators, consumers, traders and beekeepers (Sobrino-Gregorio et al., 2017). It should be noted that each honey has unique organoleptic characteristics, which are closely related to its source: botanical and geographical (Bogdanov et al., 2008).

The quality criteria for honey are set out in the EU Regulation and they include organoleptic characteristics (appearance, color, taste, consistency, aroma and taste) and physicochemical characteristics (moisture, carbohydrate content, pH, acidity, minerals, electrical conductivity, vitamins, organic acids, hydroxymethylfurfural (HMF) content, proteins) (Council, E. U., 2002). These parameters are influenced by the type of nectar, climate, soil but also by handling practices after harvesting honey (Belay et al., 2013). When we refer to the floral origin of honey, we implicitly refer to the geographical area, but also to the flora of that area during the collection period (Kaškonienė and Veskutonis, 2010). The chemical composition of honey even if it has the same floral source can be quite different, due to different climatic conditions, soil characteristics and the presence of different minerals from the soil (Persano Oddo and Piro, 2004). Honey is a complex product, whose composition varies depending on the species of bees, the type of nectar, geographical area, season and storage conditions and requires a characterization that targets various analytes: volatile compounds, phenolic acids, flavonoids, carbohydrates, amino acids and organic acids. The labeling of honey with a certain botanical or geographical origin cannot be carried out taking into account only one type of chemical markers, but rather through a combination of several.

The most known and appreciated assortment of monofloral honey produced in Central Europe is acacia honey (*Robinia pseudocacia*) (Oroian et al., 2015). Acacia honey is available on the European market, being one of the most appreciated assortments for its appearance, light yellow color, delicate fragrance and floral aroma (Schievano et al., 2019).

In order to be able to classify a sample of honey as authentic acacia honey, it must contain at least 45% granules of acacia pollen, as specified in the regulations on the quality of honey (Soares et al., 2017). Analysis of pollen grains in honey sediments is used as a benchmark to identify the botanical origin of honey (Pires et al., 2009). The main components of honey are sugars and their use as floral or geographical markers of honey is not common due to the difficulties of specifying one or more carbohydrates present in honey that can serve

this purpose (Kaškonienė et al., 2010). However, some authors have suggested the use of the amount and ratio of specific carbohydrates (fructose and glucose), as well as oligosaccharides, as useful indicators for the recognition of monofloral honey (Cotte et al., 2004). Organic acids are present in small quantities in honey but are important in establishing the freshness and authenticity of honey (Pauliuc and Oroian, 2020).

The purpose of this work was to determine for acacia honey the physicochemical parameters (melissopalynological analysis, moisture, color, pH, free acidity, electrical conductivity), hydroxymethylfurfural content, sugars content and organic acids content.

This is the first study aimed at analyzing the organic acids found in acacia honey in the N-E area of Romania.

Materials and methods

Samples

The acacia honey samples (27 samples) were purchased from the N-E part of Romania and come from the production of 2020. The samples were purchased directly from beekeepers and were liquefied at 50 ° C to be prepared for analysis.

Melissopalynological analysis

To identify the type of pollen that predominates in the honey sample, the pollen granules on a sediment spread were counted at ×40 magnification using an light microscope (Motic). Sediment spread was obtained by dissolving 10 g of honey in 40 mL of distilled water, followed by centrifugation at 4500 rpm for 15 minutes. The centrifugation process was repeated for another 15 minutes after the supernatant was removed and water was added (Von Der Ohe et al., 2004).

Physicochemical analysis

In order to determine the physicochemical parameters of acacia honey, the methods proposed by the International Honey Commission (Harmonised Methods Of The International Honey Commission, 2008) were used. With the help of these methods the following parameters were determined: moisture content (using Abbe refractometer, Leica Mark II Plus), electrical conductivity (using portable conductometer HQ14d, HACH, USA), pH (using pH meter Mettler Toledo FiveGo, Mettler Toledo, USA), free acidity (using TITROLINE easy, Schott Instruments, Germany) and color (using portable chromameter CR-400, Konica Minolta, Japan). The hydroxymethylfurfural (HMF) content was determined using a UV-VIS-NIR spectrophotometer SCHIMADZU UV-3600 (Schimadzu Corporation, Japan), according to the method proposed by White (White, 1979).

Determination of sugars composition

The sugars content was determined with a HPLC instrument (Schimadzu, Kyoto, Japan). Before being injected into the HPLC instrument the samples were prepared as follows: 5 g of each acacia honey sample were dissolved in 40 mL distilled water, mixed with 25 mL of methanol (in a 100 mL volumetric flask) and then brought to volume with distilled water (Harmonised Methods Of The International Honey Commission, 2008). The

HPLC instrument was equipped with a LC-20 AD liquid chromatograph, SIL-20A auto sampler, CTO-20AC column oven, and RID-10A refractive index detector. The separation was performed on a Phenomenex Luna® Omega 3 µm SUGAR 100 Å HPLC Column 150 x 4.6 mm. The samples were filtered through 0.45 µm PTFE membrane filters and then injected in the HPLC instrument. The sample volume injection was 10 µL. The flow rate was 1.3 mL/min and the mobile phase was acetonitrile: water (80:20, v/v); the temperature of the column and detector was 30 °C.

Determination of organic acids

Organic acids were determined using the same instrument as in the case of the sugar content. The method of analysis was described previously by Ozcelik et al., (2016). 0.5 g of acacia honey were mixed with 2.5 mL of 4% metaphosphoric acid (w/v), then the samples were vortexed. Then, the samples were centrifuged for 5 min at 3500 rpm using a Z216-MK refrigerated centrifuge (Hermle Labortechnik, Wemingen, Germany) The sample was injected in the HPLC instrument (Schimadzu, Kyoto, Japan) with a diode array detector.

Results and discussion

Melissopalynological analysis

Since the last century, the analysis of honey pollen has been used as a method of authentication, but the methodology has improved and harmonized several times over the years (Ruoff et al., 2006).

The botanical origin of all samples was confirmed using melissopalinological analysis, each sample containing more than 45% *Robinia pseudocacia* pollen granules. The percentage of 45% pollen grains is the indicator by which honey can be classified as monofloral (Consonni and Cagliani, 2015). Ma et al., (2019) detected a content of 80% pollen granules of *Robinia pseudoacacia* in honey samples from China. Dobre et al., (2013) reported a percentage of 58% *Robinia pseudoacacia* pollen in acacia honey samples from Romania.

Pollen analysis is not suitable for cases where the honey has been incorrectly filtered or has been adulterated by the addition of pollen. Therefore, melisopalinology should usually be supplemented by physicochemical and organoleptic analyzes. Thus, in order to classify honey according to its botanical origin, a global interpretation of all results is needed (Bogdanov et al., 2008).

Moisture content

The moisture of honey depends on several factors, namely: environmental factors, the moisture of the plant visited by the bees, the degree of maturity of the honey reached in the hive and the handling of beekeepers during honey harvest (Flores et al., 2015). According to Codex Alimentarius (Codex Alimentarius, 2001) moisture content should be lower than 20%. Exceeding the maximum allowed value leads to a deterioration by fermentation, due to the presence of yeasts and bacteria in honey (Sakač et al., 2019). The fermentation process results in alcohol, which in the presence of oxygen will decompose into acetic acid and water, honey thus having a sour taste (Prica et al., 2014). This parameter is important in determining quality and stability by limiting degradation during the fermentation process (El Sohaimy et al., 2015). It is considered that a moisture content lower than 18% prevents the fermentation

process. However, this possibility cannot be ruled out even when honey has a moisture content of less than 17.1%, because there are certain predisposing factors such as: yeast content, honey temperature but also the availability and distribution of water after crystallization (Prica et al., 2014). For the analyzed samples the moisture content ranged from 16.66-20.74% (table 1). The moisture content of samples of acacia honey studied by Ahamed et al., (2017) ranged between 8.8-13.85, values which were also in accordance with the Codex Alimentarius standard. In another study, Liberato et al., (2013) examined 22 samples of honey of different botanical origins from Nothern Brazil and found that the moisture percentage range was 13.63-20.4%. Juan-Borrás et al., (2014) studied acacia honey from 3 different countries and reported a moisture content of 16.9% for Romanian acacia honey, 15.9% for Spanish honey and 17% for Czech honey.

pH

The pH of honey is an important parameter because an acidic pH inhibits both the presence and growth of microorganisms and can also influence the texture of honey, its stability and shelf life (Karabagias et al., 2014). Organic acids are the compounds responsible for protecting against microbial attacks, the pH of honey beeing normally between 3.5 and 5.5. Besides the fact that pH is an indicator of a possible microbial growth (Da Silva et al., 2016) it also has a role in identifying the botanical origin of honey (Sanz et al., 2005). The geographical and floral origin influences the pH values. The acidic pH of honey depends largely on the amount of gluconic acid resulting from the oxidation of glucose under the action of glucose oxidase. In addition, different aromatic and non-aromatic acids can affect the pH of honey (Khalafi et al., 2016). The pH of acacia honey samples ranged from 3.61 to 5.33 (table 1). The values were similar to those reported by Karabagias et al., (2018) for eucalyptus, chestnut and heather honey (pH 3.62 to 4.42). Cimpoiu et al., (2013) reported that the average pH value is 4.23 for acacia honey and 4.36 for polyfloral honey.

Table 1

Physical-chemical parameters, sugars content and organic acids content of acacia honey

Variable	Minimum	Maximum	Mean	Std. deviation
pH	3.610	5.330	3.980	0.365
Acidity (meq / kg)	0.320	4.140	2.377	0.828
Conductivity µS/cm	94.800	405.000	212.322	84.159
Moisture (%)	16.660	20.740	18.093	1.126
HMF (mg / kg)	0.150	23.204	4.136	5.012
Fructose	31.986	39.818	37.062	2.148
Glucose	20.662	33.684	25.796	2.621
Sucrose	0.000	2.093	0.450	0.555
F+G	53.084	68.352	62.858	3.510
F/G	1.021	1.655	1.449	0.150
Gluconic acid (g/kg)	1.916	2.666	2.206	0.202
Formic acid (g/kg)	0.030	0.175	0.119	0.032
Acetic acid (g/kg)	0.038	0.180	0.096	0.035
Propionic acid (g/kg)	0.004	0.196	0.032	0.036
Lactic acid (g/kg)	0.000	0.041	0.002	0.008
Butiric acid (g/kg)	0.000	0.045	0.012	0.014
Succinic acid (k/kg)	0.000	0.027	0.002	0.006

Free acidity

Free acidity is given by the presence of organic acids and is the parameter that indicates the beginning of the honey fermentation process, therefore the maximum allowed value for free acidity is 50 meq acid/kg (Oroian et al., 2017; Da Silva et al., 2016). The acidity of honey is caused, in addition to the presence of organic acids (tartaric, citric, oxalic, acetic, etc.), by nectar or by secretions from bees (Yadata, 2014). The natural acidity of honey increases during the storage and maturation of honey, as well as during the fermentation of honey. The value of acidity, which is related to organic acids naturally present in honey, varies depending on the floral source and the bee species (de Sousa et al., 2016). Free acidity is also used to differentiate nectar honey from honeydew (Sanz et al., 2005). High values of free acidity indicate the sugar fermentation with the formation of acetic acid by hydrolysis of alcohol (Geană et al., 2020).

The free acidity of acacia honey analyzed in this study ranged from 0.32 to 4.14 meq/kg. (table 1) None of the analyzed samples exceeded the limit imposed by legislation. Fuentes Molina et al., (2020) reported values for free acidity of 9.5–46 meq/kg when studying polyfloral honey from Chile. Balos et al., (2018) reported values for free acidity of 5.44 meq/kg (acacia honey) – 19.33 meq/kg (forest honey).

Color

Honey color analysis is a valuable method of classifying honey as monofloral. The color varies from light yellow to dark brown with reddish hue or greenish hue, this color variation being influenced by the source of honey (Mărghitaș, 2005) The values of color parameters are presented in Figure 1.

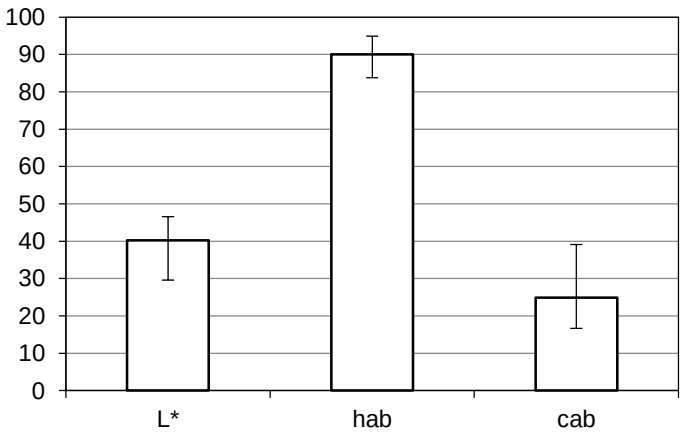

Figure 1. Color parameters (L*, h_{ab} and c_{ab}) for acacia honeys

Depending on the floral source, honey has a specific color; for example, acacia and citrus flowers produce straw-colored honey, while tilia flower produces a darker honey with a reddish hue (Siddiqui et al., 2017). The carotenoid, flavonoid and xanthophyll pigments come from nectar and pollen. The color of the pollen can vary from shades of pale yellow to dark brown, depending on the botanical species, this significantly influencing the color of

honey (dos Santos Scholz et al., 2020). Besides its botanical origin, the color of honey can also be influenced by the mineral content, the climate but also by the storage conditions (Sakač et al., 2019). The price of honey depends largely on its color, acacia honey and citrus honey obtaining the highest prices (Bogdanov et al., 2004). All honey samples showed similar lightness values (29.62–46.57). Mădaș et al., (2019) reported values for L* of 50.19-64 for *Robinia pseudoacacia* honey from Romania. Dos Santos Scholz et al., (2020) reported that the mean values of L* in Ortigueira honey was 52.65.

HMF content

The hydroxymethylfurfural (HMF) content is a parameter that indicates the degree of freshness of the honey and consequently its degree of deterioration (Onur et al., 2018). Cyclic aldehyde HMF is absent in fresh and untreated foods. HMF content is formed by caramelization and Maillard reactions or by the dehydration reaction of hexose in acidic medium (Bouhlali et al., 2019). The main causes that can lead to increased HMF in food are aging, the amount of water, free acidity, botanical origin, and stress during storage (Apriceno et al., 2018). Therefore, HMF is a compound that occurs in large quantities in damaged honey, in improperly stored honey and also in honey that has undergone a strong or prolonged heat treatment (Önür et al., 2018). The maximum limit allowed by law is 40 mg/kg (Council, E. U., 2002). The maximum limit of HMF in the samples analyzed in this study was 23.20 mg/kg thus falling within quality requirements (table 1). Juan Borras et al., (2014) identified a maximum value of 7.1 mg/kg of HMF content in tilia honey from Romania.

Electrical conductivity

Electrical conductivity is a parameter used to control the quality of honey and to differentiate honeydew from floral honey. It can be used in determining the botanical origin by correlating with the pollen content of honey (the mineral content being brought into honey along with the pollen) (Kaskoniene et al., 2010). The analysis of this parameter is very often used, being considered a good criterion to be able to identify the botanical origin and implicitly the purity of honey (Balos et al., 2018). Components of honey such as organic and mineral acids have the ability to dissociate into ions when they are in an aqueous solution or conduct electricity. Light honey usually indicates a lower conductivity value than darker honey (Kropf et al., 2008). The maximum allowed value for floral honey is 800 μS/cm and values higher than 800 μS/cm are specific to honeydew (Karabagias et al., 2018). The electrical conductivity increases with the amount of ash and acid in the honey (El Sohaimy et al., 2015). A value of less than 500 μS/cm indicates a pure floral honey, with exceptions (Saxena et al., 2010). In this study the samples of acacia honey had values between 94.8 and 405 μS/cm and this value confirm that the analyzed samples are pure floral honey (table 1). Vranić et al., (2017) reported values of electrical conductivity between 160 μS/cm and 450 μS/cm for acacia and blossom honey samples. Mărghitaș et al., (2010) reported an average value of 150 μS/cm for ten samples of *Robinia pseudoacacia* honey.

Sugars

The sugar content of honey depends on the type of flowers visited by bees and thus varies with the botanical and geographical origin of honey and climatic conditions, processing and storage (Kaškonienė et al., 2010). The sugar content of honey and even the ratios between sugars are important indicators for classifying honey according to its botanical

origin (Nozal et al., 2005). The predominant profile of sugars such as glucose, fructose, sucrose and maltose have been associated with a wide variety of properties of honey, such as viscosity, hygroscopy, granulation and energy value (Ouchemouk et al., 2009). As shown in Table 1, in the studied acacia honey a percentage of 68.35% monosaccharides was identified, with fructose reaching a maximum of 39.81% and glucose a maximum of 33.68%. All the analyzed samples showed a higher fructose content. The high proportion of d-fructose than d-glucose is related to the nectar source and suggests the possible existence of a low glycemic index (Bouhlali et al., 2019). Kamboj et al., (2020) analyzed cotton honey and reported a fructose content of 36.98% and a glucose content of 33.91%. The analyzed acacia honey samples contain a small percentage of sucrose (maximum 2.093%) which is below the 5% limit specified by Codex Alimentarius (Codex Alimentarius, 2001). A high sucrose content indicates a premature harvest of honey, which means that sucrose has not been completely transformed by the action of the enzyme invertase into glucose and fructose (do Nascimento et al., 2015). The concentration of sucrose varies with the degree of maturity and the source of nectar (Kamboj et al., 2013). Marghitaș et al., (2010) reported for Romanian acacia honey a concentration of fructose between 41.12–44.52% and for glucose 26–31.41%.

The ratio of fructose to glucose (F/G) is used to discriminate honeydew and floral honey (Dobre et al., 2012) and to predict the crystallization potential of honey (Laos et al., 2011). All types of honey that crystallize quickly have high glucose content and the F/G ratio is about 1 (Rajs et al., 2017) but the tendency of crystallization is slower with F/G values above 1.3 (Dobre et al., 2012). As shown in Table 1, the F/G ratio varied between 1.02 and 1.65 for the studied acacia samples. Juan Boras et al., (2014) reported an F/G ratio of 1.6 for acacia honey and lower values of this ratio for lime honey (1.3) and sunflower (1.06), which shows that acacia honey crystallizes more slowly.

Organic acids content

Organic acids represent a small percentage of the total components of honey (<0.5%) but define the aroma, color, pH and acidity and also play an important role in the antimicrobial and antioxidant activities of honey (Da Silva et al., 2016). The origin of aliphatic organic acids in honey is partially known, although many of them can be natural intermediates through the metabolic pathways of microorganisms, the Krebs cycle (acids: citric, succinic, glutaric, fumaric and oxaloacetic) or enzymatic reactions. The acids can also be synthesized from glucose, fructose and sucrose in the nectar, by the enzymatic action of bees or can come directly from the secretion of plants and also from the excretion of insects that reach the plants (Mato et al., 2003; Brugnerotto et al., 2019).

Determining the composition of organic acids in honey can be an important parameter used to discriminate the botanical origin of honey (Daniele et al., 2012). The acidity of honey is given by the more than 30 organic acids that are obtained directly from nectar or are formed when nectar is transformed into honey (Da Silva et al., 2016; Mato et al., 2003). Fermentation and aging processes that can occur during storage lead to an increase of total acid content (Mato et al., 2006). The citric acid content is an essential parameter in differentiating floral honey from honeydew (Suarez-Luque et al., 2012).

The non-aromatic organic acid that is predominant in the composition of honey is gluconic acid and is formed by the activity of glucose oxidase during maturation or by the metabolic activity of certain *Gluconobacter spp.* (Mato et al., 2003). Gluconic acid is also the main organic acid in the case of the samples analyzed in this study (1.916–2.666 g/kg), followed by propionic and acetic acids. Suto et al., (2020) studied acacia honey and reported a content of 1.575 g/kg gluconic acid. In a previous study, a higher amount of gluconic acid

(5.62 g/kg) was identified on sunflower honey (Pauliuc and Oroian, 2020), and the same was observed for chestnut honey (8.90 g/kg) (Sahin and Erim, 2011). In conclusion, acacia honey has a lower content of gluconic acid. As shown in Table 1, succinic acid has the lowest concentration in the studied acacia honey samples. Suto et al., (2020) reported that succinic acid was detected in 16 of 25 samples (average succinic acid concentration of 0.028 g/kg).

Conclusion

The content of pollen, the physicochemical parameters, the organic acids content and the sugar composition of Romanian acacia honey were analyzed in this study in order to classify this type of honey as monofloral honey.

All the investigated honey samples (27 samples) met the examined quality criteria (moisture, pH, free acidity, HMF content, color and electrical conductivity) and the high percentage of pollen grains of *Robinia pseudoacacia* confirmed that the analyzed honey samples were samples of monofloral acacia honey.

Acknowledgements. This work was supported by a grant of the Romanian Ministry of Education and Research,
CNCS-UEFISCDI, project number PN-III-P1-1.1-TE-2019-0583.

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Characteristics of flow and heat transfer in rotor-pulsation apparatus during delignification of wheat straw in technology of bioethanol production

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Keywords:

Wheat straw
Delignification
Rotor-pulsation apparatus
Energy dissipation

Article history:

Received 03.08.2020
Received in revised form 22.11.2020
Accepted 25.03.2021

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DOI: 10.24263/2304-974X-2021-10-1-15

Abstract

Introduction. Improving the efficiency of pretreatment of lignocellulosic raw materials is the use of physical effects that occur during the movement of viscous fluid in rotary pulsation apparatus. The aim of the research is the degree of delignification of lignocellulosic raw materials and the theoretical substantiation of its temperature increase by processing in a rotor-pulsation apparatus.

Materials and methods. The raw material for the research was wheat straw. The amount of lignin isolated was determined by the weight method. Simulation of fluid flow and heat transfer in the rotor-pulsation apparatus was performed by numerical method.

Results and discussion. It was determined that the treatment of the aqueous dispersion of straw in a ratio of 1:10 due to energy dissipation for 70 minutes leads to the release of 42% lignin. Changing the water/solid ratio from 1:10 to 1:5 leads to an increase in the percentage yield of lignin to 58%.

The results of experimental and numerical studies have shown that when processing an aqueous dispersion of straw in a rotary pulsation apparatus, this raw material for a certain period of time is heated to a temperature at which the intensive release of lignin. The changes in time of the temperature of the aqueous dispersion of straw during its processing in the rotary pulsation apparatus indicate the possibility of using rotary pulsation apparatus for heating the raw material intended for hydrolysis, instead of using external energy sources.

The results of computational studies of the dynamics of changes in the temperature of the aqueous dispersion of straw during its processing in a rotor-pulsation apparatus were compared with the results of experimental studies. Satisfactory agreement of experimental and calculated results is obtained.

Conclusions. According to the results of numerical studies of hydrodynamics and heat transfer in the rotor-pulsation apparatus, the possibility of raising the temperature of the raw material intended for hydrolysis to the required level after its processing in this apparatus has been established. It is determined that the change of the hydraulic module leads to an increase in lignin yield.

Introduction

The production of bioethanol from lignocellulosic raw materials has both a number of advantages and disadvantages compared to traditional raw materials containing starch.

Cellulosic feedstocks offer several advantages over starch- and sugar-based feedstocks. They are either waste products or purposefully grown energy crops harvested from marginal lands not suitable for other crops. Less fossil fuel energy is required to grow, collect, and convert them to ethanol, and they are not used for human food [1].

The cell wall of the plant is composed of a network of cellulose microfibrils and cross-linking glycans embedded in a highly cross-linked matrix of pectin polysaccharides. The most common additional polymer in secondary walls is lignin, a complex network of phenolic compounds [2].

Due to the complex structure and recalcitrant nature of lignocellulosic biomass, an indispensable step of its processing is pretreatment for hydrolysis [3].

As a result, of the pretreatment, complex lignocellulosic structures are converted to simple components (cellulose, hemicelluloses, and lignin) which is generally reflected by the removal of lignin, preservation of hemicelluloses, reduction of cellulose crystallinity and an increase of the material porosity [4]. The presence of lignin and hemicellulose prevents the access of hydrolytic enzymes to the surface of cellulose fibers in the technology of enzymatic hydrolysis [5]. The goal of the pretreatment process is to remove lignin and hemicellulose, reduce the crystallinity of cellulose, and increase the porosity of the lignocellulosic materials [6].

Among the many methods of influencing lignocellulosic raw materials during the preliminary preparation, several are currently commercially implemented, namely steam explosion, one- and two-stage treatment with dilute acids and ammonia treatment in combination with steam treatment [7]. The process of alkaline pre-treatment of raw materials in the pulp and paper industry is a classic [8].

The main factors that determine the effectiveness of the pre-treatment process are mechanical impact, temperature, pressure, hydraulic module, process duration, the concentration of the chemical agent. Machining increases the surface area available for cellulolytic enzymes. It is proved that fine grinding of raw materials allows increasing the yield of reducing substances during its hydrolysis [9–11].

Mechanical pretreatment leads to an increase in the surface available for cellulolytic enzymes. It is proved that the fine milling of straw allows increasing the yield of reducing substances in its hydrolysis [12].

Another factor is the temperature of the pretreatment process. Increasing the temperature from 120 to 270ºC led to greater solubilization of hemicellulose in the technology of steam explosion [13], increasing the temperature from 120 to 180 ºC led to an increase in glucose in the prehydrolyzate regardless of the concentration of sulfuric acid in preparation for hydrolysis with dilute acids [14]. A series of studies on the preliminary preparation of wheat straw is presented in [15].

It is determined that increasing the temperature of the pretreatment process with a solution of sulfuric acid, sodium hydroxide solution or hot water pretreatment leads to an increase in the degree of conversion of cellulose. The dependence of lignin yield on the temperature and processing time of wheat straw in an autoclave in 2.5% sodium hydroxide solution was studied by Asghar U, Irfan M, Iram M, et al. [16]. The results showed that the residence time for 90 min at 121 °C strongly affects the substrate, reaching a maximum cellulose content of 83%, delignification of 81%, and hemicellulose content of 10.5%.

All the above-mentioned methods of pretreatment of lignocellulosic raw materials before hydrolysis were performed with an external heat supply.

One of the ways to increase the efficiency of pre-treatment of lignocellulosic raw materials is the use of thermophysical effects that occur during the movement of a viscous fluid in rotor-pulsation apparatus [17].

Studies of the effect of temperature on the viscosity of the water-grain mixture were studied in [18]. Thermal and hydraulic characteristics of the fluid in the rotor-pulsation apparatus were given in [19]. Technical water and vegetable oil were used as model media. Experimental studies were performed on the setup, the working body of which was a rotor-pulsation unit, in the range of engine speed 0–4500 rpm. However, the physicochemical properties of the model media differed from the physicochemical properties of the aqueous dispersion of plant biomass.

The aim of the study is the degree of delignification of lignocellulosic raw materials and the theoretical substantiation of its temperature increase by processing in a rotor-pulsation apparatus.

To achieve this goal, it was necessary to perform the following tasks

- To determine the effect of the solid/water ratio of the aqueous suspension of wheat straw on the degree of delignification of lignocellulosic raw materials during its processing in the rotor-pulsation apparatus using alkali as a reagent;
- Determine the effect of temperature of the obtained suspension and its effect on the degree of lignin release;
- Theoretically substantiate the increase in temperature of the aqueous suspension of wheat straw during its processing in the rotor-pulsation apparatus.

Materials and methods

The raw material for the study was wheat straw with an average particle size of 100 microns.

Rotor-pulsation apparatus

The study was carried out on an experimental setup, the description and principle of operation of which are given in [20].

Order of study

A portion of straw in the amount determined by the experimental conditions was soaked in two liters of tap water. The remaining water was mixed with sodium hydroxide in an amount of 1 wt.%. The receiving solution and the rotary pulsation apparatus were filled with the obtained solution. The required rotor speed was set. The rest of the water was added. Turned on the engine. During a certain processing time, the obtained aqueous suspension of straw circulated in a closed circuit – receiving tank – rotor-pulsation apparatus. Samples were taken at regular intervals. The solids and the filtrate were separated by filtration.

Determination of lignin content

Sulfuric acid was added to the filtrate with stirring until a pH = 2 was reached to separate the lignin from the solution, after which the suspension was filtered through a pre-weighed paper filter. The dried filter with the remaining lignin was weighed on a scale with an accuracy of 0.001 g [21].

Modeling methods

Numerical simulation of fluid flow and heat transfer in a rotor-pulsation apparatus is performed according to the method described in [22, 23]. The flow of a viscous fluid and heat transfer in the volume of a rotor-pulsation apparatus is described by a system of Navier-Stokes differential equations together with the equation of energy conservation for fluid flow. The problem of flow and heat transfer is considered in a two-dimensional setting in a section perpendicular to the common axis of the rotor and stator. This system of equations, which describes the dynamics of fluid and energy transfer, is represented in cylindrical coordinates and has the form:

– continuity equation

$$\frac{\partial\left(r\cdot v_r\right)}{\partial r}+\frac{\partial v_\theta}{\partial\theta}=0; \tag{1}$$

– momentum transfer equation

$$\rho_l\left(\frac{\partial v_r}{\partial\tau}+\frac{1}{r}\frac{\partial\left(rv_r{}^2\right)}{\partial r}+\frac{1}{r}\frac{\partial\left(v_rv_\theta\right)}{\partial\theta}-\frac{v_\theta{}^2}{r}\right)=-\frac{\partial p}{\partial r}+\frac{2}{r}\frac{\partial}{\partial r}\left(\mu_l r\frac{\partial v_r}{\partial r}\right)-$$
$$-\frac{2\mu}{r^2}\left(\frac{\partial v_\theta}{\partial\theta}+v_r\right)+\frac{1}{r}\frac{\partial}{\partial\theta}\left\{\mu\left[\frac{1}{r}\frac{\partial v_r}{\partial\theta}+r\frac{\partial}{\partial r}\left(\frac{v_\theta}{r}\right)\right]\right\}; \tag{2}$$

$$\rho_l\left(\frac{\partial v_\theta}{\partial\tau}+\frac{1}{r}\frac{\partial(rv_\theta v_r)}{\partial r}+\frac{1}{r}\frac{\partial v_\theta{}^2}{\partial\theta}+\frac{v_\theta v_r}{r}\right)=-\frac{1}{r}\frac{\partial p}{\partial\theta}+\frac{2}{r^2}\frac{\partial}{\partial\theta}\left[\mu_l\left(\frac{\partial v_\theta}{\partial\theta}+v_r\right)\right]+$$
$$+\frac{1}{r^2}\frac{\partial}{\partial r}\left\{\mu\left[r\frac{\partial v_r}{\partial\theta}+r^3\frac{\partial}{\partial r}\left(\frac{v_\theta}{r}\right)\right]\right\}; \tag{3}$$

energy conservation equation for fluid flow

$$C_l\rho_l\left(\frac{\partial t}{\partial\tau}+\frac{1}{r}\frac{\partial\left(rv_rt\right)}{\partial r}+\frac{1}{r}\frac{\partial\left(v_\theta t\right)}{\partial\theta}\right)=\frac{1}{r^2}\frac{\partial}{\partial\theta}\left(\lambda_l\frac{\partial t}{\partial\theta}\right)+\frac{1}{r}\frac{\partial}{\partial r}\left(\lambda_l r\frac{\partial t}{\partial r}\right)+\mu_lS^2, \tag{4}$$

where

$$S=\left\{2\left[\left(\frac{\partial v_r}{\partial r}\right)^2+\frac{1}{r^2}\left(\frac{\partial v_\theta}{\partial\theta}+v_r\right)^2\right]+\left[\frac{1}{r}\frac{\partial v_r}{\partial\theta}+r\frac{\partial}{\partial r}\left(\frac{v_\theta}{r}\right)\right]^2\right\}^{0,5}. \tag{5}$$

τ, c – time; r, m – radial coordinate; θ – angular coordinate; v_r, m/s – radial velocity; v_θ, m/s – tangential velocity; p, Pa– pressure; t, °C – temperature; C_l, J/(kg·K) – heat capacity of the liquid; ρ_l, kg/m^3 – density of liquid; λ_l, W/(m·K) – thermal conductivity of the liquid; μ_l, Pa·s – dynamic viscosity coefficient.

The last term in the right part of energy equation (4) characterizes the volumetric source of heat release in a liquid medium due to the viscous dissipation of mechanical energy.

The system of equations (1) – (5) is solved numerically in the calculation domain $r_{min}\leq r\leq r_{max}$; $\theta_0<\theta<\theta_0+\Delta\theta$; $r_{min}=r_{in,\,rot}-\Delta r$; $r_{max}=r_{ex,\,st}+\Delta r$, where $r_{in,\,rot}$ is inner radius of the rotor; $r_{ex,st}$ is outer radius of the stator; Δr – width of the additional section in front of the rotor and behind the stator; $\Delta\theta$ – geometric period of working bodies of the rotor-pulsating apparatus.

The system of equations (1) – (5) is given boundary conditions: $r = r_{min}$: $t = t_{in}$; $p = p_{in}$; $\frac{\partial v_\theta}{\partial r} = 0$; $r = r_{max}$: $\frac{\partial t}{\partial r} = 0$; $p = p_{in} - \Delta p$; v_θ=0. On the stator surfaces $v_r = 0$ and v_θ=0. On the rotor surfaces $v_r = 0$, $\frac{v_\theta}{r} = \omega_0$, where ω_0 is the angular velocity of the rotor rotation.

Heat transfer through the working elements of the apparatus is described by the equations:

– for the stator:

$$C_s \rho_s \frac{\partial t}{\partial \tau} = \frac{1}{r^2} \frac{\partial}{\partial \theta} \left(\lambda_s \frac{\partial t}{\partial \theta} \right) + \frac{1}{r} \frac{\partial}{\partial r} \left(\lambda_s r \frac{\partial t}{\partial r} \right), \quad (6)$$

– for the rotor:

$$C_s \rho_s \left(\frac{\partial t}{\partial \tau} + \omega_o \frac{\partial t}{\partial \theta} \right) = \frac{1}{r^2} \frac{\partial}{\partial \theta} \left(\lambda_s \frac{\partial t}{\partial \theta} \right) + \frac{1}{r} \frac{\partial}{\partial r} \left(\lambda_s r \frac{\partial t}{\partial r} \right), \quad (7)$$

where C_s, J/(kg·K) – heat capacity of the material of the working elements; ρ_s, kg/m^3 – density of this material; λ_s, W/(m·K) – thermal conductivity of the material.

Boundary conditions of the fourth kind are set on the surfaces of working elements, which establish the equality of temperatures of the liquid and solid body, as well as the equality of heat flux densities transferred from the liquid to working elements.

Modeling of fluid flow and heat transfer is performed by numerical solution of the system of equations (1) – (7) and analysis of the obtained results on the distribution of velocity, pressure, and temperature in the fluid flow processed in the rotor-pulsation apparatus.

The system of equations (1) – (7) with the corresponding boundary conditions is solved by a numerical method. To do this, this system of equations is written in the finite-difference form. Thus, the system of differential equations is replaced by a system of algebraic equations, which is solved by known methods. In this case, the matrix run method, which is described in detail in [24], is used to solve the system of finite-difference equations. To implement this method, a computational algorithm in the DELPHI programming language has been developed. This algorithm allows determining the cross-sectional distributions of the calculated area of discrete values of fluid velocity, pressure, and temperature. It also provides the ability to graphically display velocity vectors and temperature isolines in the working area of the device. The application of the specified calculation algorithm can be carried out on a personal computer of medium power.

In order for the liquid to reach a temperature sufficient to carry out the necessary chemical transformations, the liquid must be repeatedly processed in a rotor-pulsation apparatus. The liquid after processing in the device enters the receiving tank through a system of pipelines. In pipelines and in the receiving tank, the liquid is partially cooled due to heat loss from the surfaces of the equipment. Heat loss also occurs directly from the surface of the device. To determine the nature of the change in time of the temperature of liquids that continuously enters the rotor- pulsation apparatus and is removed from it, the differential equation of heat balance is used which is given in [23] and has the form:

$$\left(C_l \rho_l V_l + C_s m_s + C_{\text{eq}} m_{\text{eq}} \right) \frac{\partial t}{\partial \tau} = Q - \alpha_{\text{ef}} F \left(t - t_\infty \right), \quad (8)$$

where V_l, is the volume of treated fluid; m_s – mass of the rotor-pulsation apparatus; C_{eq}, m_{eq} – specific heat of the material of the equipment connected to the rotor-pulsation apparatus, and its total mass; F is the total area of the outer surface of the rotor-pulsation apparatus and

equipment from which heat losses to the environment occur; α_{ef} – effective heat transfer coefficient from the surfaces of the equipment; t_∞- outside air temperature; Q is the power of the heat source in the rotor-pulsation apparatus due to the dissipation of mechanical energy. This equation of heat balance is made under the condition that the temperature of the outer surfaces is insignificantly different from the temperature of the treated liquid.

Heat transfer from the surfaces of the equipment is carried out by natural convection and radiation. Therefore, the effective heat transfer coefficient α_{ef} is defined as the sum of convection α_c and radiation α_r heat transfer coefficients. The coefficient for natural convection in turbulent flow near vertical surfaces is calculated by the formula given in [25]:

$$\alpha_c = 0,15\lambda_a \left(\frac{g\beta\left(t - t_\infty\right)}{\nu_a^2} \mathrm{Pr}_a \right)^{1/3}, \quad (9)$$

where g, m/s^2 – acceleration of gravity; β, 1/K – temperature coefficient of air expansion; λ_a – coefficient of thermal conductivity of air; ν_a – kinematic coefficient of air viscosity; Pr_a- Prandtl number for air. The radiation heat transfer coefficient is determined from the expression

$$\alpha_r = c_0 \varepsilon \frac{\left(\frac{t + 273,15}{100}\right)^4 - \left(\frac{t_\infty + 273,15}{100}\right)^4}{t - t_\infty}, \quad (10)$$

which follows from the law of Stefan – Boltzmann, where $c_0 = 5.7$ W/(m^{2}K^4) – the coefficient of radiation of an absolutely black body; ε – the degree of blackness of radiation heat transfer surfaces is given.

The power of the heat source Q in the working volume of the apparatus can be determined both by the results of the numerical solution of the system of equations (1) – (7) and by the approximate formula:

$$Q = \pi\left(r_{\text{вн.ст}} + r_{\text{зов.рот}}\right) \cdot h \cdot \mu\left(\omega_0 r_{\text{зов.рот}}\right)^2 / \delta, \quad (11)$$

where $\delta = r_{\text{in st}} - r_{\text{ex rot}}$ – the magnitude of the gap between the rotor and stator; h is the width of the working elements of the device. The approximate formula (11) is made under the condition that the dissipation of mechanical energy occurs only in the gap between the rotor and the stator. The solution of the nonlinear equation (8) is performed by the numerical method.

Results and discussion

Figure 1 shows the dynamics of changes in temperature of water and mixture over time in the setup with a speed of 47.75 rpm at different solid/water ratios.

Under these processing conditions, samples were taken every 10 minutes Data on the amount of lignin extracted are given in Table 1.

Based on the data in table 1, it can be concluded that the treatment of an aqueous suspension of straw in a rotary pulsation apparatus allows you to remove up to 42% of lignin within 70 minutes under atmospheric pressure without an external supply of thermal energy.

Changing the solid/water ratio of the aqueous suspension of straw from 1:10 to 1: 5 leads to an increase in the amount of lignin released, as the temperature of the mixture increases more intensely.

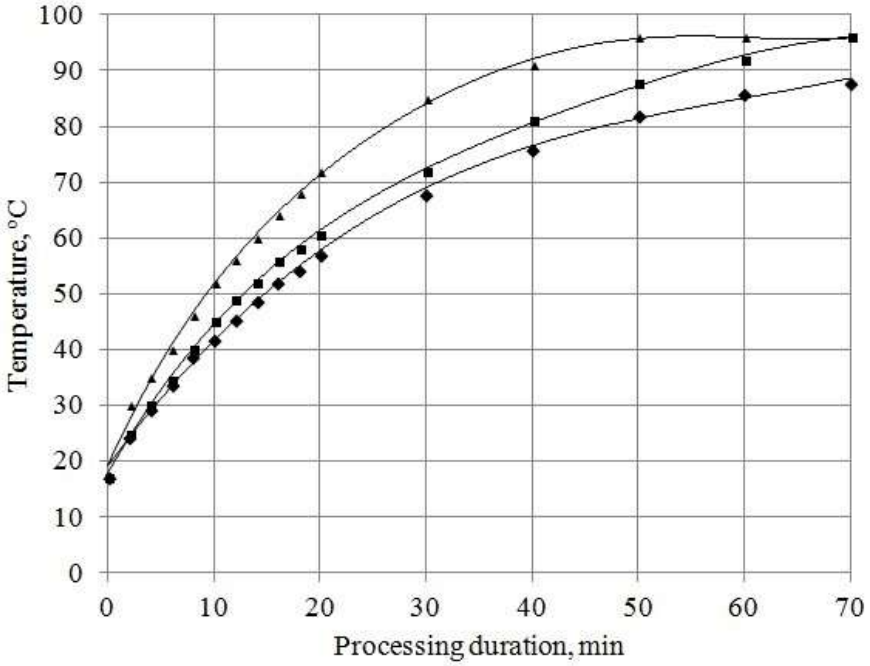

Figure 1. Dynamics of temperature change of aqueous suspension of straw in time at a solid / water ratio:
■ – 1:10; ▲ – 1: 5; ♦ – water.

Table 1

Dependence of the amount of lignin on the duration of processing at a speed of 47.75 rpm

Solid/Water ratio 1:10							
Sample	1	2	3	4	5	6	7
Processing duration, min	10	20	30	40	50	60	70
% of the total lignin content	10	18	25	32	38	41	42
Solid/Water ratio 1:5							
% of the total lignin content	18	29	36	45	50	55	58

A further increase in the straw content in the mixture leads to significant energy consumption and unstable operation for the selected design of the rotor-pulsation apparatus.

Rotor speed $\omega_0 = 2\pi \cdot 47.75\ s^{-1}$. The pressure difference Δp between the inlet and outlet cross sections of the calculated area is 5000 Pa. The picture of fluid flow and distribution of excess temperature $\Delta t = t - t_{in}$ in the investigated element of the working area is shown in Figure 2.

The increase in temperature of the medium treated in the rotor-pulsation apparatus is due to the dissipation of mechanical energy in the working volume of the apparatus. As noted in [22, 23], the most intense dissipation occurs in the gap between the rotor and the stator, where the most significant deformation of the velocity. To find out in detail the mechanism of heating the aqueous suspension of straw in the rotor-pulsation apparatus, numerical simulation of the flow and heat transfer in this apparatus is performed. By the method of numerical solution of the system of equations (1) – (7) described above, calculations of velocity fields in temperature in the working zone of the rotor pulsation apparatus are performed for two values μ = 0.09 Pa·s and μ = 0.107 Pa·s, which is approximately correspond to the viscosity of the aqueous dispersion of straw at a solid/water ratio of 1:10 and 1:5, respectively.

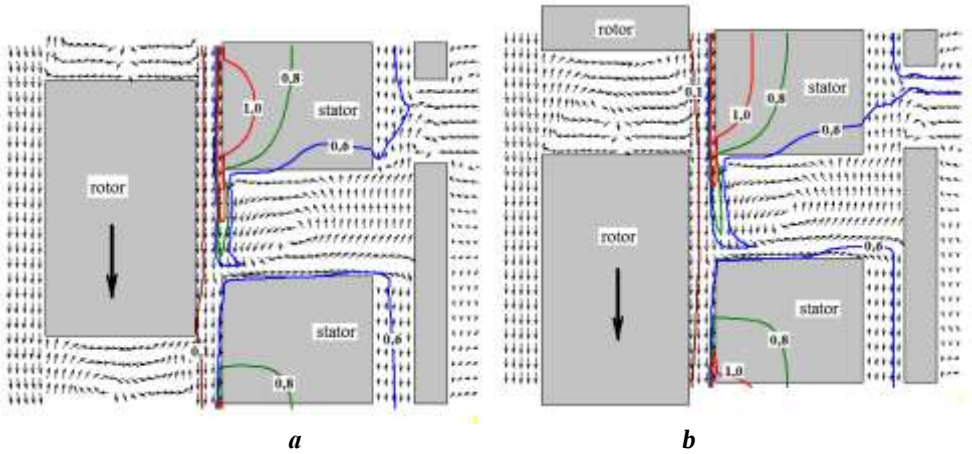

Figure 2. Velocity field and distribution of excess temperature Δt in the working zone of the rotor-pulsating apparatus during the processing of aqueous suspension of straw: a – μ = 0.09 Pa· s; b – μ = 0.107 Pa·s

As can be seen from Figure 2, the most significant overheating of the fluid occurs in the gap between the rotor and the stator near the inner surface of the stator, where the most intense heat dissipation occurs due to the dissipation of mechanical energy. From the results of numerical simulation, it follows that the maximum overheating of the liquid in the gap for one period of rotor rotation is Δt_{max} = 1.9 ºC at μ = 0.09 Pa·s and Δt_{max} = 2.2 ºC at μ = 0.107 Pa·s. The average excess temperature of the treated liquid in the rotor-pulsation apparatus outlet is Δt = 0.44 oC at μ = 0.09 Pa·s and Δt = 0.53ºC at μ = 0.107 Pa·s.

According to the results of solving equation (8), the change in time of the temperature of the aqueous suspension of straw having a volume of 10 l is determined during its processing in the rotor-pulsation apparatus. The total heat transfer surface is F = 2.8 m^2. Rotor-pulsation apparatus and additional equipment made of stainless steel.

The solution of equation (8) is performed provided that the air temperature t_∞ and the initial temperature of the liquid are equal to 20 ºC.

In fig. Figure 3 shows the dependences of the temperature of the aqueous suspension of straw on the processing duration obtained by solving the results of equation (8) using expression (11). The results were obtained for μ = 0.09 Pa·s, which corresponds to a solid /

water ratio of 1:10, and for μ = 0.107 Pa·s, which corresponds to a 1:5 ratio. The solid lines reflect the results of the numerical solution of equation (8), and the points – the results of experimental studies shown in Figure 2.

The figure shows that when processed for 60 min. the aqueous suspension of straw with a solid / water ratio of 1:10, its temperature rises from 20 °C to 87.5 °C. If the specified ratio is 1:5, then during the same time the aqueous suspension is heated to 97.7 °C. This indicates the possibility and feasibility of using for heating raw materials intended for hydrolysis, rotor-pulsation apparatus.

Figure 3 also shows the comparison of the calculated results with the experimental data. As can be seen from the figure, the agreement between the calculated and experimental results is quite satisfactory.

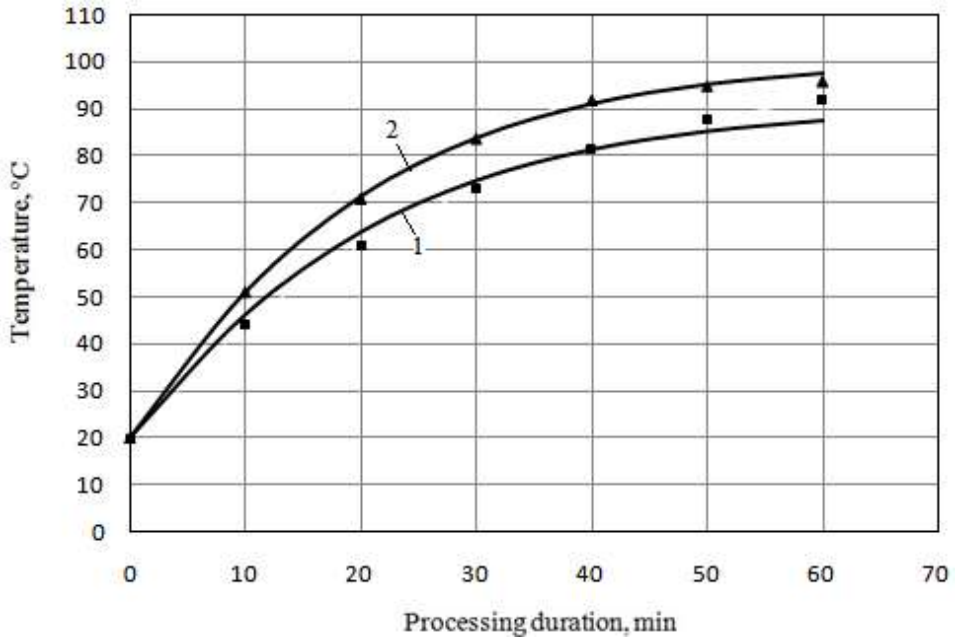

Figure 3. Changes in time of temperature of aqueous dispersion of straw during its processing in rotor-pulsation apparatus at ratios solid/water 1:10 (1) and 1:5 (2). Solid lines – the results of calculations; points – the results of experiments.

Conclusion

According to the results of computational and experimental studies, it is established that to heat the aqueous dispersion of straw to a temperature at which lignin is intensively released, it is possible to use rotary pulsation devices without external heat supply. It is determined that the change of the hydraulic module leads to an increase in lignin yield.

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Design and technological parameters of equipment influence on the lateral pressure coefficient and reduced friction coefficient of granular polyvinyl chloride

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Keywords:

Bulk
Polymer
Granule
Lateral pressure
Friction

Article history:

Received 21.04.2020
Received in revised form 29.09.2020
Accepted 25.03.2021

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DOI:
10.24263/2304-974X-2021-10-1-16

Abstract

Introduction. The dependence of the lateral pressure coefficient and the reduced friction coefficient of granular polyvinyl chloride was determined for the production of packaging material from the design and technological parameters of the feeding process.

Materials and methods. Experiments has been carried out on the stand which simulating the movement of polymer granules in the working channel of a single-screw extruder for such parameters: the polymer being researched – polyvinyl chloride (PVC); the axial pressure – 0.044–0.475 MPa; the temperature of the steel limiting surface – 20–80 °C; the speed of the steel limiting surface – 0.176–0.471 m/s; the height of the granular polymer layer – 0.015–0.025 m.

Results and discussion. The value of the lateral pressure coefficient increases under the following conditions: temperature increase; reduction of axial pressure; speed reduction; the lateral pressure coefficient does not depend on the height of the granules layer. Provided simultaneous changes in temperature and pressure to a level of approximately 0.32 MPa increase in temperature leads to increased values of lateral pressure, after it – on the contrary, to reduce. In case of speed change after point 0.15 MPa dependence also reversed.

Most on the growth of the lateral pressure coefficient of the studied polymer affects the simultaneous action of the temperature and the speed of the steel limiting surface. The central role in changing the lateral pressure coefficient is having by speed, and in changing the friction coefficient have by temperature.

In the case of simultaneous temperature rise with the speed and the layer of granules height there is a transition through the point at the level of approximately 0.32 MPa. To this point, an increase in temperature leads to an increasing in the corresponding values of the lateral pressure coefficient. After this point on the contrary – to decreasing in the corresponding values of the lateral pressure coefficient. The same in case of speed change, after the point 0.15 MPa the dependence is reversed.

In the case of simultaneous rise the speed and the temperature from 20 to 80 °C the larger values of speed correspond to the smaller values of lateral pressure coefficients. The increase in temperature leads to an increase in the lateral pressure coefficients from 0.33–0.34 to 0.42–0.46.

In the case of simultaneous rise the speed and the pressure the lower pressure values do not affect the dependence of the values of the lateral pressure coefficient on the speed. An increase in pressure from 0.044 to 0.476 MPa leads to corresponding change to increasing dependence from 0.22–0.27 to 0.34–0.46.

Subject to change in temperature, speed and granule layer height an increase in temperature leads to a corresponding increase values of the friction coefficient at the same pressure from 0.48-0.5 to 0.52-0.57 as well as increasing speed.

Conclusions. The obtained results make it possible to take into account the mutual influence of the friction and the lateral pressure coefficients of the granular polymers and the design and technological parameters of the extruder or screw feeder.

Introduction

One of the most versatile and productive methods of processing polymers into a variety of products is extrusion [1–4]. In particular, packaging materials such as polymer films and sheets are obtained by extrusion [5–8]. Polymeric raw materials intended for further processing are obtained, stored and supplied to the consumer in the form of granules of various shapes (cylindrical, cubic, spherical, elliptical, etc.) with an equivalent diameter mostly within 3–6 mm [9].

The process of screw extruder feeding with granular polymer raw material materials determines the course of all processes occurring in the following functional zones of the extruder [2]. It is in the supply zone that the pressure required for further movement of the polymer through other zones of the extruder and the extrusion head is generated. In turn, this affects the quality of the products, in particular on the stability of the thickness of the polymer films, which are widely used for packaging food, chemical and other products.

Effective treatment of granular polymers presupposes the availability of information on its physical and mechanical properties, including the friction coefficients on different surfaces, as well as the lateral pressure coefficient [10].

Extensive research carried out for thermal properties (including true density) the most common heavy-duty polymers and plastics, as well as their friction coefficient on the steel surface [11–13].

The study of external friction coefficients for polymeric materials with characteristic of modern brands specification was also carried out. However, these studies were conducted mainly for monolithic material which does not allow to use the obtained data to analyze the behavior of polymer granules [14–15]. Also conducted studies of tribological properties of granular polymers [16–17], however, they all relate to the determination of the reduced friction coefficient of the polymer granules on the limiting surface.

As you can see, if for granular polymers were studied certain physical and mechanical properties, but the data relative to the coefficient of lateral pressure, which significantly affects the accuracy of equipment design [18], almost completely absent even for the most common polymers.

The coefficient of lateral pressure K_{LP} takes into account the anisotropy of the pressure and is numerically equal to the ratio of the pressure on the side surfaces to the axial pressure. It is traditionally assumed that in static conditions the value of K_{LP} is of the order of 0.3–0.4, and in dynamic conditions (when the screw rotates) the value of K_{LP} increases to unity [19–24]. However, the operation of industrial equipment indicates [23] that the value of K_{LP} for different materials can differ essentially and depend on design and technological parameters of the equipment for treatment or processing of granules.

In view of the above, the task of a comprehensive study of the dependence of the friction coefficient and of the lateral pressure coefficient of the most commonly used granular polymers on the design and technological parameters of the equipment.

The objective of the work is establishing the dependence of such tribotechnical characteristics of granular polyvinyl chloride (PVC), as the lateral pressure coefficient and the reduced friction coefficient from design and technological parameters the process of feeding granular material, in particular the axial pressure, temperature and speed of the steel limiting surface, as well as the height of the layer of granules.

Materials and methods

Materials

Polyvinyl chloride granules (PVC) SorVyl G 2171/9005 11/01 [25] were selected for research. Polyvinyl chloride has been selected for research due to its widespread use in the packaging industry, in particular in the manufacture of packaging films and bottles of household chemicals.

Experimental installation

The designed experimental installation [26] makes it possible to researched the values of the external friction coefficient and the lateral pressure coefficient of granular materials on different surfaces, including depending on temperature, load, speed of a rotor rotation and a layer of granules height in a wide range of values.

Installation (Figure 1) consists of rotor 3, heaters 2 and vertical box 4, which is made hollow and mounted above the rotor. In the box placed pusher 5, which receiving the load through the regulator of the vertical force 6. Also in the box placed sensors of horizontal forces 11 and 13.

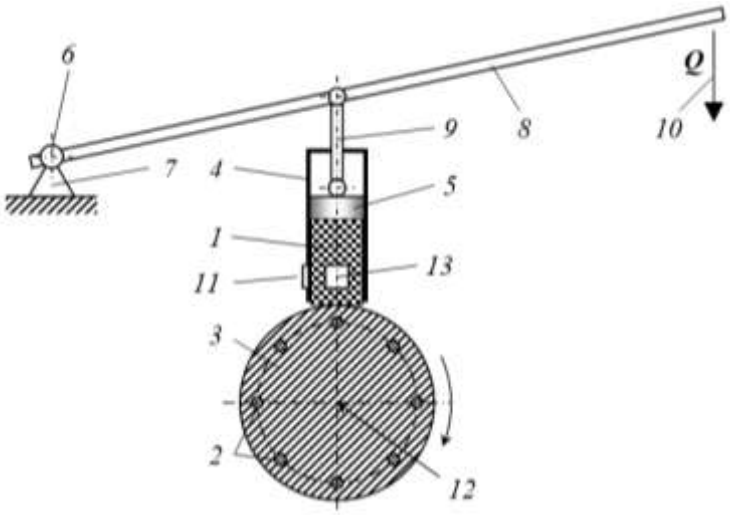

Figure 1. Scheme of an experimental installation:
***Q* – load on the rod;**
1 – installation; 2 – heaters; 3 – rotor; 4 – box; 5 – pusher; 6 – regulator of the vertical force;
7 – riser; 8 – lever; 9 – stock; 10 – counterweight; 11 – horizontal force sensor;
12 – the axis of the rotor; 13 – horizontal force sensor

The principle of operation of the installation is as follows: the granules are filled into a vertical box and pressed by the rod to the rotor, which is given the rotation of the motor. The sensor installed in the course of rotation of the rotor makes it possible to obtain the value of the friction coefficient, and the second sensor installed perpendicular to the first makes it possible to obtain the value of the lateral pressure coefficient.

By changing the speed of the rotor rotation, the temperature of the heaters or the load on the rod Q – you can get the above dependences for the desired coefficients.

Research methods

The planning of experimental researches was based on the method of a complete factorial experiment [27], which is based on a method of constructing the dependence of the determining factors influence on the optimization parameter in the form of a segment of the Taylor power series.

The optimization parameter K_{LP} (lateral pressure) will be affected by the following factors:

1. Linear speed of the rotor rotation (v);
2. Rotor temperature (t);
3. Pressure on the material (p);
4. Working channel height (h).

Given the design and technological parameters of the extrusion process for these factors, the following ranges of their change were selected:

1. Linear speed of the rotor rotation v = 0,176 –0,471 m/s;
2. Rotor temperature t = 20 – 80 °c;
3. Pressure on the material p = 0,044 – 0,476 mpa;
4. Working channel height h = 15 – 25 mm.

The results are processed according to a known algorithm [27].

The error in obtaining experimental values does not exceed 10.5% according to Fisher's criterion.

Results and discussion

Features of polymeric materials processing in single-screw extruders

As the material moves along the screw channel of a single-screw extruder, it successively changes several states: from solid at the inlet to the channel to viscous at the outlet. This channel is traditionally divided into three functional zones: feeding, melting and homogenization (Figure 2). The efficiency of screw machines is primarily determined by the processes that take place in the first two zones, as the share of power consumed here reaches 80% of the power consumed by the extruder. The productivity of the feeding zone determines the productivity of the process as a whole.

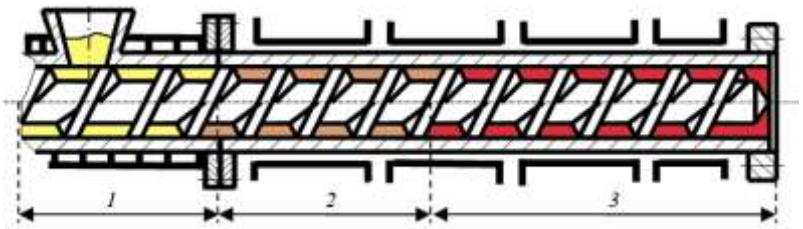

Figure 2. Scheme of one-screw extruder:
1 – feeding zone, 2 – melting zone, 3 – homogenization zone

The curves of the dependence of the specific productivity G_n on the ratio of the coefficients of friction of the polymer granules on the surfaces of the cylinder and the worm $F = f_c / f_s$ are shown in Figure 3. The specific productivity significantly depends on this ratio at its small values, and in the case of increasing the value of F, this dependence decreases and asymptotically goes to a certain value [28].

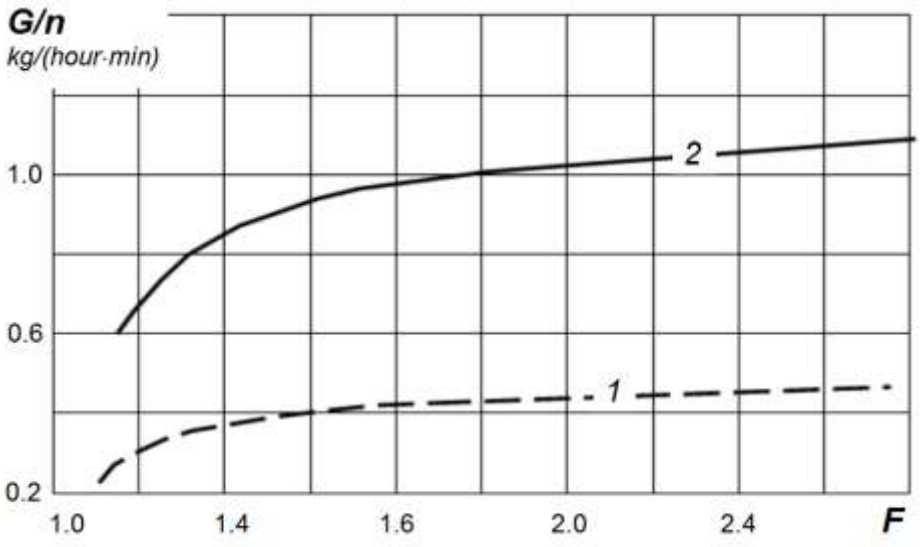

Figure 3. Curves of dependence of specific productivity on the ratio of friction coefficients:
1 – *D* = 45 mm; *H* = 4 mm;
2 – *D* = 63 mm; *H* = 5 mm

The productivity of the feeding zone is [28]

$$G = \rho_0 \left\{ \frac{\pi}{4} \left[D^2 - (D - 2H)^2 \right] - \frac{EH}{tg\varphi} \right\} \pi D n \frac{tg\omega \; tg\varphi}{tg\omega + tg\varphi} .$$

The increase in pressure from P_1 to P_2 in the selected element of the length of the feeding zone is determined by dependence

$$P_2 = P_1 \exp\left(\frac{\pi D f_c K_{LP} \Delta L}{bH} A\right) ,$$

where

$$A = \cos(\omega + \varphi) - f_s \sin(\omega + \varphi) - \frac{f_c}{f_s} \frac{(D - 2H)}{D} .$$

In the above dependencies ρ_o – polymer bulk density, kg/m^3; D – screw diameter, m; H – channel depth (thread), m; E – ridge width, m; n – frequency, s^{-1}; ω and φ – angles determined by the direction of the speed vector of the polymer; f_c, f_s – the friction coefficient of the polymer against the cylinder and the screw, respectively; $b=(S-E)\cos\varphi$ – screw channel width, m; K_{LP} – lateral pressure coefficient.

Therefore, taking into account the peculiarities of the mutual influence of structural and technological parameters of the equipment, coefficients of friction and of lateral pressure allows to clarify the results of worm extruders and screw feeders calculations.

It is established that the central role in changing the lateral pressure coefficient plays by speed, and in changing the friction coefficient plays by temperature. The mutual influence of the third order parameters is insignificant and these components can be neglected.

Using the above regression equations, graphical dependences of the coefficients of friction and of lateral pressure of the considered material on the variable parameters were obtained.

Research of the lateral pressure coefficient

For each polymer, the parameters not listed below the graphs are equal to the smallest value in the range of the complete factorial experiment for the corresponding material.

From those shown in Figure 4–5 dependences of the lateral pressure coefficient on the pressure when the temperature, speed and height of the granules layer change, the general decreasing character of these dependences is noticeable. In the case of simultaneous temperature rise with the speed and the layer of granules height (see Figure 5) there is a transition through the point at the level of approximately 0.32 MPa. To this point, an increase in temperature leads to an increasing in the corresponding values of the lateral pressure coefficient. After this point on the contrary – to decreasing in the corresponding values of the lateral pressure coefficient. The same in case of speed change, after the point 0.15 MPa the dependence is reversed, that is, to a certain value of pressure, a simultaneous increase in speed leads to a decrease in the corresponding values of the lateral pressure coefficient, and after reaching this value – on the contrary, to increasing.

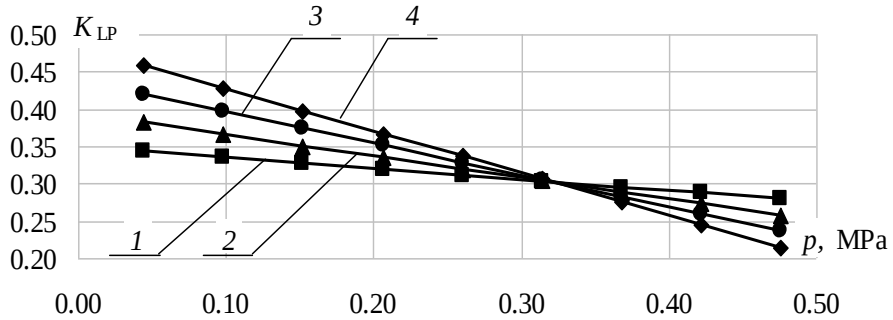

Figure 4. Dependence of the PVC lateral pressure coefficient on the pressure at different temperatures,
°C: 1 – 20; 2 – 40; 3 – 60; 4 – 80

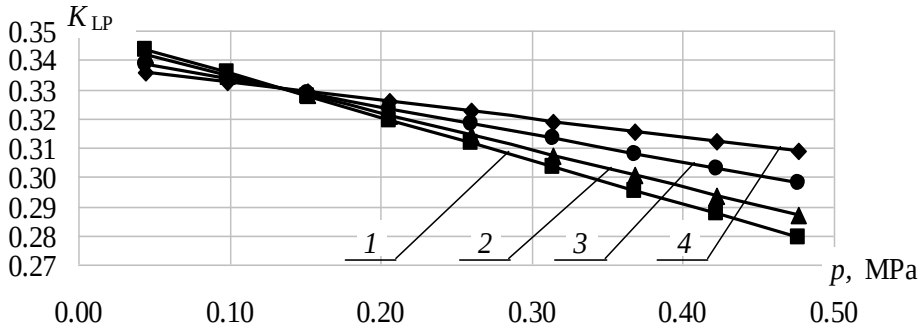

Figure 5. Dependence of the PVC lateral pressure coefficient on the pressure at different speeds, m/s:
***1* – 0.176; *2* – 0.244; *3* – 0.346; *4* – 0.448**

Such changes, in our opinion, can be explained by the beginning of the angular deformation of the granules during movement while softening the material.

In Figure 6–7 shown dependences of the lateral pressure coefficient on the speed when the temperature, pressure and height of the granules layer change. Thus, while increasing the temperature and speed (see Figure 6) larger values of speed correspond to smaller values of lateral pressure coefficient and the temperature rise (see. Figure 7) increases the lateral pressure coefficients. At simultaneous action of speed and pressure, smaller values of pressure do not influence dependence of values lateral pressure coefficient on speed, but pressure increase leads to the corresponding change to the increasing dependence.

These results can be explained by the compaction of the granule layer after compression and its transformation from bulk to a solid medium.

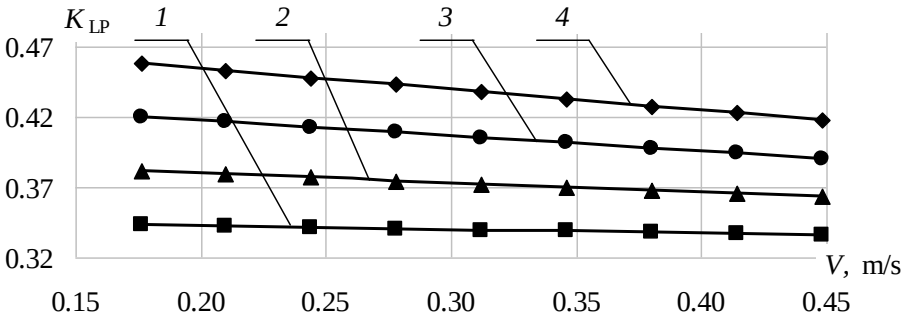

Figure 6. Dependence of the PVC lateral pressure coefficient on the speed at different temperatures, °C:
1 – 20; 2 – 40; 3 – 60; 4 – 80

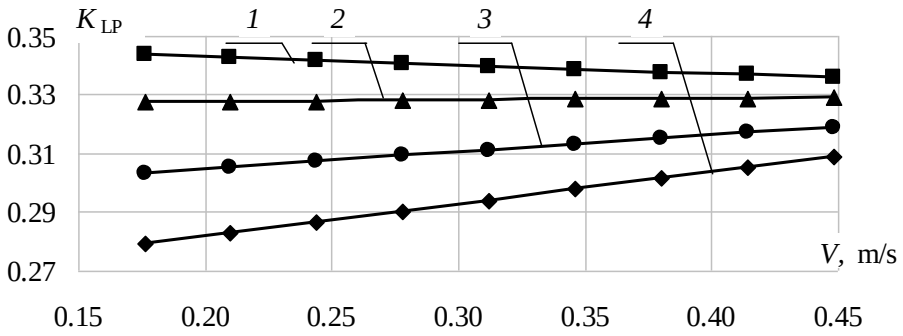

Figure 7. Dependence of the PVC lateral pressure coefficient on the speed at different pressure, MPa:
1 – 0.044; 2 – 0.152; 3 – 0.314; 4 – 0.476

In Figure 8–9 show the change of the lateral pressure coefficient from the temperature when the speed, pressure and height of the granules layer change. At the same time the general growing character of these dependences is appreciable. With at additional increase in speed (see Figure 8) there is a slight decrease in the corresponding lateral pressure coefficient, just like that with increasing pressure (see Figure 9), and the change in the height of the granules layer does not affect the dependence of the lateral pressure coefficient on the pressure.

These results can be explained by the softening of the layer of granules from temperatures and stresses that go beyond the region of elasticity of the material.

The dependences of the lateral pressure coefficient on the height of the granules layer when the speed, temperature and pressure change were also researched [18]. It is established that the increase in pressure leads to a decrease in the corresponding values of the lateral pressure coefficient as well as increasing the speed. At the same increase in temperature leads to an increase in the corresponding values of the lateral pressure coefficient.

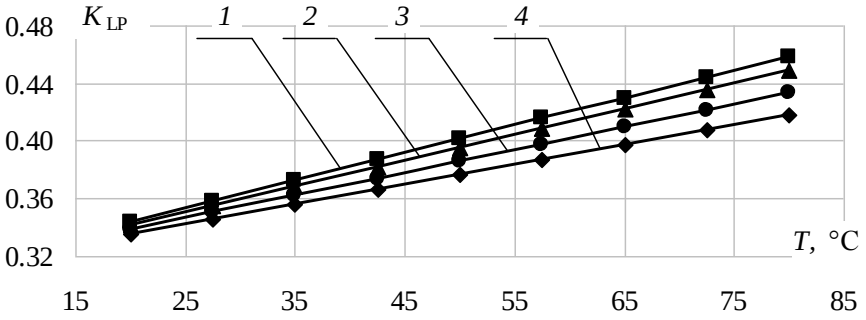

Figure 8. Dependence of the PVC lateral pressure coefficient on the temperature at different speeds, m/s:
1 – 0.176; 2 – 0.244; 3 – 0.346; 4 – 0.448

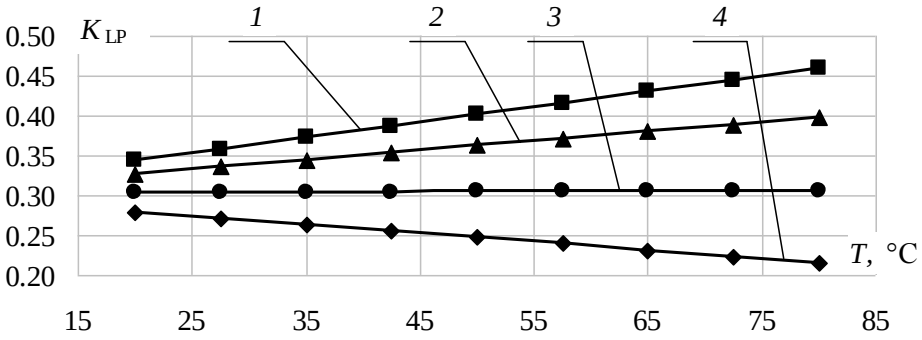

Figure 9. Dependence of the PVC lateral pressure coefficient on the temperature at different pressure, MPa:
1 – 0.044; 2 – 0.152; 3 – 0.314; 4 – 0.476

Research of the friction coefficient

In Figure 10–17 show the graphical dependences of the values of the reduced PVC friction coefficient on the steel surface from the structural and technological parameters of the extrusion process.

Parameters not listed below the graphs are the lowest values in the full factorial experiment range.

From shown in Figure 10–12 dependences, a slight decrease in the reduced coefficient of friction from pressure is noticeable subject to change in temperature, speed and height of the granules layer. The increase in temperature (see Figure 10) leads to a corresponding increase in the values of the friction coefficient at the same pressure, as well as an increase in speed (see Figure 11), and the height of the granules layer (see Figure 12) has almost no effect on the corresponding change in the coefficient of friction, except for high values of pressure.

The latter can be explained by the fact that with a larger layer of granules, the increase in pressure leads to their compaction and movement as a solid body, when the effect of rolling the granules one by one disappears, that is reducing the effect of rolling friction.

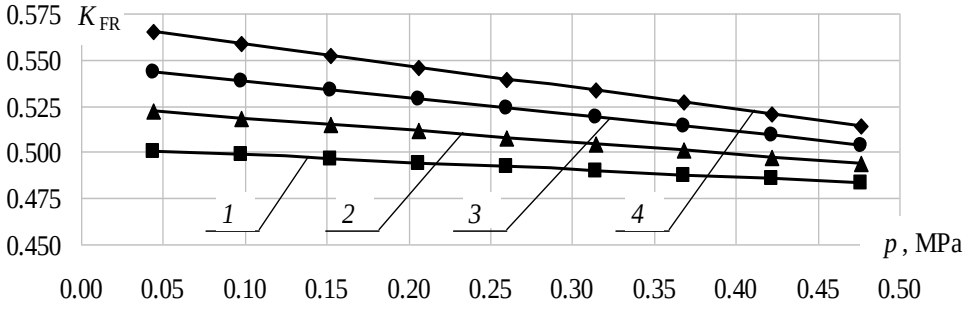

Figure 10. Dependence of the PVC friction coefficient on the pressure at different temperatures, °C:
1 – 20; 2 – 40; 3 – 60; 4 – 80

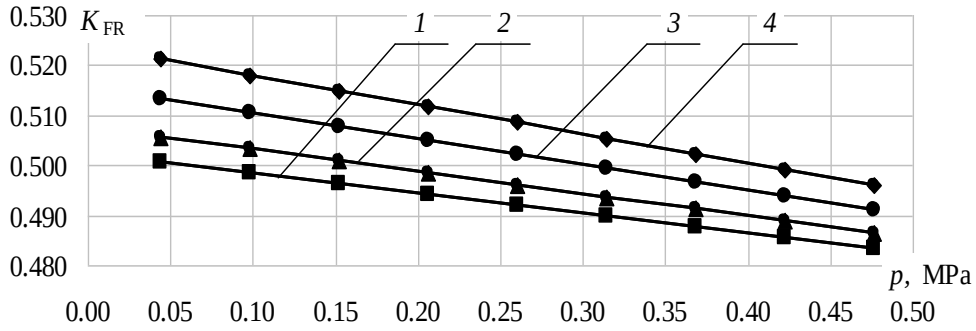

Figure 11. Dependence of the PVC friction coefficient on the pressure at different speeds, m/s: 1 – 0,176; 2 – 0,244; 3 – 0,346; 4 – 0,448

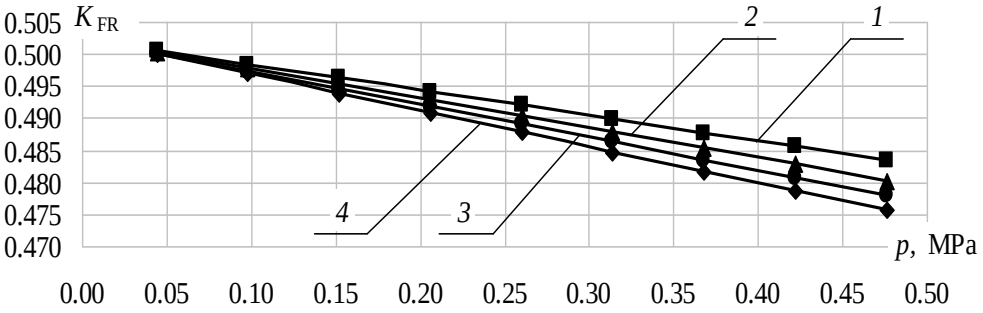

Figure 12. Dependence of the PVC friction coefficient on the pressure at different heights of the granules layer, m:
1 – 0,015; 2 – 0,019;
3 – 0,022; 4 – 0,025

From dependencies shown in Figure 13–14 a slight increase in the reduced friction coefficient from the speed of the limiting surface is noticeable under the condition of change the temperature, pressure and height of the granules layer. An increase in temperature (see Figure 13) leads to an increase in the corresponding values of the friction coefficient and increase in pressure (see Figure 14) on the contrary, leads to a decrease in the corresponding values of the friction coefficient.

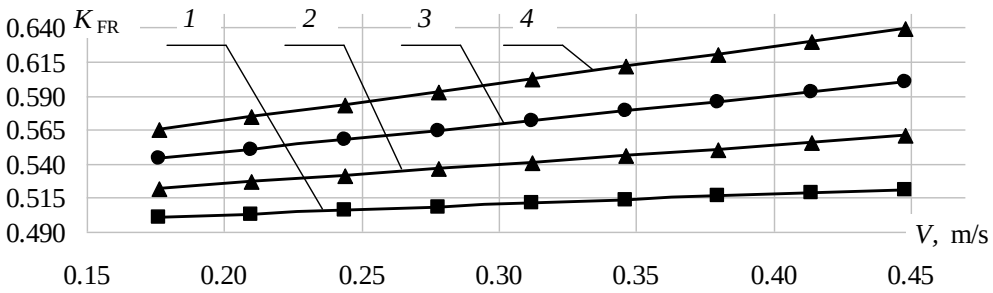

Figure 13. Dependence of the PVC friction coefficient on the speed at different temperatures, °C:
1 – 20; 2 – 40; 3 – 60; 4 – 80

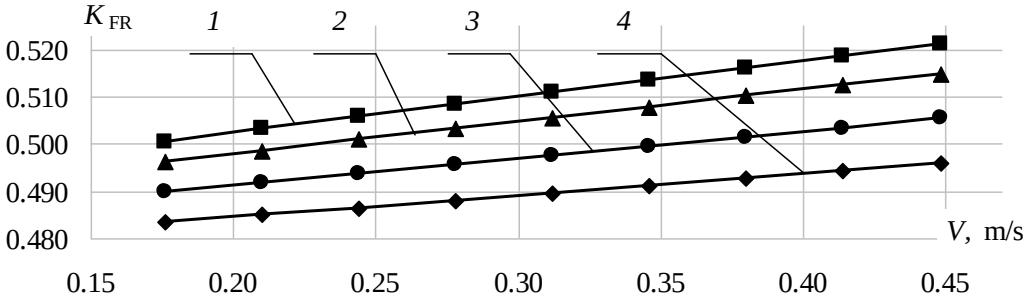

Figure 14. Dependence of the PVC friction coefficient on the speed at different pressure, MPa:
1 – 0,044; 2 – 0,152; 3 – 0,314; 4 – 0,476

These dependences can be explained by the fact that increasing the temperature increases the forces of interaction of the polymer with the surface, and increasing the pressure leads to smoothing of the surfaces of the granules.

From dependencies shown in Figure 15–16, a slight increase in the reduced coefficient of friction with temperature is noticeable under the condition of change of speed of a limiting surface, pressure and height of the granules layer. In general, the reduced friction coefficient increases with increasing temperature. In this case, increasing the speed of the limiting surface (see Figure 15) leads to an increase in the values of the friction coefficient and increasing in pressure (see Figure 16) on the contrary, leads to a decrease in the values of the friction coefficient.

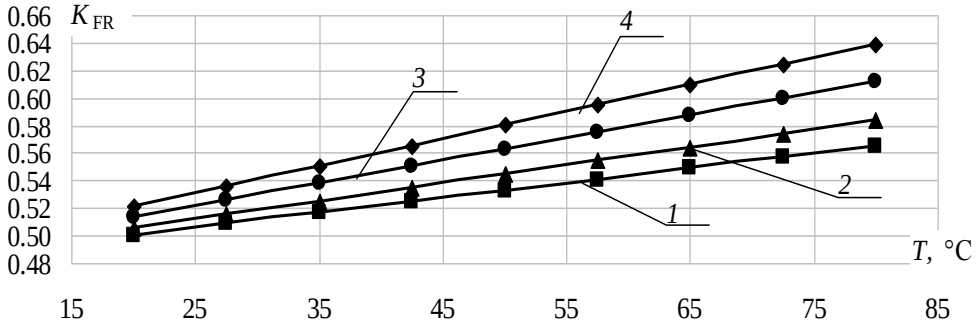

Figure 15. Dependence of the PVC friction coefficient on the temperature at different speeds, m/s: 1 – 0,176; 2 – 0,244; 3 – 0,346; 4 – 0,448

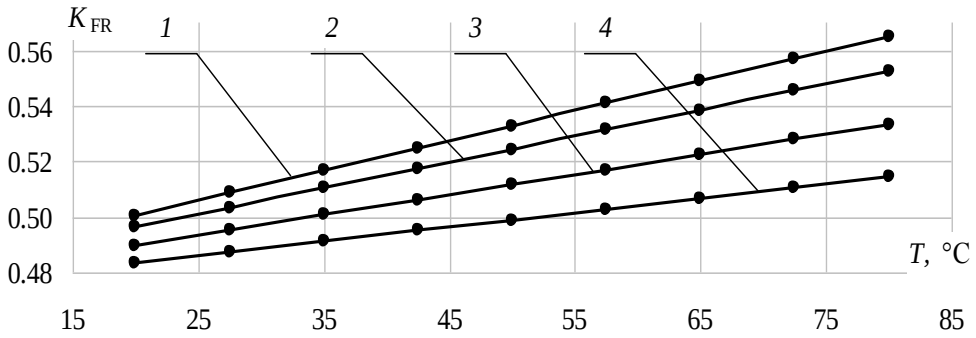

Figure 16. Dependence of the PVC friction coefficient on the temperature at different pressure, MPa: 1 – 0,044; 2 – 0,152; 3 – 0,314; 4 – 0,476

These dependences can be explained by the fact that increasing the temperature increases the forces of interaction of the polymer with the surface, and increasing the speed and pressure leads to the compaction of the surface layer of the polymer.

In Figure 17 shows the dependence of the value of the reduced friction coefficient on the height of the granules layer under the condition of pressure change. The influence of the speed of the limiting surface and temperature the reduced coefficient of friction is almost absent and the increase in pressure (see Figure 17) leads to a slight decrease in the reduced coefficient of friction.

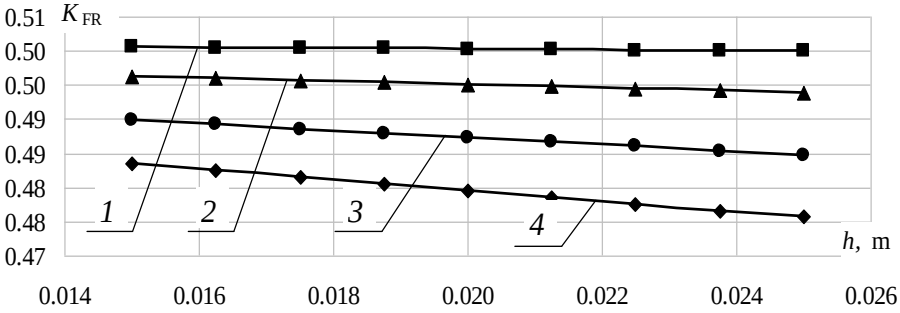

Figure 17. Dependence of the PVC friction coefficient on the height of the granules layer at different pressure, MPa: 1 – 0,044; 2 – 0,152; 3 – 0,314; 4 – 0,476

These dependences, in our opinion, can be explained by the fact that increasing the height of the granules layer of reduces the pressure transfer to the friction surface.

To verify the obtained experimental data, calculations of the process of feeding the screw extruder with polymer raw materials were performed with the initial data of the available experimental research of the authors [29] and compared the results, obtained taking into account the researched dependences of the friction coefficient and lateral pressure coefficient with their constant values (Figure 18).

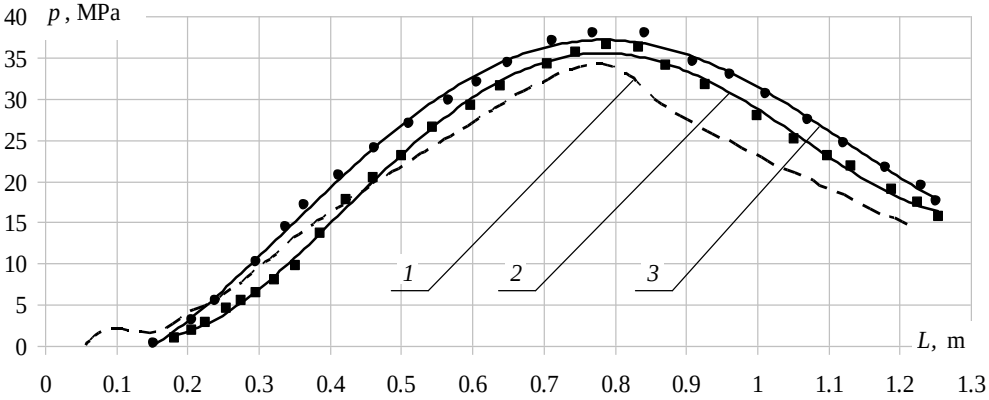

Figure 18. Pressure distribution along the extruder length:
1 – experimental data; 2 – calculation taking into account the researched dependencies;
3 – calculation at constant values of coefficients

From Figure 18 it is seen that the calculation taking into account the change of the coefficients gives the pressure values along the length of the extruder closer to the experimental values than the base, almost the entire length. In this case, in comparison with the experimental data, the maximum discrepancy of the values obtained in the basic calculation is 32% and in the proposed method is 18% (on the length interval L = 1 m).

The obtained results make it possible to take into account the mutual influence of the friction coefficient and of the lateral pressure coefficient (together with other physical and mechanical properties of the processed polymeric material) and design and technological parameters of the equipment (including the seeding zone of the screw extruder and the working channel of the screw feeder), and therefore in the case of development and modernization of technological and ancillary equipment for processing and treatment of granular polymers make it possible to determine the rational values of design and technological parameters of the appropriate equipment.

Conclusion

The following conclusions can be drawn, based on the obtained results:

1. The dependence of the reduced friction coefficient of granular polyvinyl chloride on the steel surface on the pressure (load) acting on the layer of granular polymer is determined. The decrease in the friction coefficient from the load at different values of other parameters (temperature, speed and height of the granules layer) is shown. An increase in the friction coefficient from temperature and speed, as well as a decrease from the height of the layer of granules under a certain load is proved.
2. The dependence of the reduced friction coefficient of granular polyvinyl chloride on the steel surface on the speed of the limiting steel surface is determined. The increase of the friction coefficient from speed at different values of other parameters (temperature, pressure and height of the granules layer) is shown. An increase in the friction coefficient from temperature and a decrease from pressure, as well as the absence of dependence on the height of the granules layer at a certain speed are proved.
3. The dependence of the reduced friction coefficient of granular polyvinyl chloride on the steel surface on the temperature of the limiting steel surface is determined. The increase of the friction coefficient from temperature at different values of other parameters (pressure, speed and height of the granules layer) is shown. An increase in the friction coefficient from speed and decrease from pressure, as well as the absence of dependence on the height of the layer of granules at a certain temperature are proved.
5. The dependence of the reduced friction coefficient of granular polyvinyl chloride on the steel surface on the height of the granules layer at different values of other parameters (pressure, speed and temperature) is determined. The practical absence of dependence of the friction coefficient on speed and on temperature, as well as a slight decrease on pressure is shown. An increase in the friction coefficient from speed and temperature, as well as a decrease from pressure at a certain height of the granules layer is proved.
6. It is established that the value of the lateral pressure coefficient increases under the following conditions: temperature increase; reduction of axial pressure; reducing the speed of the steel limiting surface; the lateral pressure coefficient does not depend on the height of the granule layer.
7. The growth of the lateral pressure coefficient is most affected by the simultaneous action of temperature and speed of the steel limiting surface (linear speed of the rotating working body of the equipment).

The obtained results make it possible to take into account the mutual influence of the friction coefficient and of the lateral pressure coefficient (together with other physical and mechanical properties of the processed polymeric material) and design and technological parameters of the equipment (including the seeding zone of the screw extruder and the working channel of the screw feeder), and therefore in the case of development and modernization of technological and ancillary equipment for processing and treatment of granular polymers make it possible to determine the rational values of design and technological parameters of the appropriate equipment.

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Effect of cations on the activity of NADP$^+$-dependent glutamate dehydrogenase in *Acinetobacter calcoaceticus* IMV B-7241, *Rhodococcus erythropolis* IMV Ac-5017 and *Nocardia vaccinii* IMV B-7405 grown on industrial waste

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Keywords:

Glutamate dehydrogenase
Activators
Waste
Biosurfactants

Article history:

Received 01.08.2020
Received in revised form 23.12.2020
Accepted 25.03.2021

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DOI:
10.24263/2304-974X-2021-10-1-17

Abstract

Introduction. It is studied the activity of NADP$^+$-dependent glutamate dehydrogenase in the presence of mono- and divalent cations (potential activators of this key enzyme of surface-active aminolipids biosynthesis) in *A. calcoaceticus* IMV B-7241, *R. erythropolis* IMV Ac-5017 and *N. vaccinii* IMV B-7405 during cultivation on waste of biodiesel production and sunflower oil waste.

Materials and methods. Cultivation of strains was performed in liquid mineral media using as substrates: refined and waste (after frying potato) sunflower oil, refined glycerol and waste of biodiesel production. NADP$^+$-dependent (EC 1.4.1.4) glutamate dehydrogenase activity in cell-free extracts was analyzed for glutamate formation during oxidation of NADPH at 340 nm. Monovalent (Na^+, K^+) and divalent (Mg^{2+}, Ca^{2+}, Zn^{2+}) cations in the form of salts of NaCl, KCl, $MgSO_4 \times 7H_2O$, $CaCl_2$ and $ZnSO_4 \times 7H_2O$ were added to the reaction mixture, as well as into the medium for strains cultivation.

Results and discussion. Calcium cations were found to be activators of NADP$^+$-dependent glutamate dehydrogenase activity in *R. erythropolis* IMV Ac-5017 and *N. vaccinii* IMV B-7405 grown on refined and waste sunflower oil: in the presence of 1–5 mmol Ca^{2+} in the mixture, the activity of the enzyme increased 1.3–2 times compared with that without these cations. The increase in the concentration of $CaCl_2$ to 0.2−0.4 g/l in oil-containing medium of strains IMV Ac-5017 and IMV B-7405 cultivation was accompanied by an increase in NADP$^+$-dependent glutamate dehydrogenase activity by 1.3–1.5 times compared with that on basic medium. When additional quantity of $CaCl_2$ (0.1−0.2 g/l) was introduced into the medium with purified glycerol for the cultivation of *A. calcoaceticus* IMV B-7241, an increase in NADP$^+$-dependent glutamate dehydrogenase activity was observed by almost 2.5−3 times compared with those for strain IMV B-7241 on the basic medium. There was no impact of activating cations magnesium, zinc, potassium and sodium on NADP$^+$-dependent glutamate dehydrogenase activity of all strains grown on oil-containing substrates and glycerol of different degrees of purification.

Conclusion. The results demonstrate the possibility to increase activity of key enzymes of the biosynthesis of the desired product: the composition of the medium should be modified by changing the content of enzymes' activators.

Introduction

Microbial surfactants are products of multifunctional purpose because they not only reduce the surface tension at the interface and emulsify different substrates, but also exhibit antimicrobial and antiadhesive activity (e.g. ability to destruct biofilms) [1–4]. However, under different conditions of producers cultivation, the composition of surfactants and their properties may change. This is due to the fact that microbial surfactants are secondary metabolites that are synthesized as a complex of similar compounds, the composition and ratio of which may vary depending on the growing conditions of the producer [5], which in turn will change the properties of the final product.

Earlier [5] we showed that the detection of potential activators and / or inhibitors of key enzymes (defining biosynthesis of components of the microbial surfactant complex responsible for certain properties) allows to regulate the composition and therefore the properties of the final product. This could be achieved by following modification of the nutrient composition.

A key enzyme of biosynthesis of surface-active aminolipid, responsible for the antimicrobial activity in *Acinetobacter calcoaceticus* IMV B-7241, *Nocardia vaccinii* IMV B-7405 and *Rhodococcus erythropolis* IMV Ac-5017 is $NADP^+$-dependent glutamate dehydrogenase. Its activators in IMV B-7241 strain are cations of calcium, magnesium and zinc, in IMV Ac-5017 – calcium, in IMV B-7405 – calcium, sodium and potassium [6]. Further increase of the content of enzyme's activators in the medium cultivation was accompanied by an increase of $NADP^+$ dependent glutamate dehydrogenase by 1.5–3 times compared to a basic medium.

Further experiments showed that the additional introduction of $CaCl_2$ (0.1 g/l) into the cultivation medium of *R. erythropolis* IMV Ac-5017, increasing the concentration of this salt to 0.4 g/l in the medium for growing *N. vaccinii* IMV -7405, as well as the adding of $CaCl_2$ (0.1 g/l), increasing the content of $MgSO_4 \cdot 7H_2O$ to 0.2 g/l or the adding of Zn^{2+} (38 μm) in the medium of *A. calcoaceticus* IMV B-7241 cultivation was accompanied by the synthesis of surfactants, which minimal inhibitory concentrations (MICs)relative to bacterial and yeast test cultures were 1.2–13 times lower, their adhesion on abiotic surfaces treated with the surfactants was on average 10–40% lower, and the degree of biofilm destruction was 7 −20% higher compared to the indicators for surfactants obtained on the base medium [6].

In publication [6] *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017 were grown on ethanol, while *N. vaccinii* IMV B-7405 – on purified glycerol. One of the approaches to reducing the cost of the final product is the use of industrial waste as substrates for their production. In our previous studies [7], we have established the possibility of surfactant synthesis under cultivation of strains IMV B-7241, IMV Ac-5017 and IMV B-7405 on waste oil and waste of biodiesel production. However, the antimicrobial activity of surfactants synthesized by *N. vaccinii* IMV B-7405 on the oil-based substrates depended on the quality of waste oil [8]. The biological activity of surfactants synthesized by *A. calcoaceticus* IMV B-7241 on waste of biodiesel production was lower than the surfactants obtained on purified glycerol [9].

We assumed that antimicrobial and antiadhesive activity of surfactants synthesized on industrial waste can be increased by adding of activators of $NADP^+$-dependent glutamate dehydrogenase into the medium cultivation. However, the presence of toxic substances in such substrates [7-9] may cause inhibition of the activity of this key enzyme.

The aim of this work was to determine activity of $NADP^+$-dependent glutamate dehydrogenase in the presence of mono- and divalent cations (potential activators of this key enzyme of surface-active aminolipids biosynthesis) in *A. calcoaceticus* IMV B-7241, *R. erythropolis* IMV Ac-5017 and *N. vaccinii* IMV B-7405 during cultivation on waste of biodiesel production and sunflower oil waste.

Materials and methods

Object of research

The objects of research were strains of oil-oxidizing bacteria, identified as *Nocardia vaccinii* K-8, *Acinetobacter calcoaceticus* K-4 and *Rhodococcus erythropolis* EK-1. Strains K-8, K-4 and EK-1 are registered in the Depository of Microorganisms of D.K. Zabolotnyi Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine with the numbers IMV B-7405, IMV B-7241 and IMV Ac-5017, respectively.

Medium composition and conditions of

A liquid medium of the following composition (g/l) was used for the cultivation of *N. vaccinii* IMV B-7405: $NaNO_3$ – 0.5; $MgSO_4 \times 7H_2O$ – 0.1; $CaCl_2$ – 0.1; KH_2PO_4 – 0.1; $FeSO_4 \times 7H_2O$ – 0.01; yeast autolysate – 0.5% (v/v).

Modifications of basic medium:

1 – increasing the content of $CaCl_2$ to 0.2 g/l;

2 – increasing the content of $CaCl_2$to 0.4 g/l;

3 – adding NaCl (0.5 g/L);

4 – adding KCl (0.5 g/l);

5 – adding NaCl (0.5 g/l) and KCl (0.5 g/l).

A. calcoaceticus IMV B-7241 strain was cultivated in the following medium (g/l): $(NH_2)_2CO$ – 0.35; $MgSO_4 \times 7H_2O$ – 0.1; NaCl – 1.0; Na_2HPO_4 – 0.6; KH_2PO_4 – 0.14; pH 6.8-7.0. Yeast autolysate – 0.5% (v/v) and a solution of microelements – 0.1% (v/v) were additionally included into the medium. The solution of microelements contained (g/100 ml): $ZnSO_4 \times 7H_2O$ – 1,1; $MnSO_4 \times H_2O$ – 0.6; $FeSO_4 \times 7H_2O$ – 0.1; $CuSO_4 \times 5H_2O$ – 0.004; $CoSO_4 \times 7H_2O$ – 0.03; H_3BO_3 – 0.006; KI – 0.0001; EDTA (Trilon B) – 0.5.

Modifications of the basic medium: 1) adding $CaCl_2$ (0.1 g/l), 2) adding $CaCl_2$ (0.2 g/l), 3) adding Zn^{2+} (38 μmol), 4) adding $CaCl_2$ (0.1 g/l) and Zn^{2+} (38 μmol), 5) $CaCl_2$ (0.2 g/l) and Zn^{2+} (38 μmol).

R. erythropolis IMV Ac-5017 strain was grown in the following medium (g/l): $NaNO_3$ – 1,3; NaCl – 1.0; $Na_2HPO_4 \times 12H_2O$ – 0,6; KH_2PO_4 – 0.14; $MgSO_4 \times 7H_2O$ – 0.1; $FeSO_4 \times 7H_2O$ – 0.001.

Modifications of the basic medium: 1) adding $CaCl_2$ (0.1 g/l).

Refined and waste sunflower oil (after frying potato at McDonald's restaurant chain, Kyiv, Ukraine), purified glycerol and waste of biodiesel production (biofuel plant, Poltava region, Ukraine) were used as carbon sources. The concentration of substrates was 1% (v/v).

Cultures in the exponential growth phase, grown in media of the above mentioned composition, containing 0.5% (v/v) of the corresponding substrate were used as the inoculum. The amount of inoculum (10^{-4}–10^{-5} cells/ml) was 5−10% from the volume of the nutrient medium. Cultivation of bacteria was carried out in 750 ml flasks with the volume of medium equal to 100 ml on a shaker (220 rpm) at 28-30º C until mid exponential growth phase (24-48 hours).

Enzymatic analyses

Preparation of cell-free extracts. To obtain cell-free extracts, the culture liquid was centrifuged (5000 g, 20 min, 4° C). The resulting cell precipitate was washed twice from medium residues with 0.05 mol of K^+-phosphate buffer (pH 7.0), centrifuged (4000 g, 15

min, 4° C). The washed cells were resuspended in 0.05 mol of K^+-phosphate buffer (pH 7.0) and destroyed by ultrasound (22 kHz) 3 times for 20 s at 4° C on an UZDN-1 device. The resulting disintegrate was centrifuged (12000 g, 30 min, 4° C), the precipitate was separated, and the supernatant was used for further studies as a cell-free extract.

Analysis of $NADP^+$-dependent glutamate dehydrogenase activity. NAD^+-dependent (EC 1.4.1.2), NAD $(P)^+$-dependent (EC 1.4.1.3) and $NADP^+$-dependent (EC 1.4.1.4) glutamate dehydrogenase activity was analyzed by glutamate formation during oxidation of NAD and NADPH at 340 nm [10]. During the study of the effect of cations on the activity of glutamate dehydrogenase 0.001 and 0.005 mmol Zn^{2+}, 1−10 mmol Ca^{2+}, 5 and 10 mmol Mg^{2+}, 25−100 mmol Na^+, K^+ in the form of solutions of salts of $ZnSO_4 \times 7H_2O$, $CaCl_2$, $MgSO_4 \times 7\ H_2O$, NaCl and KCl, respectively, were added to the reaction mixture.

Activity of the enzyme was expressed in nmol of the product obtained per minute of the reaction calculated per 1 mg of a protein. The protein content in the cell-free extracts was determined by Bradford [11]. Glutamate dehydrogenase activity was assayed at 28-30° C – a temperature optimal for the growth of *R. erythropolis* IMV Ac-5017, *A. calcoaceticus* IMV B-7241 and *N. vaccinii* IMV B-7405.

Statistical analysis

All experiments were performed in 3 replicates, the number of parallel determination in the experiments was 3-5. Statistical processing of experimental data was carried out as described in previous papers [6, 7]. The differences in averages were considered reliable at the significance level $p < 0.05$.

Results and discussion

Glutamate dehydrogenase is responsible for reductive amination of 2-oxoglutarate with the formation of glutamate (donor of amino groups in the subsequent biosynthesis of amino lipids) [10]. Therefore, the higher the activity of this enzyme in the cells producing surfactants, the higher the content in the surfactant complex of amino lipids responsible for the antimicrobial activity of the target product.

Activity of $NADP^+$-glutamate dehydrogenase depending on the concentration of cations in the reaction mixture.

Table 1 presents the data on $NADP^+$-dependent glutamate dehydrogenase activity in the presence of different concentrations of monovalent cations in the reaction mixture in *A. calcoaceticus* IMV B-7241, *R. erythropolis* IMV Ac-5017 and *N. vaccinii* IMV B-7405 grown on refined and waste sunflower oil.

As shown in Table 1 calcium cations at a concentration of 1-5 mmol triggered $NADP^+$-dependent glutamate dehydrogenase activity in *R. erythropolis* IMV Ac-5017 and *N. vaccinii* IMV B-7405 cells grown on both oil containing substrates. Other divalent cations (magnesium and zinc) either inhibited the activity of this enzyme in both strains, or did not influence the activity of glutamate dehydrogenase in the mixture (e.g. the same as in the control without cations). At the same time, all studied divalent cations did not have any effect on the $NADP^+$-dependent glutamate dehydrogenase activity in *A. calcoaceticus* IMVB-7241. Such trends were observed during the cultivation of the strain on both refined and spent sunflower oil.

Table 1

Effect of cations on NADP$^+$-dependent glutamate dehydrogenase activity in cell-free extracts *A. calcoaceticus* IMV B-7241, *R. erythropolis* IMV Ac-5017 and *N. vaccinii* IMV B-7405

Cation	Concentration in the reaction mixture, mmol	NADP$^+$-glutamate dehydrogenase activity (nmol·min^{-1}·mg^{-1}of protein) in cells of strains		
		IMV B-7241	IMV B-7405	IMV Ac-5017
Substrate – refined sunflower oil				
No cations	0	**486±24**	**5 73±29**	**308±15**
Ca^{2+}	1	486±24	N.d.	**615±30**
	5	486±24	**769±38**	259±13
	10	340±17	385±19	259±13
Mg^{2+}	5	486±24	145±7	154±8
	10	486±24	148±7	154±8
Zn^{2+}	0.001	470±23	192±9	259±13
	0.005	470±23	192±9	259±13
Na^+	25	486±24	473±23	154±8
	50	486±24	473±23	154±8
	100	486±24	473±23	154±8
K^+	25	486±24	473±23	154±8
	50	486±24	473±23	154±8
	100	486±24	473±23	154±8
Substrate – fried sunflower oil				
No cations	0	**579±29**	**377±19**	**555±28**
Ca^{2+}	1	579±29	N. d .	**763±38**
	5	579±29	**756±37**	555±28
	10	579±29	377±18	555±28
Mg^{2+}	5	579±29	230±11	370±18
	10	579±29	230±11	370±18
Zn^{2+}	0.001	579±29	377±18	370±18
	0.005	579±29	377±18	370±18
Na^+	25	380±19	377±18	**741±37**
	50	260±13	377±18	**741±37**
	100	198±10	189±9	**741±37**
K^+	25	N.d.	377±18	**741±37**
	50	275±14	189±9	**741±37**
	100	180±9	189±9	555±28

Note: N.d. – not determined

Monovalent cations did not affect the activity of NADP$^+$-dependent glutamate dehydrogenase during of *A. calcoaceticus* IMV B-7241 cultivation on refined oil and inhibited the activity when culturing the strain in waste oil. In the presence of potassium and sodium cations, a decrease in the activity of this enzyme was observed in *R. erythropolis* IMV Ac-5017 and *N. vaccinii* IMV-7405 cells grown on refined oil. However, underg the cultivation on waste oil monovalent cations activated NADP$^+$-dependent glutamate dehydrogenase in *R. erythropolis* IMV Ac-5017 (see Table 1).

$NADP^+$-glutamate dehydrogenase activity depending on the concentration of cations in the culture medium of the strains.

At the next step, the activity of $NADP^+$-dependent glutamate dehydrogenase was determined during cultivation of *A. calcoaceticus* IMV B-7241, *R. erythropolis* IMV Ac-5017 and *N. vaccinii* IMV B-7405 cells in basic and modified oil containing media. Mono- and divalent cations – potential enzyme activators (Table 2) – were added to the media. The choice of cations for the modification of the cultivation media of the studied strains was based on their effect on the activity of the enzyme according to the table. 1. Also the results of previous studies [6] were considered: triggers of $NADP^+$-dependent glutamate dehydrogenase were identified during cultivation of *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017 on ethanol, *N. vaccinii* IMV B-7405 – on purified glycerol.

The research results given in the Table 2, confirmed the data in Table 1: there are no positive effects of calcium and zinc cations on the enzyme activity of strain *A. calcoaceticus* IMV B-7241; calcium cations triggered $NADP^+$-dependent glutamate dehydrogenase activity in *R. erythropolis* IMV Ac-5017 and *N. vaccinii* IMV-7405 cells grown on both refined and waste oil.

At the same time adding of potassium chloride and sodium to the oil containing medium of *R. erythropolis* IMV Ac-5017 did not increase $NADP^+$-dependent glutamate dehydrogenase activity (see the Table 2). The lack of correlation between the monovalent cations' effect on the activity of the enzyme in the reaction mixture (see the Table 1) and in the culture medium (see the Table 2) can be explained as follows. Actual content of cations in bacterial cells and culture medium differs. Values of enzymatic activity in cell-free extracts do not always correspond to the speed of real process in intact cells. This speed depends not only on the content of the enzyme but also on the pool of substrates, enzyme regulations and other factors.

Previously, [6] we showed that cations of calcium and zinc were activators of NADP-dependent glutamate dehydrogenase when *A. calcoaceticus* IMV B-7241 cultivated on ethanol. While sodium and potassium cations were activators when *N. vaccinii* IMV-7405 cultivated on purified glycerol

There were no activating effects observed if oil-containing media used (see Table1 and 2). We can assume that *A. calcoaceticus* IMV B-7241 and *N. vaccinii* IMV B-7405 have several glutamate dehydrogenases, which operate during cultivation on various substrates.

For example, two NAD^+-dependent glutamate dehydrogenases were found in the extremely halophilic strain *Salinibacter ruber* M31 (DSM 13855T) [12]. The activity of one of the enzymes increased 1.4 and 67 times in the presence of 3 M sodium chloride and 3 M potassium chloride, respectively, and the activity of the other, under similar conditions, decreased 8.8 and 4 times, respectively.

It is known from the literature that monovalent and divalent cations can be both inhibitors and activators of glutamate dehydrogenase in microorganisms [13−24].

The activity of this enzyme in the archaea *Thermococcus*sp. increased by 135, 104 and 250% in the presence of 5 mmol $CaCl_2$, $MgCl_2$ and $MnCl_2$, respectively [15]. Later [18] it was found that calcium and magnesium cations are also activators of $NADP^+$-dependent glutamate dehydrogenase in archaea *Thermococcus waiotapuensis*: the presence of 10 mmol $CaCl_2$ and 10 mmol $MgSO_4$ increased an activity by 1.3 times compared to the control (without cations of metals). Divalent iron cations at a concentration of 1 mmol increased the activity of $NADP^+$-glutamate dehydrogenase in *Klebsiella pneumoniae* F-5-2 by 10% [19]. It was found in [19] that silver cations were potent inhibitors of this enzyme in the *K. pneumoniae* F-5-2 strain.

Table 2

Influence of cations in media for cultivation of *A. calcoaceticus* IMV B-7241, *R. erythropolis* IMV AC-5017 and *N. vaccinii* IMV B-7405 on $NADP^+$-dependent glutamate dehydrogenase activity

Strain	Type of oil in medium	Cultivation medium	$NADP^+$-glutamate-dehydrogenase activity ($nmol \cdot min^{-1} \cdot mg^{-1}$protein)
N. vaccinii IMV B-7405	Refined	Basic	**515±25**
		+ 0.2 g/l $CaCl_2$	**690±34**
		+ 0.4 g/l $CaCl_2$	538±26
	Fried (waste)	Basic	**329±16**
		+ 0.2 g/l $CaCl_2$	**425±21**
		+ 0.4 g/l $CaCl_2$	**488±24**
R. erythropolis IMV Ac-5017	Refined	Basic	308±15
		+ 0.1 g/l $CaCl_2$	**618±**30
		+ 0.5 g/l KCl	308±15
		+ 0.5 g/l NaCl	308±15
		+ (0.5 g/l KCl +0.5 g/l NaCl)	308±15
	Fried (waste)	Basic	**555±28**
		+ 0.1 g/l $CaCl_2$	**741±37**
		+ 0.5 g/l KCl	555±28
		+ 0.5 g/l NaCl	555±28
		+ (0.5 g/l KCl +0.5 g/l NaCl)	555±28
A. calcoaceticus IMV B-7241	Refined	Basic	**459±23**
		+ 0.1 g/l $CaCl_2$	464±23
		+ 0.2 g/l $CaCl_2$	464±23
		+ 38 μmol Zn^{2+}	464±23
		+ (0.1 g/l $CaCl_2$+38 μmol Zn^{2+})	475±24
		+ (0.2 g/l $CaCl_2$+38 μmol Zn^{2+})	471±24
	Fried (waste)	Basic	**538±27**
		+ 0.1 g/l $CaCl_2$	533±27
		+ 0.2 g/l $CaCl_2$	538±27
		+ 38 μmol Zn^{2+}	469±23
		+ (0.1 g/l $CaCl_2$+38 μmol Zn^{2+})	494±25
		+ (0.2 g/l $CaCl_2$+38 μmol Zn^{2+})	400±20

The activity of NAD^+-dependent glutamate dehydrogenase in *Laccaria bicolor* fungi increased by 50% in the presence of 1 mM calcium sulfate and magnesium chloride, but decreased by 60–70% when 0.1 mmol Cu^{2+} was added to the reaction mixture [16].

Cations of potassium and sodium at the concentration 50–200 mM are enzyme activators in aerobic hyperthermophile archaea *Aeropyrum pernix* K1 [17]. At the same time, the activity of purified $NADP^+$-dependent glutamate dehydrogenase *Pyrobaculum calidifontis* was inhibited by 50% in the presence of 100–200 mmol of potassium chloride and 100–300 mmol of sodium chloride [22].

Alba-Lois et al. [20] found that the additional of 1 M NaCl into the culture medium of halotolerant yeast *Debaryomyces hansenii* was accompanied by a fivefold increase in the activity of $NADP^+$-dependent glutamate dehydrogenase compared to the activity of the enzyme when grown on a medium without sodium chloride. At the same time, the activity of the purified enzyme did not increase in the presence of this salt. The researchers explained such unexpected results by the fact that the increased activity of $NADP^+$-dependent glutamate dehydrogenase in *D. hansenii* is a kind of defence mechanism against the inhibitory effect of high ionic strength.

It was shown in [16] that the stability of *Escherichia coli* glutamate dehydrogenase increased in the presence of lithium cations at a concentration of 1 to 10 mM, 1 M sodium phosphate, or 1 M ammonium sulfate.

Data on the effect of zinc cations on the activity of $NADP^+$-dependent glutamate dehydrogenase inmicroorganisms appeared in 1980 [13], but so far there are only a few such publications. In [13] it is reported that depending on the concentration of Zn^{2+} can be either an activator or inhibitor of this enzyme: at a concentration of less than 0.1 mmol activity of glutamate dehydrogenase of *Mycobacterium smegmatis* raised, while at concentrations above 0.1 mmol an inhibition of enzyme activity was observed. It was observed that the activity of $NADP^+$-dependent glutamate dehydrogenase *E. coli* in the presence of 1 mM Zn^{2+} decreased by 40% [14]; at a concentration of 5 mmol $ZnCl_2$ – the activity of this enzyme reduced in *Aspergillus terreus* [21].

Geotrichum sandidum S12 glutamate dehydrogenase is unique because it has substrate specificity towards glutamate, 2-oxoglutarate, hexanol and isoamyl alcohol [23]. In the presence of ADP, Fe^{2+}, K^+ and Zn^{2+} an increase in enzymatic activity towards hexanol was observed; and in the presence of EDTA, Mn^{2+} and ATF – its inhibition.

Influence of divalent cations in the culture medium of *A. calcoaceticus* IMV B-7241 with glycerol of different purification degree on the activity of $NADP^+$- glutamate dehydrogenase.

At the next step it was analyzed how activators impact $NADP^+$-dependent glutamate dehydrogenase during the cultivation of *A. calcoaceticus* IMV B-7241 on purified glycerol and waste of biodiesel production. Data in Table 3 show that after adding calcium cations into the medium with refined glycerol for *A. calcoaceticus* IMV B-7241 cultivation activity of $NADP^+$-glutamate dehydrogenase increased almost 2.5-3 times comparing to the basic medium.

However, after adding $CaCl_2$ into the media with waste of biodiesel production $NADP^+$-dependent glutamate dehydrogenase activity remained almost the same as in the basic medium. Also under cultivating *A. calcoaceticus* IMV B-7241 on oil containing substrates adding zinc cations into glycerol media did not increase activity of the enzyme (see Table 2 and 3).

Table 3

$NADP^+$-dependent glutamate dehydrogenase activity during cultivation of *A. calcoaceticus* IMV B-7241 on glycerol of various purity

Growth substrate	Cultivation media	$NADP^+$-glutamate-dehydrogenase activity (nmol · min^{-1} · mg^{-1}protein)
Purified glycerol	Basic	**159±8**
	+ 0.1 g/l $CaCl_2$	**401±20**
	+ 0.2 g/l $CaCl_2$	**476±24**
	+ 38 μmol Zn^{2+}	160±8
	+ (0.1 g/l $CaCl_2$+38 μmol Zn^{2+})	239±12
	+ (0.2 g/l $CaCl_2$+38 μmol Zn^{2+})	154±8
Wastes of biodiesel production	Basic	**526±26**
	+ 0.1 g/l $CaCl_2$	541±27
	+ 0.2 g/l $CaCl_2$	541±27
	+ 38 μmol Zn^{2+}	532±26
	+ (0.1 g/l $CaCl_2$+38 μmol Zn^{2+})	532±26
	+ (0.2 g/l $CaCl_2$+38 μmol Zn^{2+})	532±26

In our opinion, one of the reasons why Zn^{2+} activates $NADP^+$-dependent glutamate dehydrogenase [6] on ethanol media but does not on oil and glycerol may be the presence of several glutamate dehydrogenases in a strain IMV B-7241. However, in the next studies we could not identify NAD^+- or $NAD(P)^+$-dependent glutamate dehydrogenase activity in cells *A. calcoaceticus* IMV B-7241 grown on oil-containing substrates, purified glycerol and waste of biodiesel production. Probably, the strain IMV B-7241 has several $NADP^+$-dependent enzymes that function when grown on different substrates. However, the final conclusion in favor of such an assumption can be made only after the isolation of the relevant enzymes and the study of their physicochemical properties.

In addition, it is possible that in the *A. calcoaceticus* IMV B-7241 strain during cultivation on oil and glycerol, glutamate formation is not involved in glutamate dehydrogenase, but glutamine synthetase and glutamate synthase [24]. These enzymes carry out (like glutamate dehydrogenase) reductive amination of 2-oxoglutarate with the formation of glutamate as a result of two successive reactions that occur with the participation of ATP. van Heeswijk et al. [24] note that in *E. coli* and *Salmonella typhimurium* cells, glutamate dehydrogenase was characterized by high activity during exponential bacterial growth, however, as the sources of carbon and nitrogen nutrition in the culture medium were exhausted, it was decomposed by ATP-dependent proteases.

It was found in [19] that during the cultivation of *K. pneumoniae* F-5-2 under aerobic conditions, both glutamate dehydrogenase and glutamine synthetase functioned simultaneously in bacterial cells. $NADP^+$-dependent glutamate dehydrogenase catalyzed the not only amination of 2-oxoglutarate, but also 2-oxovalerate and 2-oxobutyrate and was stable in the pH range 5.5-11.5. The optimum pH of glutamine synthetase was 8.0, this enzyme was stable at pH 6.0-7.0. Unlike glutamate dehydrogenase, the activity of glutamine synthetase was strongly suppressed by ferrous iron cations. In addition to iron cations, mercury and cuprum cations were found to be inhibitors of this enzyme *in K. pneumoniae* F-5-2.

Note that the final conclusion about the functioning of or several NADP + dependent glutamate dehydrogenase, or glutamate dehydrogenase, glutamine synthetase and glutamate synthase in the *A. calcoaceticus* IMV B-7241 strain grown on oil and glycerol can be done only after the isolation of the corresponding enzymes and the study of their physicochemical properties.

Conclusion

The results of this study confirm the earlier data on possibility to regulate the activity of key enzymes of the biosynthesis of the final product. This can be achieved by modifying the composition of the medium, e.g. by changing a content of activators (inhibitors) of these enzymes.

The obtained data suggest that adding $CaCl_2$ to the oil-containing media of *R. erythropolis* IMV Ac-5017 and *N. vaccinii* IMV B-7405, as well as to the medium with purified glycerol for growing *A. calcoaceticus* IMV B-7241 will result in synthesis of surfactants with increased antimicrobial and antiadhesive activity.

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Анотації

Харчові технології

Тренди і очікувані переваги передових харчових технологій у 2021–2030рр.

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Вступ. Розглянуто основні тенденції світового розвитку інноваційних технологій харчових продуктів у 2021–2030 рр.

Матеріали і методи. Морфологічний аналіз кластерів наукових знань про харчову науку.

Результати і обговорення. Основні тенденції світового розвитку технологій харчових продуктів:

1. Більш суворі правила безпеки харчових продуктів, включаючи полімеразну ланцюгову реакцію і виявлення нових патогенів ДНК, що передаються через харчові продукти, комплексний контроль за хімічним забрудненням харчових продуктів.

2. Виробництво функціональних харчових продуктів, включаючи харчування для немовлят, дітей, вагітних жінок, людей похилого віку, для занять спортом, харчування військовослужбовців, страви, готові до вживання, збагачену мікроелементами їжу, нутрицевтики та дієтичні продукти.

3. Екологічно чисті та енергоощадні способи переробки харчових продуктів, включаючи нетеплові фізичні методи, такі як холодне оброблення плазмою, гомогенізація під високим тиском, імпульсні електричні поля, екстракція за допомогою ультразвуку, інноваційні технології сушіння продуктів, виробництва етанолу, екстракційні технології, дезінфекція і стерилізація продуктів харчування й обладнання, інноваційні технології пакування харчових продуктів, інтенсифікація перероблення харчових продуктів з використанням біотехнологічних методів, залучення нетрадиційної сировини для виробництва харчових продуктів.

4. Біотехнологічна обробка харчових продуктів з використанням ферментів протеїназ, глутамінових трансфераз, галактозидаз, ферментів екстремофільних і психрофільних мікроорганізмів, живих мікроорганізмів як пробіотиків або заквасок, мікробних метаболітів і нових джерел їжі, таких як комахи та штучне м'ясо.

5. Персоналізація перероблення і розподілу харчових продуктів, включаючи адаптацію харчової промисловості до харчових потреб різних медичних, расових, релігійних і регіональних груп споживачів, комп'ютеризація особистого виробництва та споживання їжі, проблема сприйняття споживачами нових продуктів, 3D-друк їжі. Комерційне харчування стало різноманітним, а для оптимізації виробництва та доставки їжі використовуватиметься специфічне комп'ютерне програмне забезпечення з вичерпною інформацією про харчовий продукт та особисті вимоги до раціону харчування.

Висновки. Інформація, наведена в огляді, може бути цінною для науковців та управлінців при визначенні пріоритетних напрямів досліджень та інноваційної діяльності.

Ключові слова: *харчування, безпека, оброблення, біотехнологія, пакування, функціональність, персоналізація.*

Функціональна ефективність in vitro екстрактів кавказького рододендрона (*Rhododendron caucasicum*) і вин Rkatsiteli як інгібіторів панкреатичної ліпази

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Вступ. Метою дослідження є визначення інгібувальної активності кавказьких рододендронів (Rhododendron caucasicum) і Ркацителі проти панкреатичної ліпази.

Матеріали і методи. Листя кавказького рододендрона, зібрані в регіоні Верхної Сванетії. Вина виготовляли із сорту винограду Ркацителі. Титриметричний метод використовували для визначення активності ліпази, загального вмісту фенолу (TPC). Здатність плазми до зниження заліза (FRAP) визначали спектрофотометрично.

Результати і обговорення. Продемонстровано високу кореляцію між TPC і антиоксидантною активністю (AOA) у всіх зразках. Коефіцієнт кореляції Пірсона (R2) для зразків кавказького рододендрона і зразків вина становив 0,9758 та 0,9556 відповідно. Встановлено, що середній показник TPC у кавказькому рододендроні коливається від 13,00±0,48 до 19,48±0,84% еквівалента галової кислоти (GAE) на основі вмісту сухої речовини. Третій зразок кавказького рододендрона виявив найвищий показник TPC, GAE – 19,48±0,84%, AOA – 16,10±0,32. Суттєвої різниці між третім і першим зразками (17,97±0,42% GAE та 15,35±0,74 AOA ($p < 0,05$)) не спостерігалось. Незважаючи на те, що четвертий зразок демонстрував найнижчі TPC та AOA, його інгібувальна активність ліпази дуже нагадувала орлістат. Імовірно, що поліфенол, який забезпечує антиліпазну активність кавказького рододендрона, легко окислюється в повітрі. Отже, технологія оброблення подібна до технології «Зеленого чаю», дозволяє утримувати більшу частину поліфенолу в зразку. У решті зразків ця речовина окислювалася молекулярним киснем. Результати дослідження підтвердили, що обробка зразків рододендрона може вплинути на склад біоактивних сполук. Отже, білі вина, виготовлені за кахетською технологією, багаті біоактивними сполуками та мають вищу антиоксидантну активність та інгібувальну дію ліпази порівняно з винами, виготовленими за європейською технологією.

Висновок. Екстракти кавказького рододендрона є перспективним природним інгібітором, адже зменшують всмоктування холестерину з їжею. Зважаючи на вміст сухої речовини, кавказький рододендрон має кращу інгібувальну активність.

Ключові слова: *вино, кавказький рододендрон, Ркацителі, орлістат, антиоксидант.*

Заміна хлориду натрію в промисловому тостовому хлібі

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Вступ. Ефект заміщення хлориду натрію вивчали у промисловому тостовому нарізаному пшеничному хлібі, обґрунтовуючи технологічний підхід до зменшення вмісту натрію в хлібних виробах.

Матеріали і методи. Як замінних хлориду натрію вивчали Granolife CV Sustisal 100 (GCVS100). Ферментативні властивості тіста визначалися за допомогою реоферментометра, властивості поведінки тіста під час змішування-нагрівання-охолодження – за допомогою Mixolab. Крім того, питомий об'єм хліба і профіль текстури розглядались як параметри якості випікання.

Результати і обговорення. Додавання GCVS100 або NaCl до тіста з пшеничного борошна призвело до зменшення газоутворення на етапі бродіння. Однак вони значно збільшили коефіцієнт затримки газу, сприяли вдосконаленню клейковинни та дали змогу отримати криву розвитку тіста, подібну до борошняного тіста. Крім того, обидва інгредієнти змінили кілька параметрів борошняного тіста в Mixolab. Водопоглинання зменшилося, стабільність тіста і крохмального гелю при нагріванні (C4-C3) покращилася, процес желатинізації (C3-C2) уповільнився, а ретроградація крохмалю підвищилася.

GCVS100, оцінений у формулі тостового хліба, показав подібні ефекти. Додавання GCVS100 або NaCl до тостового хліба призвело до зменшення вироблення газу під час бродіння. Коефіцієнт затримки газу не показав суттєвих відмінностей між обробками, оскільки до складу хліба входять сполуки, які стимулюють зміцнення клейковинної структури тіста, що маскує ефект NaCl і GCVS100. Отже, відповідно до меншої газоутворювальної здатності, NaCl і GCVS100 призводять до зменшення об'єму тіста.

Параметри випікання тостового хліба показали, що питомий об'єм хліба був значно вищим без NaCl або GCVS100. Аналіз профілю текстур тостового хліба не показав змін в пористості м'якуша і пружності при додаванні NaCl або GCVS100.

Висновки. Додавання GCVS100 в хліб спричиняє ефект, подібний до дії NaCl. Результати цього дослідження дають змогу припустити, що GCVS100 можна використовувати для отримання тостового хліба без натрію.

Ключові слова: *хліб, хлористий натрій, реологія, міксолаб, текстура.*

Антиоксидантна ефективність рослинних культур

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Вступ. Природні антиоксиданти мають переваги перед синтетичними, не викликаючи небажаних ефектів. Мета дослідження – виявити серед ягідних та лікарських рослин концентратори антиоксидантів і визначити їх практичне використання у харчових технологіях.

Матеріали і методи. Досліджено 4 сорти культивованих ягід та 8 видів дикоросів на загальну антиоксидантну ефективність; 10 зразків лікарських трав – на вміст і ступінь вилучення біофлавоноїдів у водно-спиртові екстракти; вміст аскорбінової кислоти, біофлавоноїдів, каротиноїдів визначали за загальновідомими методиками.

Результати і обговорення. Максимальний вміст аскорбінової кислоти виявлено в ягодах чорної смородини (234,0 мг/100 г), вишні (62,2 мг/100 г), аронії чорноплідної (129,0 мг/100 г), суниці (104,0 мг/100 г), ожини (68,8 мг/100 г). Ці ж ягоди відзначаються високим вмістом біофлавоноїдів, відповідно, 1858 мг/100 г; 1340 мг/100 г; 2460 мг/100 г; 1978 мг/100 г; 2447 мг/100 г. Спостерігається природна кореляція між вмістом цих двох груп антиоксидантів у досліджених матеріалах. Це обґрунтовує доцільність їх використання для отримання харчової продукції антиоксидантного

спрямування. Рейтинговий список ягідних культур, найбільш придатних для корегування вмісту аскорбінової кислоти, біофлавоноїдів, каротиноїдів відповідно до рекомендованих добових потреб споживання, складає: аронія чорноплідна (2600 мг/100 г), ожина (2514 мг/100 г), чорниця (2199 мг/100 г), смородина чорна (2096 мг/100 г), суниця (2084 мг/100 г), вишня (1405 мг/100 г).

Досить високий загальний вміст біофлавоноїдів, які діють як одне ціле і відіграють роль буферної антиоксидантної системи, виявлено у лікарських рослинах: звіробій (3,89 мг/100 г), материнка (2,98 мг/100 г), цмин (2,638 мг/100 г), меліса (1,685 мг/100 г), чебрець (1,470 мг/100 г). За визначених оптимальних значень основних параметрів екстрагування в екстракт переведено 85% біофлавоноїдів зі звіробою; понад 60% зі смородини, меліси, чебрецю, шавлії, цмину; до 40% л – із материнки; менш ніж 30% – із кропиви та берези. Це пояснюється певними відмінностями у їхній будові, різним вмістом харчової клітковини, що загалом впливає на коефіцієнт дифузії біофлавоноїдів при екстрагуванні.

Висновки. Рослинна сировина, характерна для помірного кліматичного поясу – культивовані та дикорослі ягоди, лікарські рослини – з високим вмістом антиоксидантів, має стати неодмінною складовою харчових продуктів і напоїв для захисту організму від вільнорадикальних впливів.

Ключові слова: *антиоксидант, окислення, ягоди, трави, екстрагування.*

Вплив термічної обробки тиском, кип'ятіння і пропарювання на колір, текстуру і вміст біоактивних сполук у коренеплодах

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Вступ. Метою дослідження було порівняння ефекту обробки тиском, варіння у воді та на парі на зміни кольору, текстури й утримання вибраних біоактивних сполук у коренеплодах.

Матеріали і методи. Морква та петрушка були піддані термічній обробці (SV) при 80 °C (SV 80) і 90 ° C (SV 90), варінні в киплячій воді (B) та варінні на парі (S) протягом 10, 20 і 30 хвилин. Властивості інструментальної текстури оцінювали за допомогою аналізу текстурного профілю (TPA). Колір вимірювали колориметром, а вміст загальних фенольних сполук і каротиноїдів визначали за допомогою спектрофотометричного методу.

Результати і обговорення. Твердість, щільність і текстура аналізованих овочів суттєво відрізнялись залежно від способу й тривалості кулінарної обробки. Найвищі значення твердості, щільності та жувальності були продемонстровані для варіантів SV 80.

Більшість колірних параметрів овочів статистично суттєво відрізнялись, залежно від способу та тривалості кулінарної обробки. Найнижча яскравість (L*) продемонстрована для петрушки B (20-хвилинна обробка), а найвище значення параметра відзначено для зразків петрушки SV 80 (10-хвилинна обробка). Найвищі значення параметра a*, який є компонентом помаранчевого кольору та визначає споживчу привабливість моркви, були зафіксовані у зразках SV 80 та SV 90. Статистично значущі відмінності були виявлені у значенні b* між видами термічної обробки. Найвище значення жовтизни відзначено для зразків R (необроблених), тоді

як зразки B (20-хвилинна обробка) мали найнижчі значення цього параметра. У випадку з морквою найвищі значення параметра b* були зафіксовані у зразках SV 90 (10-хвилинна обробка), а зразки R мали найнижче значення жовтизни.

Найвищий вміст фенольних сполук виявлено у петрушки B (20-хвилинна обробка) та моркви SV 90 (20-хвилинна обробка). Найвище значення затримки каротиноїдів зареєстровано для петрушки SV 90 (10-хвилинна обробка) та моркви SV 80 (10-хвилинна обробка).

Висновки. Порівняно з відвареними та пропареними зразками, овочі після термічної обробки тиском мають вищу твердість, щільність, жувальність і привабливий для споживача колір. Морква, оброблена цим методом, демонструє більш високе утримання каротиноїдів.

Ключові слова: *теплова обробка, кипіння, пропарювання.*

Технологічні властивості картопляного крохмалю після гідротермічного оброблення з додаванням органічних кислот

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Вступ. Крохмаль був модифікований для подолання промислових обмежень, наявних у його природному вигляді. Гідротермічне оброблення (HMT) є фізичним і термічним методом, що застосовується для модифікації крохмалю. Його можна поєднувати з деякими хімічними речовинами, щоб надати полімерам унікальних технологічних характеристик.

Матеріали і методи. Картопляний крохмаль модифікували за допомогою HMT із використанням води та 0,2 мольних розчинів L^{-1} органічних молочної та лимонної кислоти до співвідношення 22%. Досліджено склеювальні й термічні властивості картопляного крохмалю. За допомогою диференціальної сканувальної калориметрії (DSC) визначено значення початкових (To), пікових (Tp) і кінцевих температур (Tc) желатинізації, а також ентальпії (Hgel).

Результати і обговорення. Картопляний крохмаль показав тип текстури BC (тип B з характеристиками типу C). Оброблені крохмалі мають тип текстури C, з основними піками дифракції 2θ при 5,6 º, 15,3 º, 17,3 º та 23,5 º відповідно. Спостерігалось зменшення піків інтенсивності на 5,6 ° відповідно до кожної обробки. Розрахункова відносна кристалічність (RC) кожного крохмалю становила 27,2% від комерційного картопляного крохмалю. За допомогою аналізу склеювальних властивостей (RVA) виявлено, що пік в'язкості мав помітне зниження з 7824,00 мПа/с до 90,00 мПа/с (HMT+лимонна кислота). Крохмаль + HMT та оброблений 22% водою + HMT показав ентальпію 14,5 та 9,9 Дж g^{-1} відповідно. Інші крохмалі, оброблені 22% розчином молочної кислоти + HMT і 22% розчином лимонної кислоти + HMT, показали розширення між початковими і кінцевими температурами желатинізації, з різким зменшенням в'язкості.

Висновки. Основне застосування цього модифікованого крохмалю – у продуктах, що вимагають низької в'язкості (дитяче харчування, супи) та як інгредієнта для випікання печива.

Ключові слова: *крохмаль, модифікація, гідротермічний, молочна кислота, лимонна кислота.*

Масообмін під час осмотичної дегідратації айви з використанням різних видів осмосу

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Вступ. Осмотичні розчини фруктози та сахарози тестували з метою оцінки їх впливу на характеристики осмодованої айви. Вивчено вплив товщини зрізів, концентрації розчину і часу занурення на колір і хімічні характеристики зневодненої айви (Cydonia oblonga).

Матеріали і методи. Зниження ваги (WR), кольорові параметри (CIE), загальний вміст фенольної (TPC) і аскорбінової кислоти (AA) досліджували за допомогою осмотичних розчинів фруктози та сахарози протягом 3 год занурення. Вміст загальних поліфенолів (TPC) визначали за допомогою реагенту Фоліна-Чіокальтеу при 765 нм за допомогою спектрофотометра. Вміст аскорбінової кислоти (AA) відокремлювали, ідентифікували та дозували в системі HPLC SHMADZU у поєднанні з детектором UV–VIS (DAD).

Результати і обговорення. Зниження ваги (WR,%) осмодованої айви показало значні відмінності залежно від типу й концентрації осмотичного агента і часу процесу. Значно більша втрата вологи фруктози (моносахаридів) як осмотичного агента є суттєвою перевагою порівняно із сахарозою (дисахаридом). Вищі значення WR були отримані, коли зразки айви зневоднювали розчином фруктози концентрацією 80%. Було помічено, що після 180 хв осмотичної дегідратації 40% розчином фруктози тонкі зрізи (10 мм) мають вищу величину WR порівняно з товстими зрізами (20 мм). Загальний вміст поліфенолів збільшувався під час осмотичної дегідратації 80% осмотичним розчином. Вміст аскорбінової кислоти збільшився під час обробки розчином фруктози з 18,66 мг/100 г (у зразках свіжої айви) до 30,9 мг/100 г (у зразках айви після осмотичної дегідратації розчином фруктози 80%). Зразки, оброблені 80% фруктозою, мали нижчу координату L, демонструючи ферментативне забарвлення. Значення було мінімальним для зразків, оброблених 80% фруктозою. Це вказує на те, що гідратована айва має темніший колір порівняно зі свіжими зразками.

Висновки. Осмотична дегідратація айви двома осмотичними розчинами незначно вплинула на композиційні властивості, такі як вміст загальних поліфенолів та аскорбінової кислоти, а також збільшення ваги осмотично зневодненої айви.

Ключові слова: *айва, колір, поліфеноли, аскорбінова кислота.*

Харчова цінність рибного супу з культивованої форелі (*Salvelinus fontinalis*, Mitchill, 1814)

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Вступ. Метою дослідження є оцінка харчової цінності рибного супу з культивованої форелі з точки зору здорового харчування.

Матеріали і методи. Рибний суп готували з 23,65% попередньо приготовленого фаршу з форелі та 18,76% овочів протягом 35 хвилин. Кінцевий продукт аналізували на безпосередній склад, жирні кислоти та вміст мінеральних речовин, а також каротиноїди та вітаміни B1, B2, B6. Масовий спектрометр з індуктивно зв'язаною плазмою (ICP-MS) використовували для визначення вмісту мінералів після розкладання ліофілізованих зразків. Метилові ефіри жирних кислот відокремлювали за допомогою газової хроматографії за допомогою полум'яно-іонізаційного детектора (FID). Для оцінки вмісту вітамінів і каротиноїдів використовували високоефективну рідинну хроматографію (HPLC).

Результати і обговорення. Склад супу: 87,7°9% вологи, 8,18% білка, 2,89% сирого жиру, 1,17% харчових волокон та 0,03% вуглеводів. Енергетичну цінність розраховували як 58,82 ккал/100г. Значення загальних поліненасичених жирних кислот (∑PUFA) було вищим за значення загальних мононенасичених (∑MUFA) і насичених жирних кислот і становило 43,89, 34,93 та 19,83% відповідно. Основна PUFA відповідала лінолевій кислоті – 27,14%, наступною була докозагексаєнова кислота DHA – 7,92%. Загальний вміст ейкозапентаенової і докозагексаєнової кислоти (∑EPA + DHA) – 9,21%, що становило 239,04 мг/100 г супу. Порція форелевого супу (близько 200 г) задовольняла б щоденний рекомендований раціон, але для щоденного споживання потрібна дещо більша кількість EPA + DHA . Вміст мінеральних речовин варіювався в діапазоні 1,77–31,52 мг/г, тоді як результати, отримані для вітамінів B1, B2 та B6 та для каротиноїдів, були порівнянними з даними для різних типів супів у літературі.

Висновок. Поживний рибний суп може бути виготовлений із форелі та відповідає всім параметрам здорового харчування.

Ключові слова: *форель, риба, суп, жирні кислоти, мінерали, харчова цінність.*

Вилучення, реологічний і текстурний аналіз пектину із стовбурової кісточки банана

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Вступ. Мета дослідження – виявити відповідні умови для вилучення пектину із стовбурової кісточки банана та визначити, як він впливає на реологічні й текстурні властивості фруктових джемів.

Матеріали і методи. Штамбова кісточка місцевого сорту банана. Пектин екстрагували за допомогою гарячої води, підкисленою сірчаною кислотою. Вихід вивчали і виражали як відсоток використаної сушеної сировини. Ступінь метилювання

вивчали методом титрування, мономерний склад вивчали за допомогою високоефективної аніонообмінної хроматографії з імпульсним амперометричним детектором. Вплив банановго пектину на релогічні характеристики і профіль текстури ананасового джему, виготовленого з додаванням пектину, вивчали реометричним методом. Пектин класифікували методом модифікованого розподілу ліній.

Результати і обговорення. Отримано вихід 27,91%. Встановлено, що всі незалежні змінні впливають на вихід по-різному. Встановлено, що температура та pH є найважливішими умовами, що впливають на вихід, тоді як час нагрівання і SLR (відношення твердої речовини до рідини), також впливають на вихід, але меншою мірою. Найвищий вихід був при SLR 50, pH 1,5, температурі 82 ℃ і часі нагрівання 52,5 хв.

Ступінь метоксилювання становить 62% із складом, подібним до складу пектину з інших джерел, про який повідомляють інші дослідники.

Банановий пектин впливає на реологічні й текстурні властивості ананасового джему. Напруження зсуву для джему, приготованого з використанням банановго пектину, становить 113 Па, порівняно з 96 Па для контролю. У той же час найвищий модуль зсуву становив 94 Па, проти значення 60 Па для контрольного зразка. Хоча було встановлено, що банановий пектин впливає на твердість ананасового джему, для її досягнення потрібно більше банановго пектину порівняно з цитрусовим. Потрібне більше зусилля для течії джему, що свідчить про більш високий рівень виходу порівняно з цитрусовим пектином.

На текстурні властивості джему вплинуло додавання банановго пектину. Твердість зменшено із 6,18 для контролю до 2,30 для тесту, тоді як когезійність зменшено з 5,30 для контролю до 1,96 для тесту. Пружність була збільшена з 6,24 до 7,52. Клас банановго пектину становить 90,9.

Висновки. Штамбова кісточка банана є потенційною альтернативною сировиною для вилучення пектину, який як агент придатний для використання в харчових продуктах.

Ключові слова: *банан, пектин, вилучення, реологія, текстура.*

Зберігання селену та йоду у функціональних м'ясних продуктах з додаванням водоростей

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Вступ. Метою дослідження було вивчення зберігання селену та йоду в функціональних м'ясних продуктах, виготовлених з додаванням водоростей при різних температурних режимах.

Матеріали і методи. У дослідженні було використано три їстівні морські водорості *Fucus, Cystoseira* та *Laminaria*. Виготовляли різні м'ясні продукти з додаванням цих водоростей. Вміст селену вимірювали діамінонафталіновим методом. Вміст йоду визначали методом інверсійної вольтамперометрії.

Результати і обговорення. Морські водорості використовували при приготуванні м'ясних продуктів для збагачення їх йодом і селеном. *Laminaria* мала занадто високий вміст йоду та селену для виготовлення дієтичних продуктів.

Cystoseira була кращим джерелом йоду, ніж *Fucus.* Усі готові продукти з *Cystoseira* мали вищий вміст йоду, ніж ті ж самі продукти з *Fucus.* Вміст йоду в готових продуктах був вищим при температурі приготування 100–110 °C (парові котлети) порівняно зі 170 °C (смажені котлети). Спосіб приготування також мав значний вплив на втрати йоду. Найбільші втрати відмічено для продуктів, які готували в рідині: 50% йоду втрачалося при приготуванні тюфтельок, тушкованих у соусі, та 61% в фрикадельках, які варили у супі. Втрати йоду в пельменях були нижчими (38%), імовірно завдяки фаршу, який знаходиться в оболонці із тіста. Найнижчі втрати йоду, близько 17%, було відмічено для ковбасок, що готували на грилі, завдяки відносно низькій температурі приготування та відсутності рідкого середовища.

Cystoseira була дещо кращим джерелом селену, ніж *Fucus.* Усі готові продукти з *Cystoseira* мали незначно вищий вміст селену, ніж ті ж самі продукти з *Fucus.* Вплив температури та метод приготування продуктів був не настільки очевидний і втрати селену становили від 19 до 27 % для парових котлет, тюфтельок, фрикадельок і пельменів з *Laminaria.* Найнижчі втрати селену, близько 7%, було відмічено для ковбасок, що готували на грилі.

Висновки. Смажені та приготовлені на парі котлети, тюфтельки та ковбаски для грилю, в рецептуру яких входило 2% (ваг/ваг) морських водоростей *Cystoseira* або *Fucus*, рекомендовані як функціональні продукти, що містять добову потребу в йоді та селені.

Ключові слова: *функціональний, продукт, водорості, селен, йод.*

Вплив водоростей Spirulina platensis і ламінарії на вміст тіаміну та рибофлавіну в пшеничному хлібі

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Вступ. Мета статті – дослідити вплив деяких їстівних водоростей, зокрема *Spirulina platensis* та *ламінарії,* на вміст тіаміну і рибофлавіну в пшеничному хлібі.

Матеріали і методи. Хліб виготовляли з пшеничного борошна з додаванням ламінарії та *Spirulina platensis* (порошок) у кількості 2 або 4% від маси борошна. Вміст віаміну В2 оцінювали рідинною хроматографією з використанням методу мас-спектрометрії (LC-MS).

Результати і обговорення. Збагачення водоростями *ламінарії* та *Spirulina platensis* у кількості 2 і 4% від маси борошна призводить до збільшення вмісту тіаміну і рибофлавіну в пшеничному хлібі. Два типи водоростей мають різну дію. Використання 2% ламінарії призводить до збільшення на 7,35%, а 4% – на 28,27% їх кількості порівняно з контрольним зразком. Збільшення спостерігається з додаванням 4% *Spirulina platensis*. Кількість тіаміну становила 1533,75 мкг/кг хліба, що майже вдвічі більше, ніж у контрольній пробі.

Вміст вітаміну В2 у контрольній пробі хліба становить 310,5 мкг/кг. З додаванням ламінарії у кількості 2% приріст вітаміну В2 становив 81,7 мкг/кг, а за вищої дози (4%) збільшення становило 120 мкг/кг порівняно з контрольною пробою та 38,3 мкг/кг порівняно з 2% водоростей. Найвище значення було зареєстровано для хліба, збагаченого 4% *Spirulina platensis*. Вміст рибофлавіну був майже в втричі вищим, ніж

у контрольній пробі; у 2,37 раза вищим, ніж у зразку з 2% ламінарії, та в 2,16 раза вищим, ніж у зразку ламінарії із 4%.

Висновки. Збагачення пшеничного хліба деякими їстівними водоростями (особливо в кількості 4% від маси борошна) є ефективним підходом для підвищення вмісту тіаміну та рибофлавіну. Вплив *Spirulina platensis* на вміст вітамінів більш виражений.

Ключові слова: *хліб, Spirulina platensis, ламінарія, тіамін, рибофлавін.*

Вміст органічних кислот, цукрів і фізико-хімічні показники меду румунської акації

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Вступ. Деякі елементи меду вносяться бджолами, інші виникають після його дозрівання. Визначено фізико-хімічні показники, вміст пилку, вміст цукрів і вміст органічних кислот для меду румунської акації.

Матеріали і методи. 27 зразків акацієвого меду 2020 року досліджували для визначення фізико-хімічних показників: вологості, електропровідності, pH, вільної кислотності, кольору, вмісту гідроксиметилфурфуролу (HMF) і вмісту цукрів, використовуючи методи Міжнародної комісії з меду (the International Honey Commission).

Результати і обговорення. Мелісопалінологічний аналіз підтвердив ботанічне походження всіх зразків меду: кожен містив понад 45% пилкових гранул *Robinia pseudocacia*. Згідно з Codex Alimentarius, вміст вологи повинен бути нижчим 20%, а для аналізованих зразків вміст вологи коливався від 16,66–20,74%. Кислотність зразків акацієвого меду коливалася від 3,61 до 5,33. Аналізована вільна кислотність акацієвого меду коливалась від 0,32 до 4,14 мекв/кг. Жодна з аналізованих проб не перевищила встановлену межу. Усі зразки меду мали однакові значення яскравості (29,62–46,57). Максимальний вміст HMF у зразках становив 23,20 мг/кг, що відповідає вимогам якості. Значення менше 500 мкСм/см вказує на чистий квітковий мед, і в цьому дослідженні зразки акацієвого меду мали значення електропровідності 94,8–405 мкСм/см. У зразках акацієвого меду було виявлено 68,35% моносахаридів і незначний відсоток сахарози (максимум 2,093%). Співвідношення F/G коливалось від 1,02 до 1,65 для досліджуваних зразків акацієвого меду. Деякі зразки можуть швидко кристалізуватися, оскільки мають високий вміст глюкози, а співвідношення F/G становить приблизно 1. У зразках із значеннями F/G вище 1,3 тенденція кристалізації була нижчою. Глюконова кислота була основною органічною кислотою у всіх зразках (1,916–2,666 г/кг), меншим був вміст пропіонової та оцтової кислот. Янтарна кислота має найнижчу концентрацію у досліджених зразках меду.

Висновки. Досліджені зразки меду відповідали досліджуваним критеріям якості (вологість, pH, вільна кислотність, вміст HMF, колір і електропровідність), а високий відсоток пилкових зерен *Robinia pseudoacacia* підтвердив, що аналізовані зразки є монофлорним акацієвий медом.

Ключові слова: *мед, пилок, акація, Robinia pseudoacacia.*

Процеси і обладнання

Вплив конструктивно-технологічних параметрів обладнання на коефіцієнт бічного тиску та приведеного коефіцієнта тертя гранульованого полівінілхлориду

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Вступ. Визначено залежність коефіцієнта бічного тиску і приведеного коефіцієнта тертя гранульованого полівінілхлориду для виробництва пакувального матеріалу від конструктивно-технологічних параметрів процесу живлення.

Матеріали і методи. Експериментальні дослідження проведені на стенді, що імітує рух полімерних гранул у робочому каналі одночерв'ячного екструдера або шнекового живильника за таких параметрів: досліджуваний полімер – полівінілхлорид (ПВХ); осьовий тиск у шарі гранульованого полімеру – 0,044–0,475 МПа; температура сталевої обмежувальної поверхні – 20–80 °С; швидкість сталевої обмежувальної поверхні – 0,176–0,471 м/с; висота шару гранульованого полімеру – 0,015–0,025 м.

Результати і обговорення. Значення коефіцієнта бічного тиску збільшується за умов підвищення температури, зменшення осьового тиску, зменшення швидкості. Коефіцієнт бічного тиску від висоти шару гранул не залежить. За умови одночасного змінення температури та тиску до рівня приблизно 0,32 МПа, збільшення температури призводить до збільшення значень коефіцієнта бічного тиску, після нього – навпаки, до зменшення. В разі зміни швидкості після точки 0,15 МПа залежність також змінюється на протилежну.

Найбільше на зростання коефіцієнта бічного тиску досліджених полімерів впливає одночасна дія температури та швидкості сталевої обмежувальної поверхні (лінійної швидкості обертового робочого органу обладнання). Головну роль у зміні коефіцієнта бічного тиску відіграє швидкість, а у зміні коефіцієнта тертя – температура.

У разі одночасного зі швидкістю та висотою шару гранул збільшення температури відбувається перехід через точку на рівні приблизно 0,32 МПа, до якої збільшення температури призводить до збільшення відповідних значень коефіцієнта бічного тиску, після якої – навпаки, до зменшення. Так само в разі зміни швидкості – після точки 0,15 МПа залежність змінюється на протилежну. При одночасному зі швидкістю збільшенні температури від 20 до 80 °С більшим значенням швидкості відповідають менші значення коефіцієнтів бічного тиску, а збільшення температури призводить до збільшення коефіцієнтів бічного тиску від 0,33–0,34 до 0,42–0,46.

При одночасній зі швидкістю дії тиску менші значення тиску не впливають за залежність значень коефіцієнта бічного тиску від швидкості, а збільшення тиску від 0,044 до 0,476 МПа призводить до відповідної зміні на зростаючу залежність – від 0,22–0,27 до 0,34–0,46.

За умови зміни температури, швидкості та висоти шару гранул збільшення температури призводить до відповідного збільшення значень коефіцієнта тертя за однакового тиску від 0,48–0,5 до 0,52–0,57, так само, як і збільшення швидкості, а висота шару гранул майже не впливає на відповідну зміну коефіцієнта тертя, окрім високих значень тиску. Останнє можна пояснити тим, що підвищення тиску приводить до ущільнення гранул та їх руху як суцільного тіла, коли знижується вплив внутрішнього тертя.

Висновки. Отримані результати дають змогу врахувати взаємний вплив коефіцієнтів тертя й бічного тиску гранульованих полімерів і конструктивно-технологічних параметрів екструдера або шнекового живильника.

Ключові слова: *полімер, гранула, сипкий, бічний тиск, тертя.*

Біотехнологія, мікробіололія

Вплив катіонів на активність НАДФ$^+$-залежної глутаматдегідрогеназиу *Acinetobacter calcoaceticus* IMB B-7241, *Rhodococcus erythropolis* IMB Ac-5017 і *Nocardia vaccinii* IMB B-7405, вирощених на промислових відходах

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Вступ. Досліджено активність НАДФ$^+$-залежної глутаматдегідрогеназиза наявності одно- і двовалентних катіонів (потенційних активаторів цього ключового ферменту біосинтезу поверхнево-активних аміноліпідів) у *Acinetobacter calcoaceticus* IMB B-7241, *Rhodococcus erythropolis* IMB Ac-5017 і *Nocardia vaccinii* IMB B-7405 під час культивування на відходах виробництва біодизелю і відпрацьованій соняшниковій олії.

Матеріали і методи. Культивування штамів здійснювали у рідких мінеральних середовищах з використанням як субстратів рафінованої та відпрацьованої після смаження картоплі соняшникової олії, очищеного гліцерину та відходів виробництва біодизелю. НАДФ$^+$-залежну (КФ 1.4.1.4) глутаматдегідрогеназну активність у безклітинних екстрактах аналізували за утворенням глутамату під час окиснення НАДФН при 340 нм. Одновалентні (Na^+, K^+) та двовалентні (Mg^{2+}, Ca^{2+}, Zn^{2+}) катіони у вигляді солей NaCl, KCl, $MgSO_4 \cdot 7H_2O$, $CaCl_2$і$ZnSO_4 \cdot 7H_2O$ вносили у реакційну суміш, а також у середовище для культивування штамів.

Результати і обговорення. Встановлено, що катіони кальцію є активаторами НАДФ$^+$-залежної глутаматдегідрогеназної активності у *R. erythropolis* IMB Ac-5017 і *N. vaccinii* IMB B-7405, вирощених на рафінованій і відпрацьованій соняшниковій олії: за наявності 1−5 мМ Ca^{2+} в реакційній суміші активність ферменту підвищувалася в 1,3−2 рази порівняно з такою без цих катіонів. Підвищення концентрації $CaCl_2$ до 0,2−0,4 г/л в олієвмісних середовищах культивування штамів IMB Ac-5017 і IMB B-7405 супроводжувалося збільшенням НАДФ$^+$-залежної глутаматдегідрогеназної активності в 1,3−1,5 раза порівняно з такою на базовому середовищі. У разі додаткового внесення $CaCl_2$ (0,1−0,2 г/л) у середовище з очищеним гліцерином для культивування *A. calcoaceticus* IMB B-7241 спостерігали підвищення НАДФ$^+$-залежної глутаматдегідрогеназної активності майже в 2,5−3 рази порівняно з активністю під час вирощування штаму IMB B-7241 на базовому середовищі. Не виявлено активуючого впливу катіонів магнію, цинку, калію і натрію на НАДФ$^+$-залежну глутаматдегідрогеназну активність усіх штамів, вирощених на олієвмісних субстратах і гліцерині різного ступеня очищення.

Висновок. Результати дослідження підтвенрдили можливість збільшення активності ключових ферментів біосинтезу цільового продукту в результаті модифікації складу поживного середовища шляхом зміни в ньому вмісту активаторів цих ферментів.

Ключові слова: *глутаматдегідрогеназа, активатор, відходи, ПАР.*

Instructions for authors

Dear colleagues!

The Editorial Board of scientific periodical
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Requirements to all texts:
Language – English.
Recommended size of the article – 15–20 pages.
Font – Times New Roman, font size – 14, line intervals – 1, margins on both sides – 2 cm.

The structure of the article:

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All figures should be made in graphic editor, the font size 14.

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Figures and EXCEL format files with graphs additionally should be submitted in separate files.

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Шановні колеги!

Редакційна колегія наукового періодичного видання «**Ukrainian Food Journal**» запрошує Вас до публікації результатів наукових досліджень.

Вимоги до оформлення статей

Мова статей – англійська.

Мінімальний обсяг статті – **10 сторінок** формату А4 (без врахування анотацій і списку літератури)**.**

Для всіх елементів статті шрифт – **Times New Roman**, кегль – **14**, інтервал – 1.

Всі поля сторінки – по 2 см.

Структура статті:

1**.** УДК**.**
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4. *Установа, в якій виконана робота*.
5. Анотація. **Обов'язкова** структура анотації:
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   - Матеріали та методи (до 5 рядків)
   - Результати та обговорення (пів сторінки)**.**
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6. Ключові слова (3–5 слів, але не словосполучень).

Пункти 2–6 виконати англійською і українською мовами.

7. Основний текст статті. Має включати такі обов'язкові розділи:
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За необхідності можна додавати інші розділи та розбивати їх на підрозділи.

8. Авторська довідка (Прізвище, ім'я та по батькові, вчений ступінь та звання, місце роботи, електронна адреса або телефон).
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Рисунки виконуються якісно. Скановані рисунки не приймаються. Розмір тексту на рисунках повинен бути **співрозмірним** (!) тексту статті. **Фотографії можна використовувати лише за їх значної наукової цінності.**

Фон графіків, діаграм – лише білий. Колір елементів рисунку (лінії, сітка, текст) – чорний (не сірий).

Рисунки та графіки EXCEL з графіками додатково подаються в окремих файлах.

Скорочені назви фізичних величин в тексті та на графіках позначаються латинськими літерами відповідно до системи СІ.

У списку літератури повинні переважати англомовні статті та монографії, які опубліковані після 2010 року.

Правила оформлення списку літератури

В Ukrainian Food Journalвзято за основу загальноприйняте в світі спрощене оформлення списку літератури згідно стандарту Garvard. Всі елементи посилання розділяються **лише комами**.

1. Посилання на статтю:

Автори А.А. (рік видання), Назва статті, Назва журналу (курсивом), Том (номер), сторінки.

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1. Приклад:
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2. Посилання на книгу:

Автори (рік), Назва книги (курсивом), Видавництво, Місто.

Ініціали пишуться після прізвища.

Всі елементи посилання розділяються комами.

Приклад:

2. Wen-Ching Yang (2003), *Handbook of fluidization and fluid-particle systems*, Marcel Dekker, New York.

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Приклади:

1. (2013), *Svitovi naukovometrychni bazy*, Available at: http://www1.nas.gov.ua/publications/q_a /Pages/scopus.aspx
2. Cheung T. (2011), *World's 50 most delicious drinks [Text]*, Available at: http://travel.cnn.com/explorations/drink/worlds–50-most-delicious-drinks–883542

Список літератури оформлюється лише латиницею. Елементи списку українською та російською мовою потрібно транслітерувати. Для транслітерації з українською мови використовується паспортний стандарт.

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УДК 663/664

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Наукове видання

Ukrainian Food Journal

Volume 10, Issue 1
2021

Том 10, № 1
2021

Підп. до друку 31.03.2021 р. Формат 70х100/16.
Обл.-вид. арк. 15.21. Ум. друк. арк. 15.01.
Гарнітура Times New Roman. Друк офсетний.
Наклад 100 прим. Вид. № 02н/21.

НУХТ. 01601 Київ–33, вул. Володимирська, 68

Свідоцтво про державну реєстрацію
друкованого засобу масової інформації
КВ 18964–7754Р
видане 26 березня 2012 року.