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Національний університет
харчових технологій
вул. Володимирська, 68
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e-mail: ufj_nuft@meta.ua

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Editorial

The Ukrainian Food Journal is published in challenging times to present new research in food science to the world scientific community and contribute to the solving of human nutrition issues.

Russian terrorists are destroying the chain of production and transportation of grain products in Ukraine. They are mining fruitful fields, collapsing the agricultural equipment and plundering harvests. Russian missile and drone attacks are constantly destroying grain storage facilities and port infrastructure along the Black Sea and Danube coasts. Russian military blew up the Kakhovka hydroelectric power station, and cargo transportation along the Dnipro including harvested grain now is impossible. Many food industry enterprises were damaged or totally destroyed.

In these conditions, Ukrainian scientists continue to work on research related to solving pressing problems of food production. Ukraine appreciates the support of the civilized world. We also appreciate the support of the scientific community and thanks to the support of the Global Harmonization Initiative Association, the updated Editorial Board of the Ukrainian Food Journal has been operating for a year now, the quality of publications and the influence of the Journal are increasing. We gratefully accept the constructive remarks and constantly work on improving of the Journal.

We wish our respected authors, members of the editorial board and readers new creative achievements, and sincerely thank for support and attention to Ukrainian Food Journal.

We all stand together!

Editor-in-Chief,
Dr. *Olena Stabnikova*

Food industry of Ukraine during the Russian invasion: losses, experience, adaptation

Nadia Levytska, Olga Kotsiubanska

National University of Food Technologies, Kyiv, Ukraine

Abstract

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Corresponding author:

Nadia Levytska
E-mail:
nadejda_ist@ukr.net

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Introduction. During the Russian invasion of Ukraine, numerous industrial facilities were ruined, while food production in the occupied territories was stopped. This led to multifaceted consequences both for the overall food security of the world and for the Ukrainian food industry, as well as for the historical traditions of this industry. Despite the efforts of enterprises to update production, many losses are irreversible.

Materials and methods. The methodology is based on the use of comparative research and contextual analysis.

Results and discussion. The Russian invasion of Ukraine is a tough challenge in the history of Europe in the 21st century. Although world politics is still hesitant to call it World War III, it is clear that this is a renewal of the Cold War and a great threat to the issue of global nuclear security, as well as global food security.

World trade is a single system with mutually influencing, complex relationships, and Ukraine occupies an important place in it. Government policies regarding food safety have always been an integral part of a given country's survival strategy. Historical parallels are crucial for assessing changes in food safety from the 20th century to the very beginning of the 21st – in accordance with new political maps, trade routes, and the development of modern technologies.

It is hard to estimate all the losses because of occupied territories and the fact that the war goes on and the situation is unstable.

Another crucial question is losses in the case of brands that are the face and proud of Ukrainian food industry. Their traditions, technologies, and sales markets were distinguished achievements during the years of Ukrainian Independence.

What is important to realize, this article is a short note only to show a long-term perspective of future historical research on the verge of industrial history, genocide history, and the history of post-colonial studies.

Conclusions. The question of losses during the Russian invasion in the Ukrainian food industry is an immediate part of common food safety in the world. Sustainable development of the food industry, uninterrupted supply of food products never should be a question of political or war manipulation.

Introduction

With the beginning of the Russian invasion, Ukraine's food industry faced a difficult challenge. A full count of losses is currently impossible as the war continues, and the constant bombings confirm this. Many enterprises were damaged, while others remained in the occupied territories. Usual chains of supply were interrupted, substantial quantities of finished goods and raw materials were lost, and the economy found itself in a complicated unsteady position.

Research and analysis of the current situation of losses, experience and adaptation in the Ukrainian food industry is an important issue from the standpoint of studying the modern history of Ukraine as a part of Europe.

Materials and methods

The methodology is based on the use of comparative research and contextual analysis.

Results and discussion

The question of food safety is the first important question that authorities have to deal with during every war. Famine, interrupted ways of supply, and ruined food facilities always were a part of the militant policy of the Aggressor State. History of the XX century, for the first and foremost history of both World Wars full of persuasive examples of that policy. Food rationing system, food dictation of „war“ communism, and several aspects of the lend-lease, and especially the food supply of Britain by the USA during the Second World War (WWII) were essential efforts to reassure the population and to gain control of the situation. Genocide as an immediate part of colonialism (Dirk, 2008) also used a measures of famine as a strict measure to break down a resistance.

This is the most important thing humanity has to realize, facing the threat of a new Cold War (Nehring, 2023) and recreation of the former bipolar world with the right of strength, not international law.

The question of food safety in Ukraine is immediately connected with the question of world food safety because of the place of the Ukrainian State in the world sales market of food and agricultural products.

The issue of the Black Sea grain deal first emerged during the meeting, held by UN Secretary-General António Guterres and President of Turkey Recep Tayyip Erdogan in April 2022. A series of diplomatic negotiations, including the broad visit of African leaders to Moscow made obvious that the ability of Ukraine to support usual volumes of export is crucial not only for the Ukrainian economy.

Walking back to the historical parallels with the Second World War, it is crucial to stress that parallels could not be full based on two main reasons. First reason is that during the WWII territories of Ukraine were completely occupied, and then taken back by the Soviet Union. Thus, safe or partly safe rear territories were only beyond the area of nowadays Ukrainian State.

The second important reason is that the planned economy, which switched off private initiative and business, made the window of opportunity extremely narrow. It is believed that a strong feature of a planned economy is the ability of the State to use all, without exceptions, measures, and sources to renovate required branches. However, the other side of that ability is an unevenness of industrial development, violation of human rights and freedoms, deformation of the agricultural sphere, and waves of famine, immediately connected with the transfer of sources from one direction to another. This approach is brightly exemplified by the famine of 1921–1923 and 1946–1947. In the first case, it is spoken not only about the consequences of military action,

but about action of special government activities, called «food dictation», strict monopoly of the state in mastering of food sources. In the second case, we are talking about Stalin's plan for industrial renewal after World War II. It should be noted that the assessment of this as a sure way of rapid recovery after the war is clearly related to the political worldview of the enemy "New Course". Given the increasingly high level, their assessment as an example of effective economic recovery after the war is more a matter of political discourse. Franklin Roosevelt's "New Deal" and Ludwig Erhard's social economy contained elements of control over state economic processes, but did not abolish or replace their basic laws, as happened in the case of the Soviet Union. Unlike the 40s of the XX century, Ukraine entered the full-scale war as a state with a market economy and developed private initiative. The strong features of private initiative are flexibility, adaptability, speed of response, which in the conditions of martial law is one of the key factors, because exclusively state regulation always has the inertia of a bureaucratic machine. Thus, the main task of the state is not its own recovery, but the creation of appropriate conditions and mechanisms, effective interaction with private business, prevention of criminalization typical of martial law and the emergence of shadow schemes.

As of March 30, 2022, Valeriy Heyets, the director of the Institute of Economics and Forecasting of the National Academy of Sciences of Ukraine, assessed the current situation as extremely negative. In the Kyiv, Kharkiv, Sumy, Chernihiv, Donetsk, Mykolaiv, and Kherson regions, it was practically impossible to conduct economic activities, and more than 72 thousand people could lose their jobs in the industry (Heyets, 2022).

Compared to other branches of industry, the unprofitability of the food industry ranks third. The total amount of damages is almost 1,040 million US\$. This category mainly includes warehouses with significant food stocks. Only the Kyiv region lost a fifth of its warehouse space due to the war, which is about 364,000 m², including office premises and other buildings on the territory of the complexes (Heyets, 2022).

20 % of bread factories in Ukraine were destroyed. The high-tech enterprise for the production of frozen bakery products near Vyshgorod, Kyiv region, is one of such sad examples. The plant had modern equipment and high hopes for increased capacity in the near future (Lysa, 2022).

The Feraks enterprise was founded in 2011 and produced sausage products, the production capacity of the enterprise is up to 35 tons per day. The company has installed new equipment from leading companies in Austria, Italy, Switzerland and Germany. Because of an enemy projectile hitting the meat processing enterprise "Feraks", which is located in the village of Gogoliv, Kyiv region, a fire broke out (Kyiv Regional Military Administration, 2022).

The Makariv Bakery specializes in the production of bakery and confectionery products. On March 7 2022, Russian troops carried out an airstrike on the territory of the state-owned Makariv Bakery.

Another loss was the Kherson branch of Danon, a highly efficient factory in Ukraine for the production of dairy and sour milk products of the modern category. According to Ivan Khanas, director of operations, the production of key products was transferred to another plant of the company, located in Kremenchug (Poltava region), as a result of which it was possible to resume production of the main products at the new location within three to five months. However, taking into account the limited capabilities of the current plant, the assortment was partially reduced, and in some cases, the packaging format was changed.

The confectionery factory Mondelēz started operations in Ukraine in 1994. It was located in the city of Trostyanets, Sumy region, where 900 people worked, but it was destroyed and looted by invaders (State Environmental Inspection of Ukraine, 2022).

No less vivid example is Artemsil, Ukraine's largest salt producer, located in the city of Soledar, Donetsk region. Due the ongoing war, Artemsil was forced to shut down its

production, which capacity in 2020 was 1.2 million tons of salt. Buildings and equipment were destroyed, the enterprise grew into ruins, and 2,500 local residents linked to the salt production were left without job (Prasad, 2023).

In addition to the destroyed plants and factories, there are a number of brands lost due to the occupation. Among them, there are Chumak, Tavria, and Bon Kherson.

Of all the above, it should be emphasized that the situation with Chumak is the most painful. The main production facilities of Chumak are located in the temporarily occupied Kakhovka, Kherson region. The manufacturer produced its products under the trademark Chumak. This company is one of the first brands of already independent Ukraine, which has its own traditions and production quality, successfully represented the country on the domestic and import markets. Chumak Company produces ketchup, sauces, tomato paste and pasta products. Since the beginning of the war, the domestic market of 72,000 tons of tomato sauces and pastes has collapsed by 40–45%, while the share of Chumak itself has decreased by 60–70%, since before the war the manufacturer occupied about 20% of the Ukrainian market. The company was forced to stop supplies to countries in Africa, the Middle East and Europe, while the share of exports fell from 25% to zero (Stuka, 2023).

Like many other manufacturers from occupied or frontline territories, the company is trying to find a way out through relocation. "Chumak" manufactures its products at competitors' facilities throughout Ukraine and even abroad. Some of the tomato pastes, sauces, and mayonnaises are produced in Lutsk, Kyiv, Zaporizhia, and Ternopil. Despite all efforts during the year of the war, the company's profit in 2022 on the domestic market decreased from 33 mln USD to 11 mln USD (Stuka, 2023).

Production at other people's facilities is an attempt not to lose the recognition of popular product brands in supermarkets and the employees who remained in the company. The company has 110 key employees left, out of the 1,200 that worked there before full-scale war broke out.

At the beginning of the war, there were also significant problems with the products of other manufacturers. There were no enough tomato paste, ketchups and sauces on store shelves. "Sometimes we had only one brand that response to the need," recalls Auchan Ukraine public relations manager Olena Orlova (Stuka, 2023).

One of the reasons is the shortage of tomato paste, which is the basis for the production of ketchups and sauces. It arose due to the occupation of the Kherson region, where tomatoes are grown for processing and making tomato paste. The Agrofusion companies cultivated tomato are also situated on the south of Ukraine in Mykolaiv and Kherson regions. During the invasion in the Mykolaiv region, three Agrofusion factories were destroyed (Stuka, 2023).

The Coca-Cola Beverages Ukraine plant in Velyka Dymerka near Brovary, Kiev region, suffered shelling and was under Russian occupation for almost a month. To provide more than 100,000 retail outlets with at least the company's main drink, Coca-Cola began to import it from the Czech Republic and Poland. On March 9, the power plant at the enterprise was destroyed because of shelling. In Ukraine, Coca-Cola has an enterprise where, before the war, 40 types of soft drinks were bottled: branded sodas, water, juice, iced tea, and energy drinks. Among the most famous are Coca Cola, Fanta, Schweppes, Sprite, Rich, BotaniQ, water Bonaqua. More than 5.7 billion liters of beverages come off the lines every year. The plant supplied them not only to the Ukrainian market, but also to Armenia and Moldova. Currently, the plant is resuming work; in particular, exports to Moldova have been resumed.

The destruction of Europe's largest poultry farm in Chornobaivka caused huge damage of over 20 mln USD and loss more than 4 million chickens (Ukrlandfarming, 2022).



Figure 1. Mondelēz after shelling (State Environmental Inspection of Ukraine, 2022)



Figure 2. Artemsil before and after shelling (Informator.ua, 2023)



Figure 3. Chornobaivka poultry farm lost four million chickens
(Suspilne media, 2022)

Tavria distillery is located in the temporarily occupied territories in Nova Kakhovka, Kherson region. Its portfolio includes such brands as Borysfen, Tavria Premium, Georhiivskiy, Jatone, and AleXX. Tavria's production capacity is 1,400 hectares of vineyard land in the Kherson region. During the season, the enterprise processed about 17,000 tons of grapes. The capacity of the plant's bottling line is 1.6 million liters of wine and cognac per year (Agronews, 2022).

AB InBev Efes is one of the leaders of the Ukrainian brewery market, a joint enterprise with the world's largest brewing company Anheuser-Busch InBev. InBev EFES produces the brands Chernihivske, Rogan, Yantar, Staropramen, Stella Artois on the factories located in Chernihiv, Kharkiv, and Mykolaiv and also imports brands Bud, Corona Extra, Stella Artois, Hoegaarden, Leffe InBev Efes products occupied 31.7% of the Ukrainian beer market (Agronews, 2022). Currently, many factories were forced to stop beer production due to bombing or shelling. The facilities of non-alcoholic beverages production Sandora were also partially damaged (Ministry of Infrastructure of Ukraine, 2023).

Another recognizable brand that is nowadays not in production is Mivina. The company is located in Kharkiv and its work is now suspended. Before the war, the assortment included more than 42 types of products. In addition to vermicelli, the factory produced soy sauces and seasonings. The products were supplied to all supermarket chains. More than 40% of products were exported to the countries of the European Union and the whole world. With the beginning of large-scale shelling, work stopped, products from warehouses were distributed to volunteers in agreement with the company's management.

According to the Minister of Agrarian Policy and Food of Ukraine, Mykola Solskyi, 470,000 hectares of agricultural land in Ukraine now require inspection and demining. So far, only 17.5% of the mined territories have been surveyed, of which 57,000 hectares are for agricultural purposes.

Even more questions are caused by the consequences of the environmental disaster from the explosion of the Kakhovka Hydroelectric Power Plant. Losses of cultivated areas, pollution, water supply problems, erosion of cattle burial grounds, soil and water pollution. Many settlements are still flooded. In many cases, the functioning of enterprises in the front-line and front-line territories is not possible.

On the left bank of the Kherson region, as a result of the explosion of the Kakhovka Hydroelectric Power Plant, 107 hydrotechnical structures of the State Agency of Land Reclamation and Fisheries of Ukraine were flooded and completely destroyed (Ministry of Agrarian Policy and Food, 2023).

The victim of the disaster was the only state sturgeon farm in Ukraine - Production-experimental Dnipro Sturgeon Breeding Plant named after Academician S.T. Artichoke (Pavlysh, 2023).

According to the Institute for Economic Research and Policy Consulting, the food industry is one of the few industries maintaining production compared to its critical decline in the metallurgical, chemical, and construction materials industries (Angel and Gulyk, 2022).

The analysis of the current state of the Ukrainian food industry and the conducted historical analogies allow us to draw the following conclusions. The adaptability and flexibility of the market system made it possible to avoid total starvation or limited use of food resources. In most territories, except for the front-line ones, it was possible to preserve the post-war production volumes and assortment.



Figure 4. Consequences of Kakhovka Dam destruction
(Ecopolitic.com.ua, 2023; Reuters.com, 2023)



Figure 5. Kherson sturgeon breeding plant
(Ministry of Agrarian Policy and Food, 2023)

At the same time, the situation with the front-line, occupied territories and territories that suffered the consequences of the explosion of the Kakhovka Hydroelectric Power Plant remains steadily difficult. A large part of the lost or temporarily non-functioning enterprises, preserving ancient production traditions, represented Ukrainian products on the world and domestic markets. Most enterprises try to correct the situation with the help of relocation. Moral motivation is also of great importance in the reconstruction and functioning of enterprises, food supply of territories. A significant number of entrepreneurs are involved in the volunteer movement, and there are a number of examples of direct support for the population of the frontline areas.

References

- Agronews. (2022), The list of products that disappeared from the shelves due to the war was announced, Available at: <https://agronews.ua/news/ozvucheno-perelik-produktiv-yaki-znykly-z-polycz-cherez-vijnu>
- Damaged in UA. (2023), Available at: <https://damaged.in.ua>
- Dirk M. (2008), *Empire, colony, genocide*, Berghann books, New York.
- Angel Ye., Gulyk A. (2022), How the Ukrainian industry dealing with war challenges, Available at: <https://zn.ua/ukr/promyshliennost/jak-ukrajinska-promislovist-dolaje-vojenni-vikliki.html>
- Heyets V. (2022), On the assessment of Ukraine's economic losses as a result of the armed aggression of the Russia. Transcript of the report at the meeting of the Presidium of the National Academy of Sciences of Ukraine on March 30, 2022, Available at: <http://dspace.nbuv.gov.ua/handle/123456789/185175>
- Karpov R. (2023), UNIAN How. Ukrainian enterprises survived in war, Available at: <http://businessua.com/produkti-harchuvannya/83042yak-vistoyali-ukrainski-harchovi-pidpriemstva-pid-chas-viini.html>
- Kyiv Regional Military Administration (2022), Available at: <https://t.me/s/kyivoda?before=2030>
- Lysa A. (2022), More than 20% of bread making facilities were destroyed in Ukraine, Available at: <https://landlord.ua/news/v-ukraini-blyzko-20-khlibozavodiv-zruinovani-abo-ne-vidnovlyi-vyrobnnytstvo/>
- Ministry of Agrarian Policy and Food. (2023), Available at: <https://minagro.gov.ua/news/107-gidrotehnychnih-sporud-derzhribagentstva-zatopleno-na-livoberezhzhi-hersonshchiny>
- Ministry of Infrastructure of Ukraine. (2023), Report on the direct damage to the infrastructure from the destruction caused by Russia's military aggression against Ukraine a year after the start of the full-scale invasion (March 2023).
- Nearing H. (2023), Reconstructing a nuclear peace, *Current History*, 122, pp. 118–120, <https://doi.org/10.1525/curh.2023.122.842.118>
- Pavlysh P. (2023), The only state sturgeon plant is completely flooded, Available at: <https://www.epravda.com.ua/news/2023/06/7/700917/>
- Petrenko I. (2021), Industry of Ukraine in 2016-2020. Statistical collection, State Statistics Service of Ukraine, Kyiv.
- Prasad A. (2023), Artem salt, Forbes, Available at: <https://forbes.ua/news/artem-sil-zibrala-585-mln-grn-vid-prodazhu-soli-mits-ukrainska-kamyana-na-droni-dlya-gur-27032023-12646>
- Reuters.com (2023), Available at: <https://www.reuters.com/authors/sergiy-chalyi>
- State Environmental Inspection of Ukraine. (2022), Information on the negative impact on the environment because of the actions of the Russian occupiers on the territory of PJSC "Mondelēz Ukraine", Available at: https://www.facebook.com/hashtag/%D1%88%D1%82%D0%B0%D0%B1%D0%B4%D0%B5%D1%96/?source=feed_text&epa=HASHTAG
- Stuka N. (2023), Chumaks way, Available <https://forbes.ua/company/chumatskiy-shlyakh-110-spirovbitnikiv-z-1200-padinnya-na-70-chastki-rinku-ta-vitorgu-yak-vizhivae-virobnik-ketchupiv-ta-konservatsii-chumak-z-kakhovki-09022023-11622>
- Stuka N. (2023), War caused deficit, Available at: <https://forbes.ua/inside/viyna-povertae-defitsit-chumak-chemigivske-coca-cola-i-pepsi-znikayut-z-polits-supermarketiv-yaki-produkti-shchev-zoni-riziku-04052022-5811>
- Ukrlandfarming. (2022), Press service of "Ukrlandfarming", Available at: <https://suspilne.media/238480-zbitki-vid-znisenna-vijskovimi-rf-ptahofabriki-cornobaviska-stanovlat-800-miljoniv-griven>
- Reuters.com (2023), Available at: <https://www.reuters.com/authors/sergiy-chalyi>

Physico-chemical and rheological properties of meat pates with corn starch suspensions prepared on electrochemically activated water

Andrii Marynin, Vladyslav Shpak, Vasyl Pasichnyi,
Roman Svyatnenko, Yevgenia Shubina

National University of Food Technologies, Kyiv, Ukraine

Abstract

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Corresponding author:

Andrii Marynin
E-mail:
andrii_marynin@
ukr.net

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Introduction. The aim of the study was to determine the influence of electrochemically activated water application on the rheological properties of corn starch suspensions and pates prepared with them.

Materials and methods. Corn starch suspensions were prepared on artesian water, which was activated by electrochemical treatment on a diaphragm electrolyzer. Physico-chemical characteristics of electrochemically activated water were determined using a Palintest 7500 photometer. Morphological studies of starch granules were carried out by scanning electron microscopy, and granulometric composition was determined by laser diffraction method. The rheological indicators of starch suspensions and pates were studied using a Kinexus Pro+ rheometer.

Results and discussion. The physico-chemical characteristics of electrochemically activated water except pH met the requirements of the European Parliament and Council Directive on the quality of water intended for human consumption. The water absorption capacity of starch when preparing its suspensions on a catholyte (obtained by passing a direct electric current through water in the cathode chamber of an electrolyzer) decreased by 26%, and when prepared on an anolyte (obtained in the process of water oxidation reactions on the anode) increased by 18%. The moisture holding capacity of hydrated starch decreased by 10% when using catholyte, and increased by 36% when using anolyte. Electrochemically activated water had a significant effect on the rheological characteristics of starch suspensions: with an increase in the percentage of complex shear strain, the shear stress increased proportionally for samples of suspensions made on electrochemically activated water. The viscoelastic properties of starch suspensions prepared using electrochemically activated water both at 25 °C and at 68 °C tended towards an ideally elastic gel, that is, they had more elastic structure than the control samples. Under the action of shear deformation, the elastic properties were lost, while the suspensions acquired viscosity (phase angle values increased). The maximum value of the moisture binding capacity of pates was observed when using a starch suspension prepared on anolyte. The best values of rheological characteristics of pates were obtained when using starch suspension with 2% starch prepared on anolyte.

Conclusions. Electrochemically activated water had a significant effect on the physicochemical and rheological properties of semi-finished products, in particular corn starch suspensions, and pates prepared with them, and contributed to better structuring of the studied food systems.

Introduction

Water is a part of any raw material used in food industry. It is often the main component of a food product and determines its properties. Electrochemical treatment of water to give it new valuable for preparation of food qualities belongs to new environmentally friendly technologies (Ivanov et al., 2021).

When a direct electric current is passing in the cathode chamber of the electrolyzer, water is saturated with products of electrochemical reactions such as hydroxide ions, hydrogen, metal hydroxides, which are formed from water-soluble salts, resulting in activated water, defined as a catholyte (Figure 1).

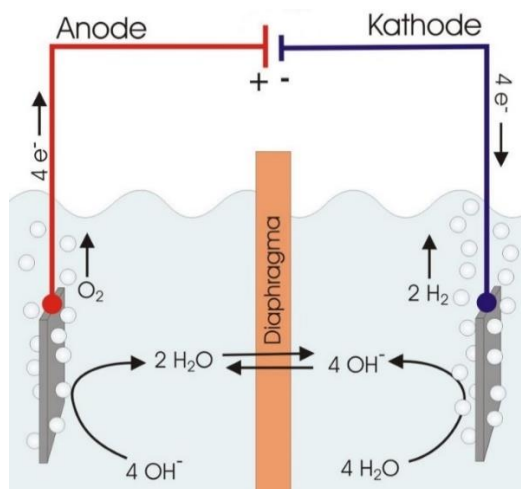


Figure 1. Scheme for the process of electrochemical activation of water

At the anode, water is oxidized with subsequent release of oxygen and chlorine gas, and the resulting liquid is called anolyte (Jiang et al., 2021). The catholyte, which consists of negatively charged OH^- ions and H_2 molecular hydrogen formed in the electrolysis process, received a negative potential. Anolyte is obtained in the process of electrolysis near the positive electrode, the anode, where oxidants are accumulated, and as a result a positive oxidation-reduction potential (ORP) is formed. These are primarily positively charged ions (cations) of hydrogen H^+ , as well as hypochlorous acid HClO , oxygen O_2 , and hydrogen peroxide H_2O_2 (Wulan and Notodarmojo, 2020).

Redox reactions are the basis of life on Earth (Scheibe et al., 2011). Water is responsible for redox processes, as the main solvent (in the chemical sense) and as the main structural element of the body (in the biological sense). The redox potential of the internal medium of a healthy person is always below zero and ranged from -90 to -225 mV (Matsiyevska, 2017), but for industrially purified artesian water – from +100 to +400 mV. Such differences in the ORP of water means that the activity of electrons in the internal medium of the human body is much higher than the activity of electrons in artesian water. To use water rationally in exchange processes of the body, it must be conditioned according to ORP values, which are achieved by applying electrochemical activation technologies (Goncharuk et al., 2010).

In food products, water is used not only for adding to the recipe in its pure form, but also for the preparation of semi-finished products, such as starch suspensions. Starch is a part of the most food products; in addition, it is widely applied as an emulsifier. Starch could be in native or modified forms. Native starch is obtained in the process of wet grinding of grain, and modified starches are obtained from native starch by chemical or physical methods. Starch in its native form has certain limitations compared to modified starch: the lower the hydration level, lower thermal stability, lower shear resistance, and higher retrogradation rate (Dru et al., 2018; Kaur et al., 2019).

During the preparation of food products, starches are subjected to mechanical action, as a result of which their rheological characteristics change, which have a significant impact on the quality of the finished product, in particular on its structural and mechanical properties, such as consistency, water-retaining and moisture-binding capacity (Montes et al., 2022).

To modify corn starch, its suspension is treated with heat, which means heating the suspension before the start of gelatinization. As an environmentally friendly modification method, this method attracts much attention because it involves only water and heat without the use of chemical reagents (Guo et al., 2020). Compared to native starch, heat-treated starch is better for use in the recipes of hard-to-digest foods, namely meat and pasta products, and canned food (Chung et al., 2009).

Meat pates are popular, widely demanded products, the formulation of which includes corn starch to regulate their structural and mechanical properties (Song and Jane, 2000).

Electrochemical water treatment is used to convert water into a metastable excited state. This technology is one of the most accessible methods, which is based on a unipolar electrochemical effect on water (Li et al., 2023).

The use of electrochemically activated water (anolyte or catholyte) to prepare suspension of thermally modified starch can change its rheological properties, which will allow it to regulate the quality of food products.

The aim of the present study was to determine the influence of electrochemically activated water on the rheological characteristics of corn starch suspensions and pates prepared with them.

Materials and methods

Obtaining electrochemically activated water

Electrochemically activated water was obtained using diaphragm electrolyzer by electrochemical treatment of artesian water with initial characteristics of pH 6 and ORP +224 mV. After setting the operating mode of the electrolyzer, two experimental water samples were obtained: catholyte with ORP -542±20, pH 10 and anolyte with ORP +767±15, pH 3. The required amount of water consumption was set at the level of 18 l/h.

Determination of physico-chemical characteristics of electrochemically activated water

Samples of waste were taken from containers that were previously washed with the same water to minimize the error. Physical and chemical parameters were determined using the Palintest 7500 photometer. Test tubes with a volume of 10 ml were filled with a water sample and Palintest water test tablets were added for each type of element: Mg, Fe, Mn, Cl, F, Al, K, NO₂, NO₃, PO₄, and SiO₂. After that, it was left at room temperature for a certain

time to fully develop color. Results were recorded on the photometer display (Al-Mahrabi et al., 2021).

Determination of pH. Determination of pH of water was carried out using an I-160MI ionometer by potentiometric method of measurement. Water was added to a clean, dry glass up to the mark of 40 cm³, the electrodes were immersed in it, and after 10-15 s the indicators were taken on the scale of the device (Lahav et al., 2001).

Determination of redox potential. Redox potential was measured using an Oakton ORP Tester 10 (Cole-Parmer, UK). The principle of the method was based on measuring the potential difference on two electrodes (Pt-electrode and a reference electrode with a double Ag/AgCl connection). Resolution was ±1 mV with accuracy ±2 mV. The electrode was placed in a fixed position with the sample above the bottom of the container. The output signal was established within 5 minutes (Tantra et al., 2012).

Determination of electrical conductivity. The electrical conductivity of water was measured by inducing a small alternating current through a precise volume of the studied liquid. Readings were recorded with a measuring device (McCleskey et al., 2011).

Preparation of starch suspensions

Corn starch produced by AS GROUP, LLC (Ukraine) was used for the research.

Suspensions were prepared in the ratio of corn starch: water as 1:10 at water temperature $t = 23 \pm 2$ °C (the temperature at which starch modification began) and kept for 2 hours to ensure starch hydration. The control was a sample of starch suspension on artesian water.

Water absorption capacity of starch

To determine the water absorption capacity (WAC), 0.5 g of starch was dispersed in 50 ml of distilled water and left for 30 min at room temperature in pre-weighed centrifuge tubes. The obtained suspension was centrifuged for 30 min at 3500 rpm. The upper layer with water was removed and dried for 25 min at a temperature of 50 °C to remove excess moisture. Then it was re-weighed.

WAC was expressed in %, which was determined by the formula:

$$\text{WAC} = [(W2 - W1)/W_s] \cdot 100,$$

where $W1$ is the weight of the test tube with a dry sample;

$W2$ is the weight of the sample with the tube after centrifugation;

W_s is the weight of the sample.

Moisture retaining capacity of starch

To determine the moisture retaining capacity (MRC), 0.5 g of starch was dispersed in 50 ml of distilled water and left for 30 minutes at room temperature in a heat-resistant glass. The suspension was subjected to heat treatment with constant stirring for 30 min to a temperature in the center of 95 ± 2 °C, cooled to a temperature of 10 ± 2 °C, placed in pre-weighed centrifuge tubes and centrifuged for 30 min at 3500 rpm. The upper layer with water was removed and dried for 25 min at a temperature of 50 °C to remove excess moisture and re-weighed.

MRC was expressed in %, which was determined by the formula:

$$\text{MRC} = [(W_2 - W_1)/W_s] \cdot 100,$$

where W_1 is the weight of the test tube with a dry sample;

W_2 is the weight of the sample with the tube after centrifugation;

W_s is the weight of the sample.

Determination of morphology and granulometric composition of starch grains

Morphology and surface relief of starch granules were studied by scanning electron microscopy using a Tescan MIRA 3 LMU microscope (Niamat et al., 2019). The sample was applied to a conductive carbon substrate and coated with a 30 nm layer of gold using a PECS 682 precision sputtering system. When measuring, an area with a field of view of 1.5 mm was selected.

The study of the dispersed composition of starch powder was carried out using a laser particle size analyzer (Bettersizer 2600; Dandong Baxter Instrument Co., Ltd., China) in the dry dispersion mode, using the laser diffraction method (Changgao et al., 2022).

Determination of rheological indicators of starch suspensions

Determination of the rheological indicators of corn starch suspensions was carried out on a Kinexus Pro+ rheometer. Starch Paddle Plastic 2 Blade geometry was used – a coaxial cylinder with a paddle stirrer (PC34 SL0007 SS) mounted on a vertical shaft. Suspensions were analyzed at the temperature at which the modification of starch began – 25 °C, and at the temperature of gelatinization of corn starch – 68 °C. The suspension was placed in a cylinder to a height of 70 mm; the stirrer was lowered and brought to a temperature of 25 °C. The complex shear stress (σ), shear viscosity (η), phase angle (σ) was determined depending on the change in the complex shear strain with its gradual increase (0 – 100%). Each step was maintained until a steady state was reached in a minimum time. Similarly, the experiment was conducted after heating the suspension to 68 °C (Alvarez et al., 2015).

Preparation of meat pates

Onions and carrots were peeled and sent for grinding; the liver was washed with cold water and cut into pieces, blanched at a temperature of 98-100 °C for 15-30 minutes, cooled; wheat flour bread was soaked in milk; chicken eggs were washed, the starch suspension was prepared in a starch:water ratio of 1:10.

The prepared raw materials were loaded into a mixer and crushed until a homogeneous mass was obtained. Pates were packaged in glass jars, which were loaded into equipment for pasteurization. After that, they were cooled and labeled.

Determination of rheological characteristics of meat pates

Determination of the rheological characteristics of pates was carried out on a Kinexus Pro+ rheometer (Malvern Instruments Ltd., Great Britain). The geometry used was a circular plate with a diameter of 40 mm (PU40 SR5040 SS: PL61 ST), fixed on a vertical shaft. The prepared sample was placed on the lower platform; the shaft with the plate was lowered to a gap of 1 mm. The complex shear stress (σ), shear viscosity (η), phase angle (σ) was

determined depending on the change in the complex shear strain with its gradual increase (0 – 100%). Each step was maintained until a steady state was reached in a minimum time (Marynin et al., 2023).

Determination of the moisture binding capacity of meat pate

To determine the moisture binding capacity of pates, the moisture content in the product was previously determined. Moisture content was determined by drying 5 g sample to constant weight at a temperature of 105 °C. The moisture binding capacity relative to the moisture content (MBC_m) and to the weight of the sample (MBC_s) was determined by pressing 0.30 g of minced meat and calculating the ratio of the area of the wet spot to the weight of the minced meat or moisture in the sample (Strashynskiy et al., 2016).

The size of the wet spot (external) was calculated by the difference between the total area of the spot and the area of the spot formed by minced meat. It was experimentally established that one cm² of the area of a wet filter spot corresponded to 8.4 mg of moisture.

The mass fraction of bound moisture in the samples was calculated by the formulas:

$$\begin{aligned} \text{MBC}_s &= (M - 8.4S) \cdot 100/m_0 \\ \text{MBC}_m &= (M - 8.4S) \cdot 100/M \end{aligned}$$

where M is the total weight of moisture in the sample, mg;

S is the wet spot area, mg;

m₀ is the weight of minced meat, mg.

Statistical analysis

All experiments were performed in three replicates. Results were shown as mean ± standard deviation. Statistical analysis was performed using the XLstat software (version 2020).

Results and discussion

Physico-chemical characteristics of electrochemically activated water

Physico-chemical characteristics of water, as a component of food products, were important from the point of view of its safety and quality. The determined characteristics were compared with norms for artesian water (The Drinking Water Directive, 2020) (Table 1).

Electrical conductivity was determined as a measure of electrolytic properties of water. In the catholyte, the value was higher by 19.1% than in the control, in the anolyte it was lower by 17.0%. These data correlated with water hardness values: in the unfiltered catholyte obtained after passing artesian water through the electrolyzer, both hardness and electrical conductivity were higher than for the control. This is explained by the presence of Ca²⁺ and Mg²⁺ salt ions, which have the ability to conduct an electric current. The level of total hardness of the water samples correlated with the obtained data on the content of calcium and magnesium, which, together with other minerals were present in both artesian and electrochemically activated water (Table 2).

Table 1

Physico-chemical characteristics of water

Characteristics	Type of water		
	Artesian	Catholyte	Anolyte
pH	7.34±0.1	10.15±0.1	3.16±0.1
Total hardness, mmol/l	2.42±0.1	Unfiltered – 4.02±0.1 Filtered – 2.12±0.1	2.06±0.1
Total alkalinity, mmol/l	4.6±0.1	4.7±0.1	4.0±0.1
Electrical conductivity, µS/cm	470±1.1	560±10	390±10
RP, mV	246±10	-562±20	767±15

Table 2

Content of mineral substances in water

Mineral substances	Content of mineral substances, mg/l, in water		
	Artesian	Catholyte	Anolyte
Calcium (Ca)	56.40±0.1	Unfiltered – 113.60±0.1 Filtered – 37.60±0.1	45.60±0.1
Magnesium (Mg)	24.0±0.1	28.0±0.1	22.0±0.1
Total iron (Fe)	0.02±0.0001	Not found	0.01±0.00001
Manganese (Mn)	0.001±0.00001	Not found	0.002±0.00001
Chlorine (Cl)	17.5±0.1	1.7±0.001	15.5±0.1
Fluorine (F)	0.24±0.001	0.19±0.0001	0.38±0.0001
Aluminum (Al)	0.16±0.001	0.097±0.0001	0.16±0.0001
Potassium (K)	3.2±0.01	6.8±0.1	2.3±0.01

The content of total iron in the electrochemically activated water decreased: in the anolyte by 50%, and it was not detected in the catholyte. Since the content of Ca²⁺ and Mg²⁺ salt ions in the anolyte was lower than in the control, its electrical conductivity was also lower (Table 1).

The content of oxides in the studied water samples is given in Table 3.

Table 3

The content of oxides in water

Oxides	The content of oxides in water, mg/l		
	Artesian	Catholyte	Anolyte
Nitrites (NO ₂ ⁻)	0.02±0.0001	0.017±0.0001	0.007±0.0001
Nitrates (NO ₃ ⁻)	2.79±0.01	1.16±0.01	3.34±0.01
Phosphates (PO ₄ ³⁻)	0.09±0.0001	0.05±0.0001	0.15±0.0001
Silicon (SiO ₂ ⁻)	12.25±0.1	13.0±0.1	16.0±0.1

The content of nitrites in water after its electrochemical treatment decreased significantly, namely by 15% in catholyte and 65% in anolyte compared to artesian water. The content of sulfates in the anolyte was lower by 33.3%, and no sulfates were detected in the catholyte. Chloride content in catholyte was significantly lower than in artesian water – by 90.3%, and in anolyte by 11.4%. This was because inorganic compounds, including toxic ones (nitrites and sulfates), underwent cathodic and anodic oxidative destruction, and strong inorganic oxidants, including chlorine, were inactivated during chemical reactions (Lu and Zhang, 2022).

Electrochemically activated water with acidic (anolyte) and reducing (catholyte) properties will have a positive effect on the rheological indicators of the semi-finished products with it. When using water for the preparation of starch suspensions and their subsequent use in the formulation of food products, it was important to determine the morphological features and relief of the surface of starch grains.

In the study of starch by scanning electron microscopy, lamellar large and rounded small grains with a porous surface were found in the form of granules of various shapes and sizes (Figure 2a), which was confirmed by the distribution of particles obtained by laser diffraction (Figure 2b).

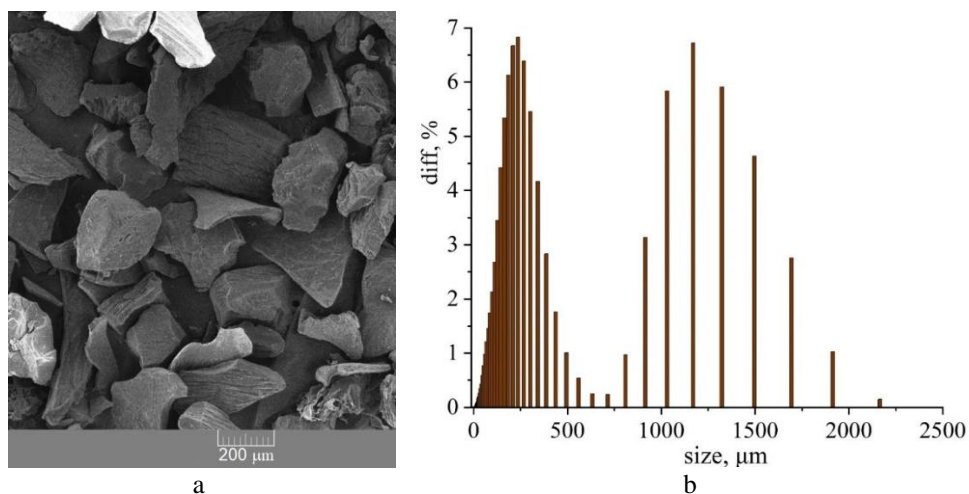


Figure 2. Scanning electron microscopy of corn starch granules and particle size distribution

To characterize the size distribution of particles, the values D10, D50 and D90 were used, which were equivalent volume diameters at 10%, 50% and 90% of the total volume, respectively.

The analysis of the obtained results of particle size distribution indicated the polydispersity of the system and the wide distance between the D10 and D90 points (Table 4).

Table 4

Particle size distribution

Sample	D10, μm	D50, μm	D90, μm	Specific surface area SSA, m^2/kg
Corn starch	102 \pm 5	270 \pm 5	1364 \pm 8	11.22 \pm 1

Note: Each value is the mean of triplicate measurements \pm SD.

Depending on the type of water used, hydrated starch will have different functional and technological properties. So, when using activated water for the preparation of starch suspensions in the case of catholyte, the water-absorbing capacity of starch decreases by 26%, and when prepared on anolyte, it increases by 18%. Therefore, when using anolyte, the amount of added water in the formulation for the manufacture of products with starch suspensions should be increased (Figure 3).

When using catholyte to obtain a suspension of starch, the water-holding capacity of starch is reduced by 10%, and anolyte is increased by 36%. Such changes are explained by different acidity of aqueous media, and the salts present in catholyte prevent its absorption of catholyte by starch granules (Han et al., 2009).

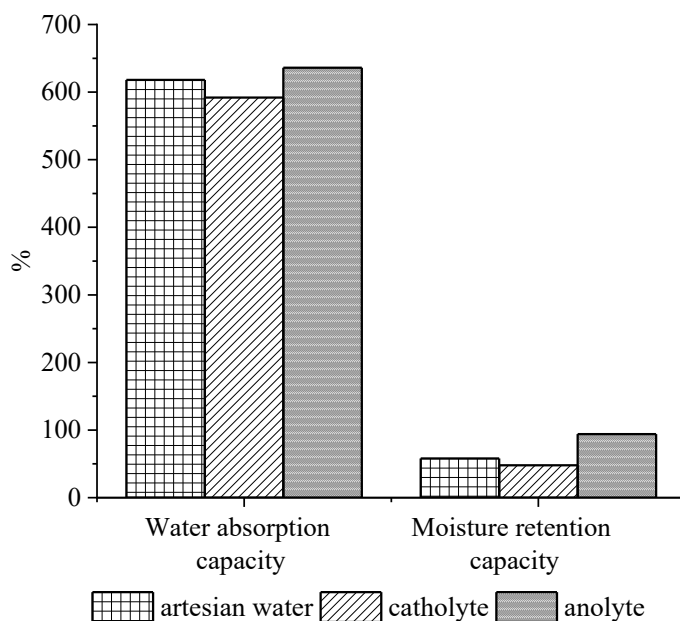


Figure 3. Functional and technological properties of hydrated starch depending on the water used

The use of electrochemically activated water can be effective in controlling the structure-forming and thickening properties of starch. During the technological processes of manufacturing food products, mechanical work is applied (transactions of mixing, molding), as a result of which the structure of starch grains is deformed. The effect of electrochemically activated water (catholyte and anolyte) on the change in the rheological characteristics of corn starch suspensions at different temperatures was determined.

The dependence of the change in the complex shear stress (σ) of corn starch suspensions on the complex shear strain (Y) is shown in Figure 4. It was found that with an increase in the percentage of shear strain, the shear stress increased proportionally for all samples. Moreover, for the suspension of corn starch on the catholyte at 25 °C, this change was the most rapid (0–2.5 Pa), somewhat less on the anolyte (0–2 Pa), and in the control with artesian water – 0–1 Pa.

When the temperature of gelatinization (68°C) was reached, the change in the complex shear stress were much lower, but the trend of the change remained: the least rapid increase in the complex shear stress was observed for the control – 0–0.4 Pa, for suspensions on the catholyte and anolyte, this indicator was slightly higher, however practically coincided in the entire range of action of shear deformation – 0–0.6 Pa and 0–0.7 Pa, respectively.

The change in the viscous properties of the starch suspension estimated by the index of complex shear viscosity during deformation is shown in Figure 5.

It was established that the complex shear viscosity of all samples decreased in the entire range of deformation influences. The initial values for the samples on the catholyte and anolyte at 25 °C and at 68 °C were higher than the corresponding values of these parameters for the control sample. Moreover, this tendency was observed in the entire range of deformations, because viscous properties were manifested to a greater extent in the case of preparation of starch suspension on electrochemically activated water. This was explained by the fact that the nature of the contact of hydrophilic groups changed, namely, the contact zone between swollen starch granules decreased, which provided more opportunities for starch to bind water molecules (Chen et al., 2020; Chen et al., 2018).

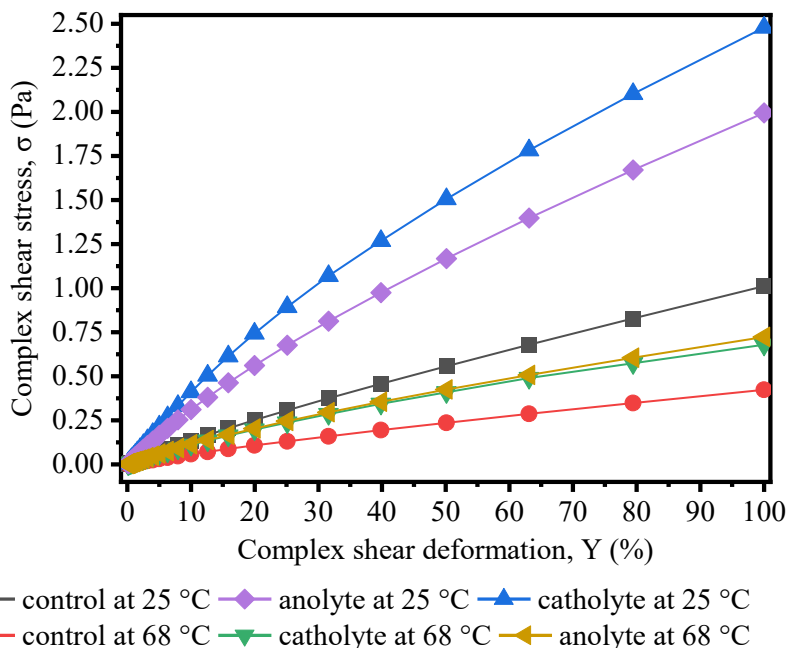


Figure 4. Dependence of the change in the complex shear stress (σ) of corn starch suspensions at temperatures of 25 °C and 68 °C, prepared with electrochemically activated water, on the complex shear strain (Y)

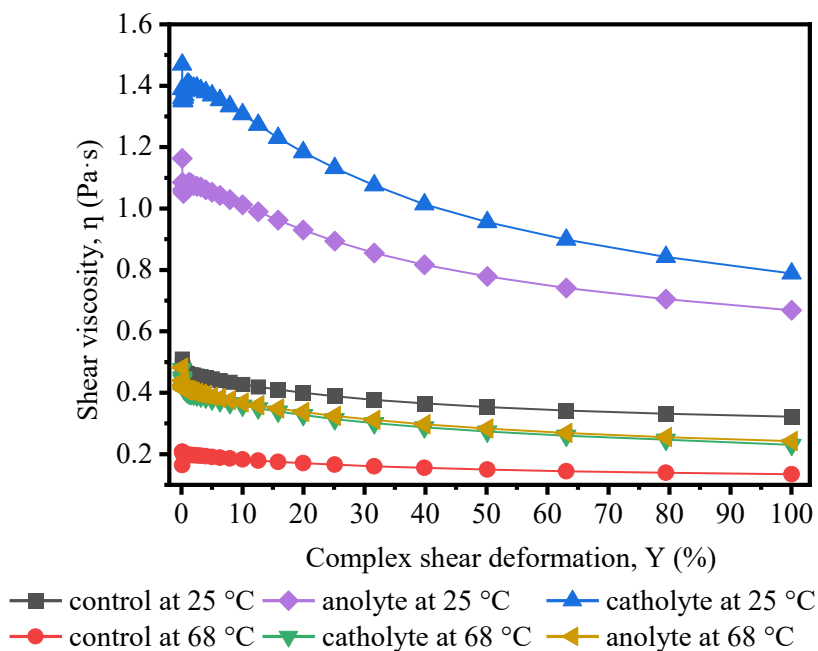


Figure 5. Dependence of shear viscosity of corn starch suspensions at temperatures of 25 °C and 68 °C, prepared with electrochemically activated water, on the complex shear strain (Y)

To characterize the effect of electrochemically activated water on the viscoelastic properties of starch suspensions, the change in the phase angle was determined, which is the angle between the light incident on the object and the reflected light (Figure 6).

The phase angle of the suspension on the catholyte at 25 °C is lower than on the anolyte and with artesian water: 29.13°, 34.28° and 51.29°, respectively, in the absence of deformation. At the temperature of pasteurization, the sample with artesian water also had the highest value - 55.46°, but the values of the phase angle of the samples on the catholyte and anolyte coincided - 39.05°, and this trend was observed in the entire range of deformation influence. The values of the phase angle of the suspensions on the catholyte and anolyte both at 25 °C and at 68 °C tended to 0 (ideally elastic gel), that is, they had more elastic structure than the control samples. At the same time, with the effect of shear deformation, the elastic properties were lost, while the suspensions acquired viscosity (phase angle values increased).

Changes in functional and technological and rheological indicators of starch suspensions when using electrochemically activated water should affect the properties of pates with their content, in particular, functional and rheological indicators.

Determination of the moisture binding capacity of the pates in relation to the weight of the sample (MBCs) and the weight of moisture in the sample (MBCm) showed that the anolyte had greater influence on the studied indicators (Table 5).

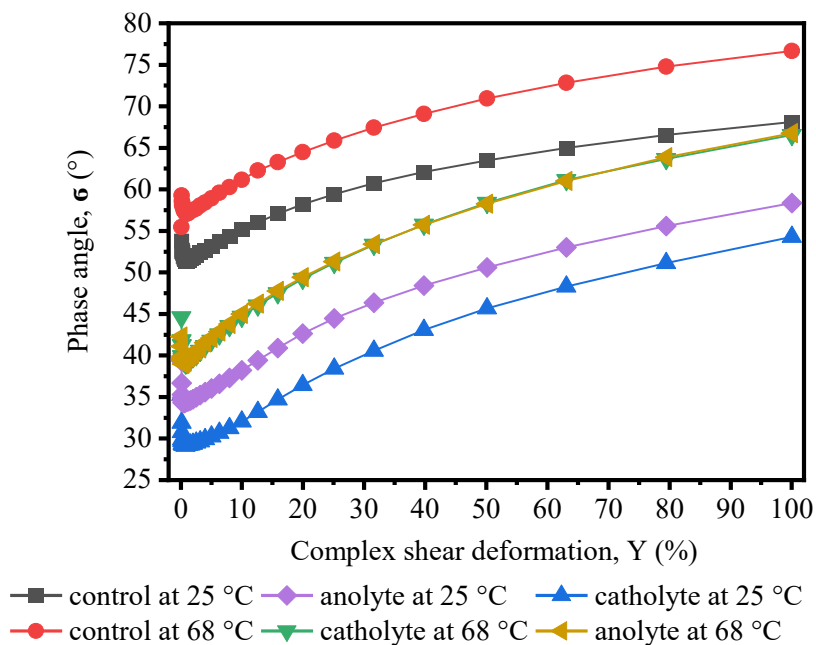


Figure 6. Dependence of the phase angle during the study of suspensions of corn starch at temperatures of 25 °C and 68 °C, prepared with electrochemically activated water, on the complex shear deformation

Table 5

Moisture binding capacity (MBC) of the pates

Moisture binding capacity	Type of water used		
	Artesian	Catholyte	Anolyte
2% corn			
MBCs before pasteurization, %	67.61	70.02	100
MBCs after pasteurization, %	66.95	68.66	100
MBCs after 21 days after pasteurization, %	58.65	62.28	93.79
MBCm before pasteurization, %	96.29	96.12	100
MBCm after pasteurization, %	96.59	94.33	100
MBCm after 21 days after pasteurization, %	90.95	91.45	96.47
5% corn			
MBCs before pasteurization, %	70.59	87.57	86.25
MBCs after pasteurization, %	86.36	90.00	73.71
MBCs after 21 days after pasteurization, %	70.88	83.10	71.27
MBCm before pasteurization, %	73.83	88.80	96.70
MBCm after pasteurization, %	98.76	90.00	96.85
MBCm after 21 days after pasteurization, %	94.90	94.67	96.06

With the content of 2% starch in the suspension on the anolyte and catholyte, the MBCs of the pates increased by 32.39% and 2.41% before pasteurization, compared to the control, and by 33.05% and 1.71% after pasteurization. When using anolyte, this indicator reached a maximum value of 100%. Electrochemically activated water helped to increase the hydrophilic properties of starch granules and slowed down their reduction over time (Akua et al., 2022). An increase in the proportion of starch helped to increase the moisture binding capacity of the pates in relation to the weight of the sample.

In the samples of pates with 2% starch in the suspension on the anolyte, the moisture binding capacity of the pate in relation to the mass of moisture in the sample increased by 3.71% before pasteurization, compared to the control, the catholyte almost did not affect the value of this indicator. After pasteurization of pate on the anolyte, the moisture binding capacity in relation to the mass of moisture in the sample reached 100%, on the catholyte it decreased by 2.26% compared to the control.

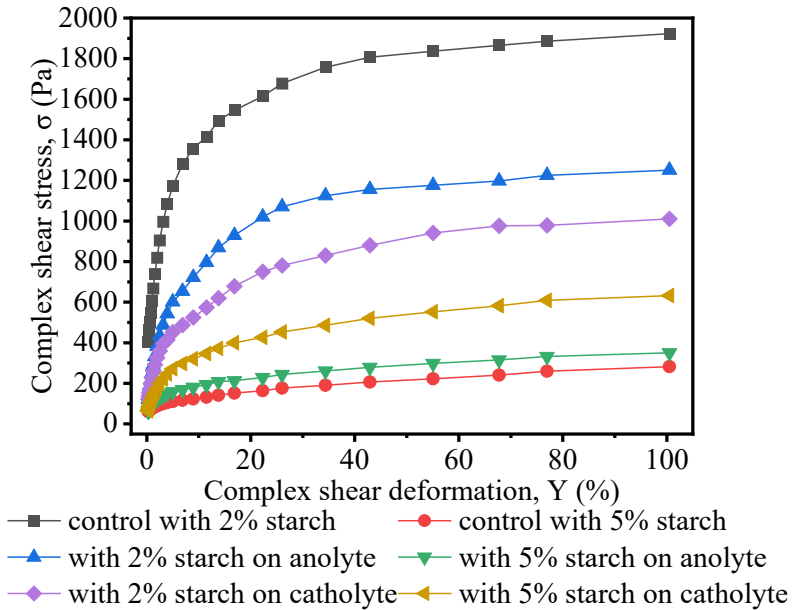
In the process of storage, MBCs and MBCm decreased in all samples. However, in pates with electrochemically activated water, the percentage of reduction was lower, compared to the control, which indicated better moisture binding and preservation of the texture of the products.

When starch suspension was used in the production of pates, the food system was partially distorted. Studies of the dependence of the change in the complex shear stress of pates with a suspension of corn starch on the complex shear deformation showed that with an increase in the percentage of deformation, the shear stress increased proportionally for all samples (Figure 7). Moreover, for samples with the suspension with 2% starch, this change was the most rapid in the control – 33–1623 Pa, somewhat less in the anolyte – 21–1156 Pa, and in the catholyte – 18–946 Pa.

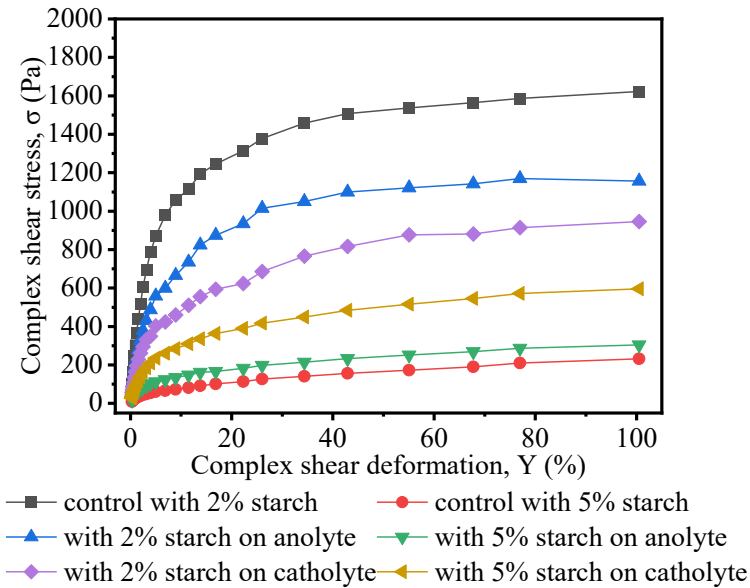
With an increase in the concentration of corn starch in the suspension, the values of the complex shear stress of the pates was significantly lower and the trend changed: the least rapid increase in the complex shear stress was observed for the control – 3–232 Pa, for pates with suspensions on the anolyte and catholyte this indicator was slightly higher – 5–305 Pa and 8–596 Pa respectively. The nature of the curves of these samples tended to samples of pure starch. However, the values of shear stress in the same strain range for pate samples were much larger: in the range of 3–1623 Pa compared to 0–2.5 Pa for samples of starch suspensions. This was explained by a change in the structure of the studied systems, namely an increase in viscosity and density (Amann-Winkel et al., 2013). In the process of storage, the complex shear stress decreased in all samples, which was explained by the fact that the pate structure became less strong over time and broke down faster at smaller deformations.

The change in the viscous properties of pates was determined by the index of shear viscosity during the deformation process (Figure 8).

Shear viscosity of all samples decreased in the entire range of deformation influences. The initial values for samples of pates with 2% starch in suspension were higher than the corresponding values of these parameters for pates with 5% starch in suspension: on artesian water, anolyte and catholyte – 5290 Pa·s, 3316 Pa·s and 2925 Pa·s, respectively compared to 472 Pa·s, 878 Pa·s and 1409 Pa·s for samples with 5%. In samples with 2% starch, the control had the highest values of shear viscosity in the entire range of deformations. Moreover, the systems did not stabilize until the end of the deformation influences both with suspensions on artesian and electrochemically activated water. This was due to the fact that with a low starch content, suspensions couldn't provide a stable pate viscosity, despite their strength (Montes et al., 2022). However, in this case, electrochemically activated water affected to a greater extent the nature of the contact of hydrophilic groups, which provided more opportunities for starch to bind water molecules.



a



b

Figure 7. The dependence of the change in the complex shear stress of pates with different concentrations of corn starch in the suspension prepared with electrochemically activated water on the complex shear strain:

a – after pasteurization; *b* – after 21 days after pasteurization

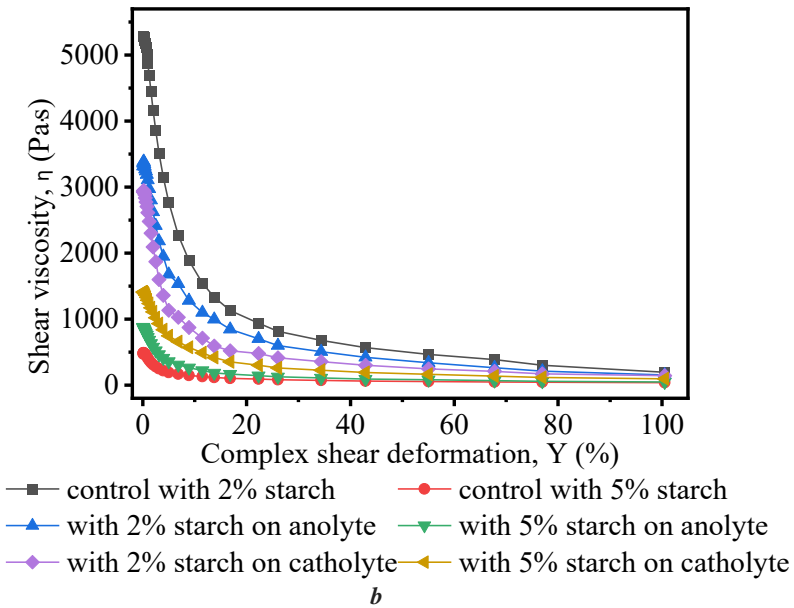
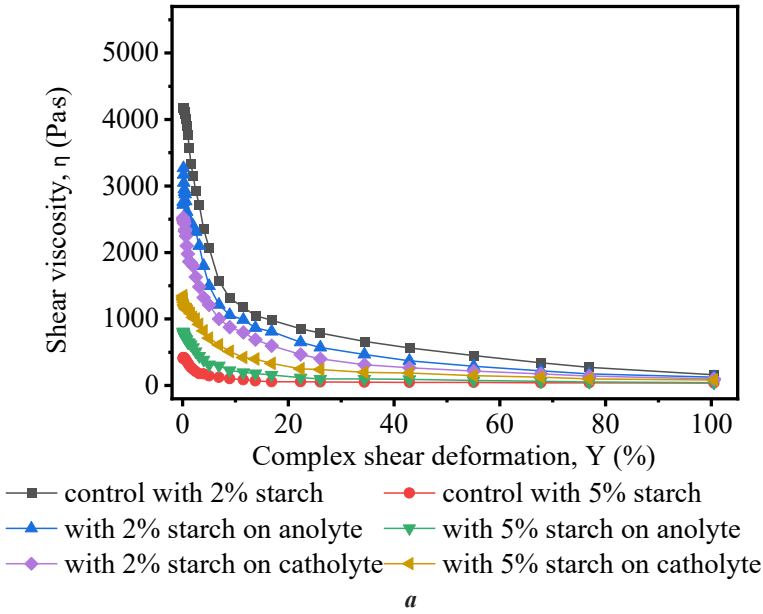


Figure 8. The dependence of the change in the shear viscosity of pates with different concentrations of corn starch in the suspension prepared with electrochemically activated water on the complex shear strain:
a – after pasteurization; *b*– after 21 days after pasteurization

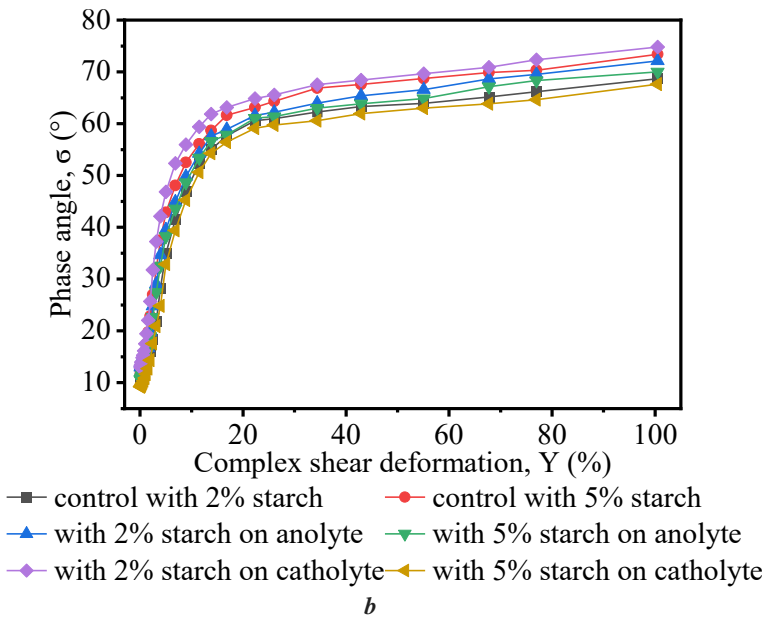
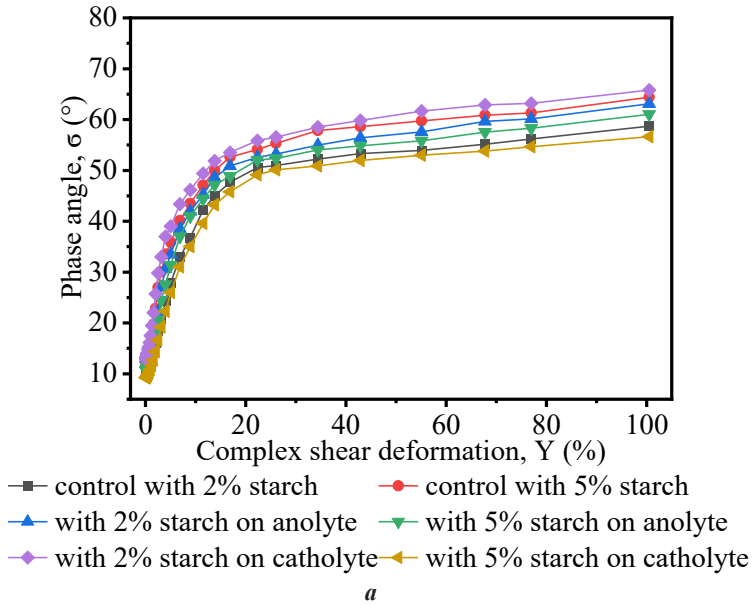


Figure 8. The dependence of the change in the phase angle during pates investigation with different concentrations of corn starch in the suspension prepared with electrochemically activated water on the complex shear strain:

a – after pasteurization; *b*– after 21 days after pasteurization

In samples of pates with a suspension with 5% starch, the trend was opposite: the lowest values of shear viscosity over the entire range of deformation were in the control sample, slightly higher with suspensions on the anolyte and catholyte. However, the change of the investigated parameter was not as rapid as for samples with 2% starch. This was explained by the lower strength of the system (Boonkor et al., 2022). Pates acquired a stable viscosity after 80% deformation, both with artesian and with electrochemically activated water. In the process of storage, the shear viscosity increased in all samples, which correlated with the change values of this indicator depending on the change in the shear rate and was explained by the decrease in the strength of the pate system over time.

To characterize the effect of electrochemically activated water on the viscoelastic properties of pates and their internal structure, the change in the phase angle was determined, which was the angle between the light falling on the object and the light reflected from it (Figure 9). The curves of changes in the phase angle of pate samples were completely different in nature from the curves of pure suspensions, which indicated the influence of the formulation components on this indicator and the influence of electrochemically activated water on the structure of the pates.

The internal structure of the pate samples changed under the influence of electrochemically activated water on the starch suspension included in their composition, as evidenced by the phase angle parameters, because the changes were followed (that is, the phase angle/shear deformation curves of different dough samples did not match) (Cristiano et al., 2019).

In the absence of deformation, the phase angle of all samples was practically the same and was about 10°. Pates with a suspension with 5% starch prepared on catholyte and anolyte had a lower phase angle than the control sample in the entire range of deformations and tended to 0, that is, they provided a more elastic structure to the pates than the control sample. However, under the action of shear deformation, the elastic properties were lost, while the suspensions acquired viscosity (phase angle values increased) (Maurice, 2019).

Samples of pates with 2% starch prepared on catholyte and anolyte had a higher phase angle than the control sample, which indicated that this amount of starch when interacting with electrochemically activated water due to the much higher strength of the starch system was not able to ensure the elasticity of the minced meat system and helped to increase their viscosity. In the process of storage, the phase angle increased for all samples, which was explained by the increase in the viscosity of the pates and the loss of elasticity at smaller deformations.

Conclusions

1. Physico-chemical indicators of the quality of electrochemically activated water, except pH, met the requirements of regulatory documentation for potable artesian water.
2. When starch suspensions were prepared on the catholyte, the water absorption capacity of starch was slightly reduced by 26%, and when prepared on the anolyte, it increased by 18%. The values of moisture retention capacity changed similarly: in the case of catholyte, it decreased by 10%, and in the case of anolyte, it increased by 36%.
3. Electrochemically activated water had a significant effect on the rheological indicators of starch suspensions. As the percentage of complex shear strain increased, the shear stress increased proportionally for all samples on electrochemically activated water. Moreover, for the suspension on the catholyte at 25 °C, this change

was the most rapid (0–2.5 Pa), somewhat less on the anolyte (0–2 Pa), with artesian water (control) – 0–1 Pa. The shear viscosity of all samples decreased in the entire range of deformation influences. The effect of electrochemically activated water on the viscoelastic properties of starch suspensions showed that they tended to 0 (perfectly elastic gel) at 25 °C and at 68 °C, that is, they had more elastic structure than the control samples. At the same time, with the effect of shear deformation, the elastic properties were lost, while the suspensions acquired viscosity (phase angle values increased).

4. The moisture binding capacity of pates was maximum when preparing starch suspension on the anolyte. The best values of the rheological indicators of pates were obtained when the starch suspension prepared on the anolyte with 2% corn starch was added to the recipe.

References

- Akua Y., Boakye O., Bertoft E., Annor G. (2022), Temperature of plasma-activated water and its effect on the thermal and chemical surface properties of cereal and tuber starches, *Current Research in Food Science*, 5, pp. 1668–1675, <https://doi.org/10.1016/j.crfs.2022.09.020>
- Al-Mahrabi F.A.M., Abdelrahim A.M.Y., Abdullah M.D. (2021), Determination of fluoride in drinking water in Al-Hussein district, Al-Dalea Governorate, Yemen by using palintest photometer 7500, *Humanitarian & Natural Sciences Journal*, 2(10), <https://doi.org/10.53796/hnsj21012>
- Alvarez M.D., Fuentes R., Canet W. (2015), Effects of pressure, temperature, treatment time, and storage on rheological, textural, and structural properties of heat-induced chickpea gels, *Foods*, 4(2), pp. 80–114, <https://doi.org/10.3390/foods4020080>.
- Amann-Winkel K., Gainaru C., Handle P.H., Loerting T. (2013), Water's second glass transition, *PNAS*, 110(44), pp. 17720–17725, <https://doi.org/10.1073/pnas.1311718110>
- Boonkor P., Sagis L.M.C., Lumdubwong N. (2022), Pasting and rheological properties of starch paste/gels in a sugar-acid system, *Foods*, 11, 4060, <https://doi.org/10.3390/foods11244060>
- Changgao S., Olkhovikov O., Xiaojin G., Marynin A., Sichen Z., Shevchenko A., Botong S., Yue Z. (2022), Research and comparative analysis of the qualitative parameters of food powders produced from grain raw materials using an improved jet mill, *Technology Audit and Production Reserves*, 6(3(68)), pp. 36–43, <https://doi.org/10.15587/2706-5448.2022.271557>
- Chen D., Fang F., Federici E., Campanella O., Jones O.G. (2020), Rheology, microstructure and phase behavior of potato starch-protein fibril mixed gel, *Carbohydrate Polymers*, 239, 116247. <https://doi.org/10.1016/j.carbpol.2020.116247>
- Chen L., Tian Y., Bai Y., Wang J., Jiao A., Jin Z. (2018), Effect of frying on the pasting and rheological properties of normal maize starch, *Food Hydrocolloids*, 77, pp. 85–95, <https://doi.org/10.1016/j.foodhyd.2017.09.024>
- Chung H., Liu Q., Hoover R. (2009), Impact of annealing and heat-moisture treatment on rapidly digestible, slowly digestible and resistant starch levels in native and gelatinized corn, pea and lentil starches, *Carbohydrate Polymers*, 75, pp. 436–47, <https://doi.org/10.1016/j.carbpol.2008.08.006>
- Copelli D., Cavecchi A., Merusi C., Leardi R. (2018), Multivariate evaluation of the effect of the particle size distribution of an active pharmaceutical ingredient on the

- performance of a pharmaceutical drug product: a real-case study, *Chemometrics and Intelligent Laboratory Systems*, 178, pp. 1–10, <https://doi.org/10.1016/j.chemolab.2018.04.013>
- Cristiano M.C., Froiio F., Costanzo N., Poerio A., Lugli M., Fresta M., Britti D. Paolino D. (2019), Effects of flour mean particle size, size distribution and water content on rheological properties of wheat flour doughs, *European Food Research and Technology*, 245, pp. 2053–2062, <https://doi.org/10.1007/s00217-019-03315-y>
- Directive (EU) 2020/2184 of the European parliament and of the council on the quality of water intended for human consumption (2020), <https://eur-lex.europa.eu/eli/dir/2020/2184/oj>
- Dru B., Agnieszka O., Worobiej E., Ostrowska-lig E. (2018), Effect of hydrothermal modifications on properties and digestibility of grass pea starch, *International Journal of Biological Macromolecules*, 118, pp. 2113–2120, <https://doi.org/10.1016/j.ijbiomac.2018.07.063>
- Goncharuk V.V., Bagrii V.A., Mel'nik L. A., Chebotareva R.D., Bashtan S.Y. (2010), The use of redox potential in water treatment processes, *Journal of Water Chemistry and Technology*, 32(1), pp. 1–9, <https://doi.org/10.3103/s1063455x10010017>
- Guo B., Wang Y., Pang M., Wu J., Hu X., Huang Z., Wang H., Xu S., Luo S., Liu C. (2020), Annealing treatment of amylose and amylopectin extracted from rice starch, *International Journal of Biological Macromolecules*, 164, pp. 3496–3500, <https://doi.org/10.1016/j.ijbiomac.2020.08.245>
- Han Z., Zeng X., Zhang B., Yu S. (2009), Effects of pulsed electric fields (PEF) treatment on the properties of corn starch, *Journal of Food Engineering*, 93(3), pp. 318–323, <https://doi.org/10.1016/j.jfoodeng.2009.01.040>
- Ivanov V., Shevchenko O., Marynin A., Stabnikov V., Gubenia O., Stabnikova O., Shevchenko A., Gavva O., Saliuk A. (2021), Trends and expected benefits of the breaking edge food technologies in 2021–2030, *Ukrainian Food Journal*, 10(1), pp. 7–36, <https://doi.org/10.24263/2304-974X-2021-10-1-3>
- Jiang H., Wang L., Gao B., Li Y., Guo Y., Zhuo M., Sun K., Lu B., Jia M., Yu X., Wang H., Li Y. (2021), The anolyte matters: Towards highly efficient electrochemical CO₂ reduction, *Chemical Engineering Journal*, 422, 129923, <https://doi.org/10.1016/j.cej.2021.129923>
- Niamat M., Sarfraz S., Shehab E., Ismail S. O., Khalid Q. S. (2019), Experimental characterization of electrical discharge machining of aluminum 6061 t6 alloy using different dielectrics, *Arabian Journal for Science and Engineering*, <https://doi.org/10.1007/s13369-019-03987-4>
- Kaur M., Singh S. (2019), Influence of heat-moisture treatment (HMT) on physicochemical and functional properties of starches from different Indian oat (*Avena sativa L.*) cultivars, *International Journal of Biological Macromolecules*, 122, pp. 312–319, <https://doi.org/10.1016/j.ijbiomac.2018.10.197>
- Lahav O., Morgan B., Loewenthal R.E. (2001), Measurement of pH, alkalinity and acidity in ultra-soft waters, *Water S.A.*, 27(4), pp. 423–431, <https://doi.org/10.4314/wsa.v27i4.4954>
- Li D., Liu R., Tao Y., Shi Y., Wang P., Han Y. (2023), Enhancement of the carboxymethylation of corn starch via induced electric field, *Carbohydrate Polymers*, 121137, <https://doi.org/10.1016/j.carbpol.2023.121137>
- Lu S., Zhang G. (2022), Recent advances on inactivation of waterborne pathogenic microorganisms by (photo) electrochemical oxidation processes: Design and

- application strategies, *Journal of Hazardous Materials*, 431, 128619, <https://doi.org/10.1016/j.jhazmat.2022.128619>
- Marynin, A., Pasichnyi, V., Shpak, V., Svyatnenko, R. (2023). Influence of electrochemically activated water on the physical properties and rheological indicators of meat pates, *Technology Audit and Production Reserves*, 2(3(70)), pp. 41–46, <https://doi.org/10.15587/2706-5448.2023.278113>
- Matsiyevska O. (2017), Influence of redox potential of different water quality on the human blood, *Technology audit and production reserves*, 33, pp. 34–38, <https://doi.org/10.15587/2312-8372.2017.93633>
- Maurice P. (2019), *Structure and properties of water in its various states. encyclopedia of water (science, technology, and society)*, John Wiley & Sons, pp. 1–19, <https://doi.org/10.1002/9781119300762.wsts0002>.
- Mcclesley R.B., Nordstrom D.K., Ryan J.N. (2011), Electrical conductivity of natural waters, *Applied Geochemistry*, 26, pp. 227–229, <https://doi.org/10.1016/j.apgeochem.2011.03.110>
- Montes L., Rosell C. M., Moreira R. (2022), Rheological properties of corn starch gels with the addition of hydroxypropyl methylcellulose of different viscosities, *Frontiers in Nutrition*, 9, <https://doi.org/10.3389/fnut.2022.866789>
- Scheibe R., Dietz K. (2011), Reduction–oxidation network for flexible adjustment of cellular metabolism in photoautotrophic cells, *Plant, Cell & Environment*, 35(2), pp. 202–216, <https://doi.org/10.1111/j.1365-3040.2011.02319.x>
- Song Y., Jane J. (2000), Characterization of barley starches of waxy, normal, and high amylose varieties, *Carbohydrate Polymers*, 41, pp. 365–377, [https://doi.org/10.1016/S0144-8617\(99\)00098-3](https://doi.org/10.1016/S0144-8617(99)00098-3)
- Strashynskiy I., Fursik O., Pasichniy V., Marynin A., Goncharov G. (2016), Influence of functional food composition on the properties of meat mince systems, *Eastern-European Journal of Enterprise Technologies*, 6(11(84)), pp. 53–58, <https://doi.org/10.15587/1729-4061.2016.86957>
- Tantra R., Cackett A., Peck R., Gohil D., Snowden J. (2012), Measurement of redox potential in nanoecotoxicological investigations, *Journal of Toxicology*, 270651, <https://doi.org/10.1155/2012/270651>
- Wulan D.R., Notodarmojo S. (2020), Effect of catholyte concentration on current production during chocolate industry wastewater treatment by a microbial fuel cell, *Makara Journal of Technology*, 24(2), pp. 53–58, <https://doi.org/10.7454/mst.v24i2.418>

Bioactive profile of carob (*Ceratonia siliqua* L.) cultivated in European and North Africa agrifood sectors

Tatiana Capcanari, Eugenia Covaliov, Aurica Chirsanova, Violina Popovici, Oxana Radu, Rodica Siminiuc

Technical University of Moldova, Chisinau, Republic of Moldova

Abstract

Keywords:

Carob bean
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activity

Introduction. Studies of the physico-chemical composition of the carob beans and the pulp of the pods originating from four countries are present.

Materials and methods. The physicochemical properties of carob (*Ceratonia siliqua* L.) cultivated in different countries, Moldova, Algeria, Italia and Spain, were characterized in terms of mineral (Ca, Mg, and Fe), carotenoids (β -carotene, lycopene, and zeaxanthin) and chlorophyll (a chlorophyll and b chlorophyll) content. The antioxidant activity of biologically active compounds was determined using simulated gastrointestinal digestion.

Results and discussion. The samples of Moldovan carob compared to those grown in Algeria, Spain, and Italy contain higher amounts of biologically active compounds, some positions far exceeding those of carob from the mentioned regions. Thus, the mineral content in terms of Ca, Mg and Fe in Moldovan carob samples was 1.1–1.7 times higher. The same trends were recorded for the content of carotenoids in Moldovan carob beans: β -carotene, 13.610 mg/100 g of dry matter (DM); lycopene, 19.882 mg/100 g DM, and zeaxanthin, 20.709 mg/100 g DM, which were much higher in comparison with samples from Algeria, Spain, and Italy. The differences concerning the amounts of biologically active compounds between Moldovan and other regions of carob beans were significant. Samples from Italy were distinguished by the highest content of chlorophyll and it was up to 1.1 mg/100g DM. The evolution of the antioxidant activity of biologically active compounds, which was done via gastrointestinal digestion, confirmed the functional profile of carob pods and beans. Thus, the DPPH (2,2-Diphenyl-1-picrylhydrazyl) antioxidant activity of bioactive compounds in carobs from different regions of the world, during gastric digestion simulation, increased from 38–48% to 60–74%.

Conclusions. The studied four carob bean and pod samples originating from different world regions were similar by their bioactive potential. Nevertheless, it was found that Moldovan carob is the best in terms of the content of minerals, β -carotene, lycopene, zeaxanthin, and antioxidant activity.

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Corresponding author:

Tatiana Capcanari
E-mail:
tatiana.capcanari@
toap.utm.md

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Introduction

Carob (*Ceratonia siliqua* L.) is a tree that can grow in varied latitudes, from those with temperate climates to tropical ones (FAOSTAT, 2022) (Figure 1).

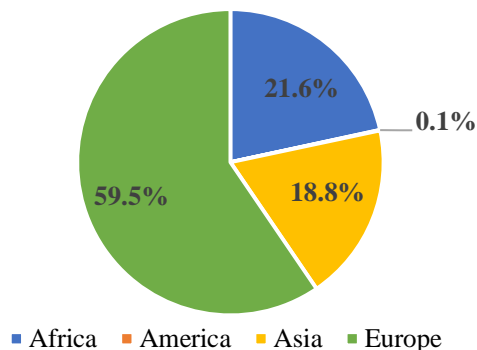


Figure 1. Production share of carob beans by region, sum 1994 – 2021 (FAOSTAT, 2022)

According to the 2022 data of Food and Agriculture Organization, Portugal takes the first place for carob tree cultivation. The second largest producer in the world is Italy, although Morocco, Turkey, and Greece also produce carob on a large scale (FAOSTAT, 2022). Among European countries with a Mediterranean climate, Romania, the Republic of Moldova, and Ukraine are geographically advantageous in terms of favorable climatic conditions for carob cultivation (Capcanari et al., 2022). Unfortunately, according to the same report carob production is decreasing around the world (Figure 2).

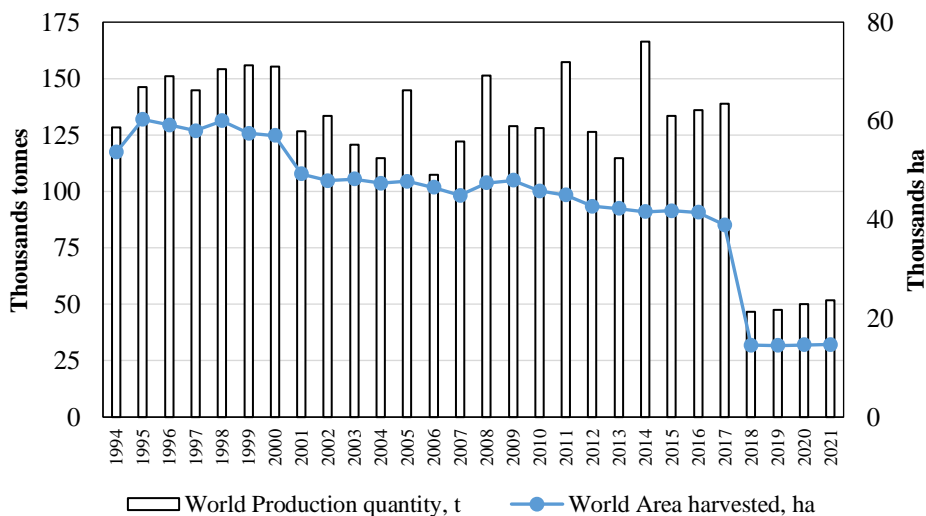


Figure 2. Area harvested/ Production quantity of carob beans in the World, 1994 – 2021 (FAOSTAT, 2022)

This fact is probably caused by several factors, among which those ones can be mentioned: (1) ignorance of nutritional and biological potential of fruits; (2) reduced individual consumption; (3) low fruit prices (FAOSTAT, 2022; Şahin et al., 2016).

It was reported that carob fruits (pod pulp and beans) contain biological active substances, such as polyphenols, vitamins, and minerals (Bouzdoudi et al., 2012; Santonocito et al., 2020; Vekiari et al., 2012). Carob beans could be considered as a valuable source of such minerals as K (850.8–1169.3 mg/100 g dry matter (DM), Ca (237.1–350.6 mg/100 g DM), and Mg (45.30–137.70 mg/100 g DM) (Fidan et al., 2015). According to Fidan and Sapundzhieva (2015), the content of mineral elements varies from one grade to another, respecting the following series: K > Ca > Mg > P > Fe > Zn > Mn. Large variations between results can be caused by environmental factors including climate, soil, and growing region.

Due to its chemical composition, the carob has many nutraceutical uses. The antioxidant activity of carob is primarily attributed to polyphenolic constituents, including ellagitannins present mainly in carob pod pulp (Chait et al., 2020). However, most of the existing research is devoted to the chemical composition, technological properties, transformation processes and culinary processing of carob beans, while there is much less studies on the pulp of the pods. Currently, the beans from the carob pods are transformed into flour that closely resembles cocoa powder, naturally sweet, aromatic and ideal in sweet dishes, being one of the natural and healthy additives (unfortunately too little used), in bakery products, ice cream, salad dressings and other food products (Loullis et al., 2018; Stabnikova and Paredes-Lopez, 2023).

It was shown that carob powder has certain advantages over cocoa because it contains a lower amount of fat and significantly higher amounts of dietary fiber. The lower fat content adds fewer calories, while the high dietary fiber content with its unique composition along with the polyphenolic compounds offers numerous health benefits. The key to the potential of carob in substituting cocoa is the cocoa-like aroma and flavor. Overall, the nutritional and economic advantages that carob presents make it a great candidate for the substitution of cocoa (Gunel et al., 2020). There is experience in the incorporation of carbon flour in the recipes of nutritious snacks for children (Aydın et al., 2017), milk and dark compound chocolate (Akdeniz et al., 2021), pastry sauces (Capcanari et al., 2022), and muffins (Pawłowska et al., 2018).

The aim of the present paper was to study the bioactive profile of carob cultivated in European and North Africa agrifood sectors in terms of mineral (Ca, Mg, Fe), carotenoids (β -carotene, lycopene, and zeaxanthin) and chlorophyll (a chlorophyll and b chlorophyll) content. The antioxidant activity of biologically active compounds was determined using simulated gastrointestinal digestion.

Materials and methods

Materials

Carob samples from different world regions (Republic of Moldova, Italy, Algeria, and Spain) have been used in the study. The unroasted carob bean samples from Spain, Italy and Algeria were bought from supermarkets in Italy and Romania. The pod pulp is not commercially available for the above-mentioned samples, as it is considered a by-product. The Moldovan samples (pods pulp and beans) were collected from different country geographical regions (Center, South and East).



Figure 3. Carob samples used in the present study

In order to avoid major experimental errors in the comparative analysis, Spanish, Algerian, and Italian unroasted carob samples were selected with the same particles size ($\leq 90 \mu\text{m}$).

Preparation of carob powder extracts

In order to assess the bioactive profile of carob, hydroalcoholic extracts were obtained according to the technological scheme shown in Figure 4. It should be noted, that after Moldovan carob beans and pod pulp grinding, particles with the same particles size ($\leq 90 \mu\text{m}$) as the bought ones were selected for determination. The extracts were further used as raw material for laboratory determinations.

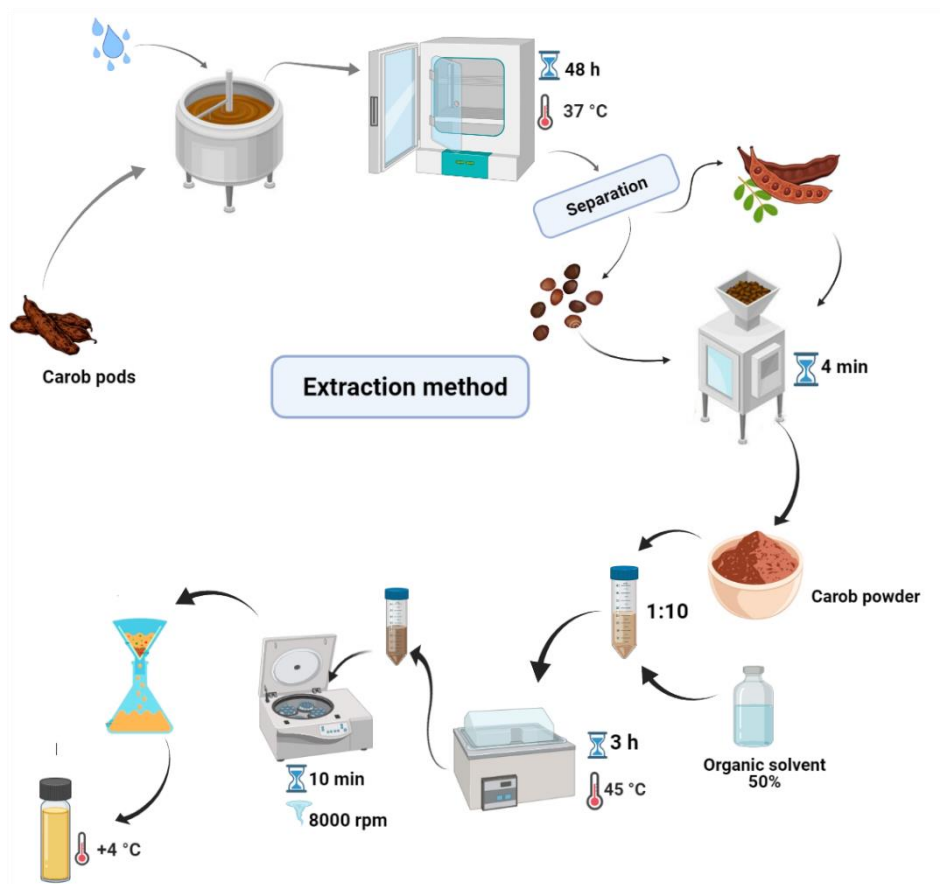


Figure 4. The technological flow of carob extract preparation

Mineral content

The content of minerals, Ca, Mg and Fe, was determined from the resulting solution using AOAC (Association of Official Analytical Chemists) methods as described by (James, 1995).

Chlorophyll and carotenoid content

Chlorophylls a and b were extracted with refined sunflower oil from carob powder and the absorbance of the extract solutions was measured in a spectrophotometer (Shimadzu UV-1800, Kyoto, Japan) at 661.6, 644.8 and 470 nm. Pigment contents were estimated as described by (Lichtenthaler, 1987). Carotenoids lipid extractions were analysed in the range of 448 and 480 nm wavelength for determining β -carotene, lycopene and zeaxanthin content (Meléndez-Martínez et al., 2007).

Antioxidant activity expressed via gastrointestinal *in vitro* digestion

Product samples were dissolved in HCl (pH 1.5–2.0), pepsin being added. Similar product samples were dissolved in NaHCO₃ (pH 8.2), and trypsin was added. The samples were incubated at 37°C by continuous stirring at 95 rpm for 60 minutes. Aliquots were extracted during different incubation periods (0 h, 1 h, 2 h, and 3 h). Each aliquot was centrifuged for 10 minutes at 6000 rpm then the upper phase was isolated and further used for the determination of antioxidant activity using DPPH (2,2-Diphenyl-1-picrylhydrazyl) free radical. DPPH (60 μM) was added to the sample filtrate (0.1 mL). Methanol was used as a reference solution. The samples with DPPH were incubated for 30 min in a dark place. The absorbance was recorded on the spectrophotometer LLG uniSPEC-2 at the wavelength λ = 515 nm (Pavan et al., 2014).

Statistical analysis

All experiments were carried out in triplicate. The results were given as mean standard deviation (SD). Statistical analysis was performed using XLstat (2020 version) software.

Results and discussion

Mineral content

For the quantitative analysis of mineral compounds there were selected 10 lots of Moldovan carob products and 5 lots of carob beans samples from different regions of Spain, Italy and Algeria (Table 1).

Table 1

Mineral content in carob cultivated in European and North Africa agrifood sectors, mg/kg dry matter (DM)

Sample	Ca		Mg		Fe	
	Min-Max	Mean	Min-Max	Mean	Min-Max	Mean
Spanish carob beans (n=5)	2519.3–2526.4	2522.5 ^b	1232.4–1239.4	1235.5 ^{ab}	39.48–46.12	42.57 ^{ab}
Italian carob beans (n=5)	2698.7–2705.4	2702.2 ^b	1042.5–1052.2	1047.1 ^a	42.39–48.71	45.42 ^b
Algerian carob beans (n=5)	2174.3–2181.6	2178.5 ^a	1344.7–1352.4	1348.1 ^b	34.89–43.75	39.65 ^a
Carob pods pulp from Moldova (n=10)	4501.2–4511.5	4506.7 ^d	1860.7–1869.2	1864.4 ^c	75.17–82.94	78.19 ^d
Carob beans from Moldova (n=10)	3813.7–3819.6	3816.4 ^c	1483.9–1491.4	1487.7 ^{bc}	58.24–64.62	61.28 ^c

^{a-d} The means in columns followed by the same letter are not statistically different ($P \leq 0.05$); n is number of samples from each region.

Data concerning the mineral content of carob pods is practically lacking in literature, a part of Ayaz et al. (2007), who stated that the mineral amount of Ca, Mg and Fe in Anatolian carob pods was 3000, 600 and 18.8 mg/kg, respectively. Later, the same author reported an amount of 3040, 554 and 15.1 mg/kg of Ca, Mg and Fe, respectively, in home-prepared carob pods flour (Ayaz et al., 2009). It is worth mentioning that carob pods pulp had a greater amount of each determined element, emphasizing once again the importance of the valorization of these by-products.

The amount of Ca, Mg and Fe in Moldovan carob pods is almost twice higher (4506.7, 1864.4 and 78.19 mg/kg DM, respectively) than Spanish, Italian or Algerian beans (Table 1).

According to studies (Fidan et al., 2020; Musa Ozcan et al., 2007; Pazir et al., 2018), the amount of Ca, Mg and Fe in carob beans consists 2510–4207 mg/kg, 630–894 mg/kg and 12.5–42.57 mg/kg, respectively. The studies have mainly focused on the mineral content of Turkish carob, while data for European carob are lacking. Thus, the obtained data are valuable for the European agrifood sector. Analyzing and comparing the content of the selected elements in the studied samples the following chains can be formed:

- $Ca_{MD} > Ca_{IT} > Ca_{ES} > Ca_{DZ}$;
- $Mg_{MD} > Mg_{DZ} > Mg_{ES} > Mg_{IT}$;
- $Fe_{MD} > Fe_{IT} > Fe_{ES} > Fe_{DZ}$.

(MD, Republic of Moldova, IT, Italy, ES, Spain, DZ, Algeria)

Carotenoid content

Table 2 provides data on the carotenoid content of carob cultivated in European and North Africa agrifood sectors.

Table 2

Carotenoid content in carob cultivated in European and North Africa agrifood sectors, mg/100 g DM

Sample	β-carotene		lycopene		zeaxanthin	
	Min–Max	Mean	Min–Max	Mean	Min–Max	Mean
Spanish carob beans (n=5)	0.534–0.612	0.542 ^b	0.639–0.659	0.647 ^{ab}	0.712–0.718	0.715 ^{ab}
Italian carob beans (n=5)	1.118–1.161	1.126 ^{ab}	1.549–1.569	1.558 ^b	1.451–1.457	1.453 ^b
Algerian carob beans (n=5)	0.258–0.279	0.267 ^a	0.458–0.483	0.467 ^a	0.421–0.433	0.427 ^a
Carob pods pulp from Moldova (n=10)	2.648–2.841	2.749 ^c	3.874–3.892	3.879 ^c	3.787–3.817	3.804 ^c
Carob beans from Moldova (n=10)	13.541–13.724	13.610 ^d	19.869–19.897	19.882 ^d	20.681–20.735	20.709 ^d

^{a-d} The means in columns followed by the same letter are not statistically different ($P \leq 0.05$); n– number of samples from each region.

Carotenoids are the most common pigments in nature, also known as the source of dietary vitamin A (Delgado-Vargas et al., 2000). However, data concerning the carotenoid content in carob are practically lacking. According to Khatib and Vaya (2010), carob pods from the Mediterranean region contained 0.2 mg total carotenoids/100 g DM, with α - and β -carotene (0.08 mg/100 g DM), lycopene (0.03 mg/100 g DM), and lutein (0.02 mg/100 g DM).

In the present study, major carotenoids present in carob included β -carotene, zeaxanthin and lycopene. Lycopene was the most abundant carotenoid in Italian carob beans (1.558 mg/100 g DM), while in Spanish and Algerian samples this indicator had a mean value of 0.647 and 0.467 mg/100 g DM, respectively. Italian carob beans were most abundant in carotenoids (β -carotene 1.126 mg/100 g DM, lycopene 1.558 mg/100 g DM, zeaxanthin 1.453 mg/100 g DM), while in Spanish and Algerian samples these indicators had mean values of 0.267 mg/100 g DM β -carotene for Algerian samples, 0.542 mg/100 g DM β -carotene for Spanish; 0.467 mg/100 g DM lycopene for Algerian samples, 0.647 mg/100 g lycopene for Spanish; 0.427 mg/100 g DM zeaxanthin for Algerian samples, 0.715 mg/100 g DM zeaxanthin for Spanish.

The data presented in Table 2 confirm that the difference between Moldovan and rest of the samples was significant ($P \leq 0.05$). The carotenoid content in samples of Moldovan carob beans was more than 10 times higher than the biological potential of industrial bought samples, especially in the case of zeaxanthin, which concentration was up to 20.71 ± 0.42 mg/100 g DM.

This can be explained by different conditions of pre-treatment of raw materials. Nevertheless, it is necessary to mention the biological potential of carob pod pulp originated from Moldova. Although this product is considered as industrial waste and does not find use in the food industry, it contains high amounts of β -carotene, lycopene and zeaxanthin.

Chlorophyll content

Chlorophyll has antioxidant properties and on a par with vitamins A, C and E could neutralize the damaging effect of free radicals (Inanc, 2011; Lanfer-Marquez et al., 2005; Queiroz Zepka et al., 2019). There are two main types of chlorophyll: chlorophyll a and chlorophyll b, the contents of which in carob samples are shown in Table 3.

Taking into account that chlorophyll is not an essential parameter in carob pods or beans, its presence in these products has not been studied before. The data presented in Table 3 show that in all beans samples, except for Algerian carob beans, chlorophyll b is more abundant than chlorophyll a, and its content values ranging within the limits from 0.122 to 0.605 mg/100 g DM.

The content of chlorophyll in Moldovan beans is comparable to the data of other carob bean samples. However, the content of these compounds is higher in carob pod pulp and is up to 0.749 ± 0.04 for chlorophyll a and 0.482 ± 0.02 mg/100 g DM for chlorophyll b. This indicates the antioxidant potential of Moldovan carob, including carob pod pulp. It is worth mention that in Moldovan carob pods the content of chlorophyll a prevails over chlorophyll b. The content of a chlorophyll from pods (0.749 mg/100 g DM) was higher than the one from all beans.

Evaluation of antioxidant activity by simulated *in vitro* gastrointestinal digestion

In order to evaluate the antioxidant activity of carob samples, a simulation of gastrointestinal digestion in time was performed. The evaluation of the antioxidant activity following induced gastric digestion (in acid medium) (Figure 5) shows lower values for the

Spanish samples (40.00–64.25)±0.1%, Italy (38.15–60.25)±0.2% and Algeria (39.28–62.61)±0.6% compared to the Moldovan carob samples, which values vary between (47.00–70.07)±0.05% for carob pods pulp and (48.00–74.24)±0.03% for carob beans.

Table 3

Chlorophyll content in carob cultivated in European and North Africa agrifood sectors, mg/100 g DM

Sample	Chlorophyll a		Chlorophyll b	
	Min–Max	Mean	Min–Max	Mean
Spanish carob beans (n=5)	0.149–0.157	0.152 ^a	0.231–0.237	0.234 ^a
Italian carob beans (n=5)	0.497–0.507	0.501 ^b	0.602–0.608	0.605 ^b
Algerian carob beans (n=5)	0.139–0.156	0.146 ^a	0.116–0.129	0.122 ^c
Carob pods pulp from Moldova (n=10)	0.742–0.758	0.749 ^c	0.478–0.487	0.482 ^d
Carob beans from Moldova (n=10)	0.275–0.283	0.278 ^d	0.347–0.359	0.352 ^{ad}

^{a-d} The means in columns followed by the same letter are not statistically different ($P \leq 0.05$); n—number of samples from each region.

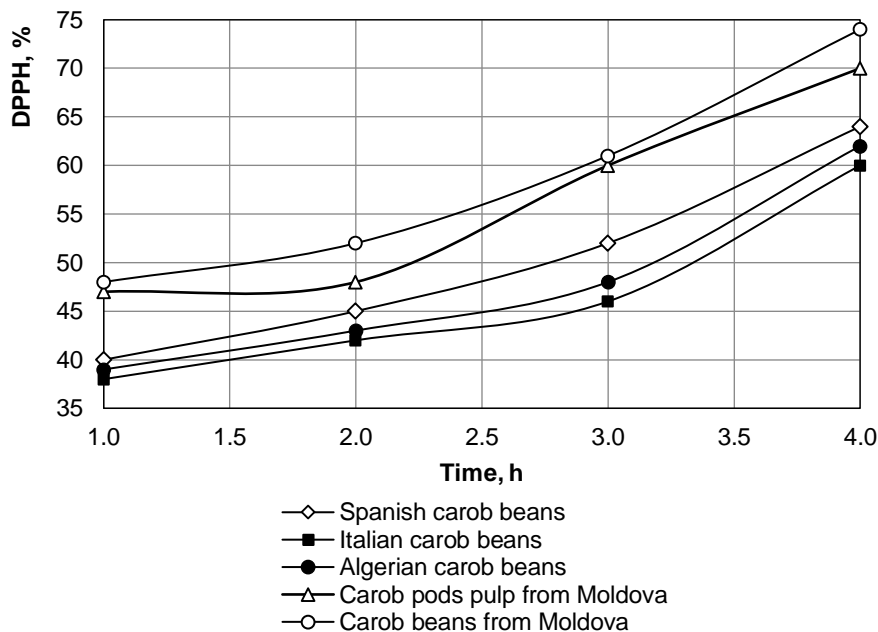


Figure 5. Antioxidant activity expressed via gastric digestion of carob powders

The gradual increase of antioxidant activity in 3 hours is explained by the gradual release of biologically active compounds in the process of gastric digestion. Another important factor was the influence of the pH of the solution and the enzymatic interactions in the studied samples. It was shown that changes in the content of biologically active compounds with antioxidant capacity, including polyphenols, flavonoids, tannins and other compounds such as carotenoids and chlorophyll groups can increase the antioxidant capacity of the analyzed samples. The data showed that gastric digestion did not essentially change the qualitative and quantitative composition of biologically active compounds with the antioxidant capacity of the studied carob samples. These compounds demonstrated high stability under low pH (acidic medium) conditions. The acidic environment along with the digestive enzymes favored the release of the biologically active compounds, which also affects the increase in the antioxidant capacity of the studied carob samples.

Following the gastrointestinal digestion process, the simulation of the intestinal digestion phase induced by incubating the samples in an alkaline environment (pH=8.2) (Figure 6) and determining the antioxidant activity in time for a period of 3 hours was performed. The data showed that the antioxidant activity of both types of Moldovan carob samples (from pod pulp and beans) was higher compared to the carob beans samples from Spain, Algeria and Italy. The values obtained varied between $(40.05-19.89)\pm 0.06\%$ for Moldovan carob pod pulp; $(35.25-18.69)\pm 0.12\%$ for Moldovan carob beans; $(31.22-14.65)\pm 0.08\%$ for Italian carob beans; $(30.04-17.47)\pm 0.34\%$ for Algerian carob beans and respectively $(32.56-16.78)\pm 0.58\%$, for Spanish carob beans.

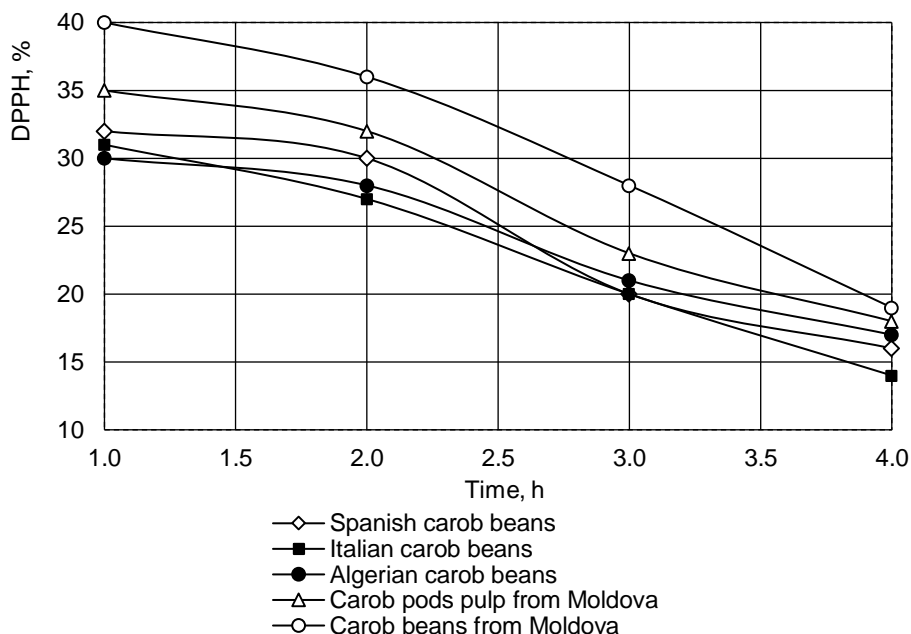


Figure 6. Antioxidant activity expressed via intestinal digestion of carob powders

Following intestinal digestion, there was a gradual decrease in antioxidant activity within 3 hours for both Moldovan carob samples and samples from Spain, Italy, and Algeria. This can be explained by the low stability of biologically active compounds in alkaline conditions (pH=8.2) and the formation of metabolites that inhibit their antioxidant activity in the analyzed samples. This is a normal physiological process. Absorption of biologically active substances occurs within 2–3 hours during the period of their maximum antioxidant activity.

Conclusions

The obtained results demonstrated the biological profile and antioxidant potential of carob pods and beans, originating from different world regions. The content of minerals Ca, Mg, and Fe in Moldovan carob pods and beans was found to be 1.1–1.7 times higher than in samples from Algeria, Spain, and Italy. The content of biologically active compounds β -carotene, lycopene and zeaxanthin in Moldovan carob pods, which are usually considered as food residue, is higher than in carob beans originating from Italy, Spain and Algeria. In the same time, the content of β -carotene, lycopene, and zeaxanthin (13.610, 19.882 and 20.709 mg/100 g DM respectively) in Moldovan carob beans exceeds by over 10 times the content of these substances in Italian (1.126, 1.558 and 1.453 mg/100g DM), Spanish (0.542, 0.647 and 0.715 mg/100 g DM), and Algerian (0.267, 0.467 and 0.427) carob beans. The biologically active compounds from carob pods and beans are stable at low pH values, exhibiting an increase in the antioxidant capacity from 38 - 48% to 60 - 74 % during the simulated gastric digestion.

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References

- Akdeniz E., Yakışık H., Rasouli Pirouzian S., Akkın B., Turan E., Tipigil O. S., O. Ozcan O. (2021), Carob powder as cocoa substitute in milk and dark compound chocolate formulation, *Journal of Food Science and Technology*, 58(12), pp. 4558–4566, <https://doi.org/10.1007/s13197-020-04943-z>.
- Ayaz F. A., Torun H., Ayaz S., Correia P. J., Alaiz M., Sanz C., Grúz J., Strnad M. (2007), Determination of chemical composition of Anatolian carob pod (*Ceratonia siliqua* L.): sugars, amino and organic acids, minerals and phenolic compounds, *Journal of Food Quality*, 30(6), pp. 1040–1055, <https://doi.org/10.1111/j.1745-4557.2007.00176.x>.
- Ayaz F.A., Torun H., Glew R.H., Bak Z.D., Chuang L.T., Presley J. M., Andrews R. (2009), Nutrient content of carob pod (*Ceratonia siliqua* L.) flour prepared commercially and domestically, *Plant Foods for Human Nutrition*, 64(4), pp. 286–292, <https://doi.org/10.1007/s11130-009-0130-3>.
- Aydın S., Özdemir Y. (2017), Development and characterization of carob flour based functional spread for increasing use as nutritious snack for children, *Journal of Food Quality*, 2017, pp. 1–7, <https://doi.org/10.1155/2017/5028150>.

- Bouzdoudi B.E., Ammouri N., Joly N., Martin P., Rabah S., Nejjar El Ansari Z., Bouras M., Badoc A., Lamarti A. (2017), Total polyphenols and gallic acid contents in domesticated Carob (*Ceratonia siliqua* L.) pods and leaves, *International Journal of Pure & Applied Bioscience*, 5(4), pp. 22-30, <http://dx.doi.org/10.18782/2320-7051.5344>.
- Capcanari T., Chirsanova A., Covaliov E., Radu O., Siminiuc R. (2022), Pastry sauce with carob (*Ceratonia siliqua*) powder, *Ukrainian Food Journal*, 11(2), pp. 235–258, <https://doi.org/10.24263/2304-974X-2022-11-2-4>.
- Capcanari T., Chirsanova A., Radu O., Covaliov E., Popovici V., Siminiuc R. (2022), Functional profile of carob (*Ceratonia siliqua* L.) beans and pod pulp originated from the Republic of Moldova, *Czech Journal of Food Sciences*, 40(6), pp. 465–473, <https://doi.org/10.17221/139/2022-CJFS>.
- Chait Y. A., Gunenc A., Bendali F., Hosseinian F. (2020), Simulated gastrointestinal digestion and in vitro colonic fermentation of carob polyphenols, bioaccessibility and bioactivity, *LWT*, 117, Article 108623, <https://doi.org/10.1016/j.lwt.2019.108623>.
- Delgado-Vargas F., Jiménez A.R., Paredes-López O. (2000), Natural pigments: carotenoids, anthocyanins, and betalains-characteristics, biosynthesis, processing, and stability, *Critical Reviews in Food Science and Nutrition*, 40(3), pp. 173-289, <https://doi.org/10.1080/10408690091189257>.
- FAOSTAT (2022), *Crops Statistics*, Available at: <https://www.fao.org/faostat/en/#data/QCL/visualize> (last access: Apr 05, 2023)
- Fidan H., Stankov S., Petkova N., Petkova Z., Iliev A., Stoyanova M., Ercisli, S. (2020), Evaluation of chemical composition, antioxidant potential and functional properties of carob (*Ceratonia siliqua* L.) seeds, *Journal of Food Science and Technology*, 57(7), pp. 2404–2413, <https://doi.org/10.1007/s13197-020-04274-z>.
- Fidan H., Sapundzhieva T. (2015), Mineral composition of pods, seeds and flour of grafted carob (*Ceratonia siliqua* L.) fruits, *Scientific Bulletin. Series F. Biotechnologies*, 19, pp. 136–139, ISSN 2285-1364
- Gunel Z., Torun M., Sahin-Nadeem H. (2020), Sugar, D -pinitol, volatile composition, and antioxidant activity of carob powder roasted by microwave, hot air, and combined microwave/hot air, *Journal of Food Processing and Preservation*, 44(4), <https://doi.org/10.1111/jfpp.14371>.
- Inanc A. (2011), Chlorophyll: structural properties, health benefits and its occurrence in virgin olive oils, *Akademik Gida / Academic Food Journal*, 9, pp. 26–32.
- James C.S. (1995), *Analytical Chemistry of Foods*, Springer, Boston, <https://doi.org/10.1007/978-1-4615-2165-5>.
- Khatib S., Vaya J. (2010), Fig, carob, pistachio, and health, in bioactive foods in promoting health, In: Watson R.R., Preedy V.R. (eds.), *Bioactive Foods in Promoting Health*, pp. 245–263, Elsevier, <https://doi.org/10.1016/B978-0-12-374628-3.00017-7>.
- Lanfer-Marquez U.M., Barros R.M.C., Sinnecker P. (2005), Antioxidant activity of chlorophylls and their derivatives, *Food Research International*, 38(8–9), pp. 885–891, <https://doi.org/10.1016/j.foodres.2005.02.012>.
- Lichtenthaler H.K. (1987), *Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes*, In: *Methods in Enzymology*, pp. 350–382, Academic Press, Plant Cell Membranes, [https://doi.org/10.1016/0076-6879\(87\)48036-1](https://doi.org/10.1016/0076-6879(87)48036-1).
- Loullis A., Pinakoulaki E. (2018), Carob as cocoa substitute: a review on composition, health benefits and food applications, *European Food Research and Technology*, 244(6), pp. 959–977, <https://doi.org/10.1007/s00217-017-3018-8>.

- Meléndez-Martínez A.J., Britton G., Vicario I.M., Heredia F.J. (2007), Relationship between the colour and the chemical structure of carotenoid pigments, *Food Chemistry*, 101(3), pp. 1145–1150, <https://doi.org/10.1016/j.foodchem.2006.03.015>.
- Musa Özcan M., Arslan D., Gökçalık H. (2007), Some compositional properties and mineral contents of carob (*Ceratonia siliqua*) fruit, flour and syrup, *International Journal of Food Sciences and Nutrition*, 58(8), pp. 652–658, <https://doi.org/10.1080/09637480701395549>.
- Pavan V., Sancho R.A.S., Pastore G.M. (2014), The effect of in vitro digestion on the antioxidant activity of fruit extracts (*Carica papaya*, *Artocarpus heterophyllus* and *Annona marcgravii*), *LWT - Food Science and Technology*, 59(2), pp. 1247–1251, <https://doi.org/10.1016/j.lwt.2014.05.040>.
- Pawłowska K., Kuligowski M., Jasińska-Kuligowska I., Kidoń M., Siger A., Rudzińska M., Nowak J. (2018), Effect of replacing cocoa powder by carob powder in the muffins on sensory and physicochemical properties, *Plant Foods for Human Nutrition*, 73(3), pp. 196–202, <https://doi.org/10.1007/s11130-018-0675-0>.
- Pazir F., Alper Y. (2018), Carob bean (*Ceratonia siliqua* L.) and its products, *Anadolu Ege Tarımsal Araştırma Enstitüsü Dergisi*, 28(1), pp. 108–112.
- Queiroz Zepka L., Jacob-Lopes E., Roca M. (2019), Catabolism and bioactive properties of chlorophylls, *Current Opinion in Food Science*, 26, pp. 94–100, <https://doi.org/10.1016/j.cofs.2019.04.004>.
- Şahin G., Taşlıgil N. (2016), Agricultural geography analysis of carob tree (*Ceratonia siliqua* L.) from Turkey, *Turkish Journal of Agriculture - Food Science and Technology*, 4(12), pp. 1192–1200. <https://doi.org/10.24925/turjaf.v4i12.1192-1200.979>.
- Santonocito D., Granata G., Geraci C., Panico A., Siciliano E. A., Raciti G., Puglia C. (2020), Carob seeds: Food waste or source of bioactive compounds? *Pharmaceutics*, 12(11), Article 1090, <https://doi.org/10.3390/pharmaceutics12111090>.
- Stabnikova O., Paredes-Lopez O. (2023), Plant materials for the production of functional foods for weight management and obesity prevention, *Current Nutrition & Food Science*, <https://doi.org/10.2174/1573401319666230705110854>.
- Vekiari A.S., Ouzounidou G., Gork G., Ozturk M., Asfi M. (2012), Compositional changes of major chemical compounds in Greek carob pods during development, *Bulletin of the Chemical Society of Ethiopia*, 26(3), pp. 343-351, <http://dx.doi.org/10.4314/bcse.v26i3.3>.

Proteolytic activity of the Carpathian traditional liquid milk coagulant

Volodymyr Yukalo, Olha Krupa,
Kateryna Datsyshyn, Liudmyla Storozh

Ternopil Ivan Puluj National Technical University, Ternopil, Ukraine

Abstract

Keywords:

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substrates
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Introduction. In the Ukrainian Carpathians, a special Carpathian liquid milk (CLM) coagulant is used for the production of traditional soft cheeses such as brynza and budz. Since CLM coagulant has not been studied before, the aim of the work was to characterize its milk coagulant and proteolytic properties.

Materials and methods. Milk coagulants were obtained from farms in Vorokhta, Carpathians. Milk-clotting activity was examined by the Soxhlet method. The content of proteins was determined using the Kjeldahl method. Homogeneous α_{S1} -, β - and κ -casein substrates were separated by preparative electrophoresis. The concentration of the products of casein substrates proteolysis was determined spectrophotometrically. The specificity of casein fractions proteolysis was established by analytical electrophoresis.

Results and discussion. CLM coagulant has high milk coagulant activity, which is slightly lower than of the standard rennet enzyme and is close to the solid milk coagulant preparation Glek. It was found that the activity of CLM coagulant increases during its storage, which is apparently achieved through the proteolytic enzyme extraction from calf's stomach cells. The milk clotting time for fresh CLM coagulant is 159 min, and after 18 months of storage it is reduced to 49 min. Milk-clotting activity of the fresh CLM coagulant is 2515 Soxhlet Units (SU), whereas after storage during 18 months the milk-clotting activity increased to 8100 SU.

In the production of Carpathian brynza and budz, it is recommended to use the amount of CLM coagulant, which provides the beginning of milk coagulation at 33–35 °C after 20–25 min. The analysis of the casein substrates proteolysis products by electrophoretic and spectrophotometric methods shows the high specificity of CLM coagulant to κ -casein. Such specificity is characteristic for the purified natural milk coagulant enzyme - chymosin. Along with that, CLM coagulant, unlike pepsin and, to a lesser extent, standard rennet, practically does not cleave α_{S1} - and β -casein substrates.

Conclusions. Milk-clotting activity of CLM coagulant during its storage for 18 months increases 3 times. According to the specificity of α_{S1} -, β - and κ -casein substrates proteolysis, CLM coagulant is close to chymosin.

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Corresponding author:

Olha Krupa
E-mail:
cmakota@ukr.net

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Introduction

In the Carpathian ecologically clean region of Ukraine, traditional products, which are able to provide full-valuable nutrition, have been produced since ancient times. The original waste-free technologies of their production allow the use of all milk components. Such products have good sensory properties (Cais-Sokolińska et al., 2020; Weichselbaum et al., 2009), as well as high nutritional and biological value (Blazic et al., 2017; Rybak 2016). These include guslyanka, pretaned milk, urda, budz, and Carpathian brynza (Braichenko et al., 2020). The interest in these products has significantly grown (Slyvka et al., 2018). They can be of great importance for the development of tourism in the Carpathians. Their production will become highly profitable if they get international recognition and the status of National Product.

It is worth noting that traditional products are characteristic of many countries. Some of them are protected by EU regulations (Commission regulation (EC) No 642, 2007; Commission Regulation (EC) No 676, 2008). In particular, Bryndza Podhalanska (Poland) was granted the Protected Designation of Origin (PDO) of EU as well as Slovenská Bryndza was granted the Protected Geographical Indication (PGI) as they are produced in a defined mountainous regions of Poland and Slovak Republic, respectively (Najgebauer-Lejko et al., 2022; Semjon et al., 2018).

Traditional soft cheeses are considered to be produced locally or regionally, usually seasonally, for many generations, and they have an important place in rural regional food culture (Hernik et al., 2022; Weichselbaum et al., 2009). Furthermore, they reflect the country's history, geography, climate and agriculture (Braichenko et al., 2020; Trichopoulou et al., 2007). In many central European countries varieties of brynza are made. In different countries this cheese is designated with different names: Bryndza Podhalańska in Poland (Nalepa et al., 2023), Bryndza in Slovakia (Kačániová et al., 2020), Brânză de burduf in Romania (Donnelly et al., 2016), tulum cheese in Turkey and Bulgaria (Dimitrova et al., 2018; Karagozlu et al., 2009).

Another cheese is urda, which has a long tradition of production in mountainous areas of Montenegro (Rasovic et al., 2017) as well as in high mountainous areas of the region of Konitsa, Epirus in Greece (Pappa et al., 2016). However, in Bulgaria it is called the izvara, in Italy ricotta (El-Sheikh et al., 2010; Kamel et al., 2013). Despite this, in different regions the production of urda is similar and based on warming the whey and separating its proteins (Law et al., 2010; Rasovic et al., 2017).

It is known that coagulants play an important role in the clot formation, as well as in the rheological and sensory properties of cheeses (Law et al., 2010; Walstra et al., 2006). In our laboratory, the milk coagulant properties, as well as the proteolytic activity of the solid milk coagulant Glek (Iukalo, 2015) were studied. The regularities of changes of the coagulant proteolytic activity during storage and the specificity of its action in relation to the main casein fractions were established. However, the authors' expedition to the Carpathians showed that today, for the production of budz and Carpathian brynza, more often is used a Carpathians liquid milk (CLM) coagulant, but not Glek. This coagulant differs in manufacturing technology and can be used for cheese production during three years after its manufacture, and according to some reports, up to five years. The properties of CLM coagulant have not been investigated for today and required to be studied.

In this regard, the aim of this work was to study the milk coagulant properties, proteolytic activity and specificity of the Carpathian liquid milk coagulant, which is used for the production of budz and Carpathian brynza.

Materials and methods

Materials

CLM coagulants of different life shelf were used for the study. They were obtained from farms in the urban-type village of Vorokhta, Ivano-Frankivsk region (Figure 1). Also, standard rennet enzyme and pepsin (Kaunas, Lithuania) were used to compare the proteolytic action. Skimmed milk (18 °T) for determination of milk coagulant activity and fresh whole milk for isolation of casein substrates were obtained from the Ternopil Milk Factory. Buffer solutions and reagents for electrophoresis were prepared from reagents of the Reanal Company (Hungary) and domestic reagents of a high degree of purification.

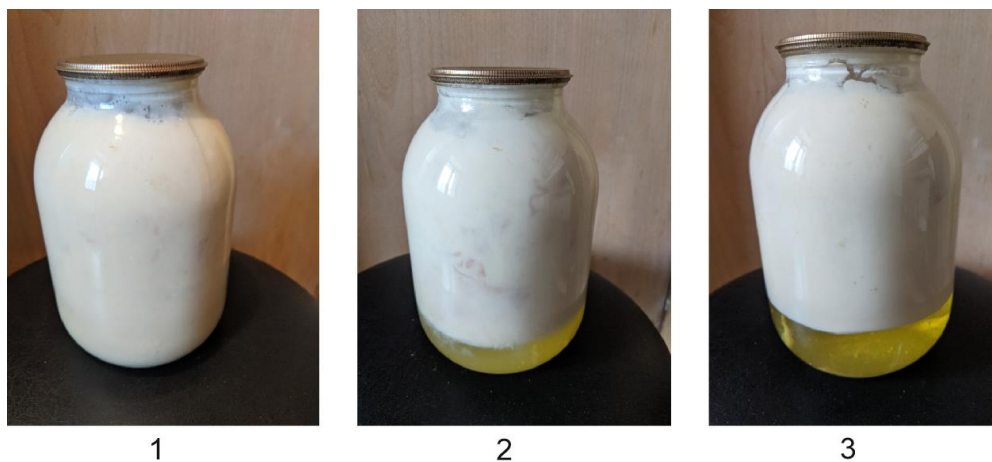


Figure 1. CLM coagulant of different shelf life: 1, freshly isolated; 2, during 6 months; 3, during 18 months

Characteristics of CLM coagulant

Milk-clotting activity of the CLM coagulant was the Soxhlet method as previously described in (Hyslop, 2003; Teplý et al., 1976).

Determination of moisture content in CLM coagulant was carry out by the drying of samples to a constant mass at 100 °C.

The protein content of CLMC was determined according to the Kjeldahl method, using the conversion coefficient 6.25 for the calculation of protein's content (Fox et al., 2015).

The formation and quality of a milk clot's structure during coagulation were visually determined.

Casein fraction proteolysis

Proteolysis of each casein fraction was carried out with enzyme preparations: CLM coagulant, standard rennet enzyme and pepsin. The amounts of enzyme preparations was

taken in such way that they had the same milk coagulant activity. At the same time, the enzyme: substrate ratio for pepsin was set at 1:100.

Proteolysis occurred at a temperature of 35 °C and a pH value of 7.2. The same conditions were used earlier in the study of the action of the solid preparation Glek (Iukalo, 2015). During proteolysis, after 15, 30, 60 and 120 min, samples were taken to determine the cleavage products of the casein fraction, soluble in 12% solution of trichloroacetic acid (TCA). The concentration of proteolysis products was represented as the optical density determined at a wavelength of 280 nm by a SF-46 spectrophotometer (JSC "Lomo", Russia). For greater objectivity, when comparing the proteolysis of casein fractions, the difference in the values of their absorption coefficients at 280 nm was taken into account. Also, at the 60th min of proteolysis, samples were taken for electrophoretic analysis.

Concentration of total casein and casein fractions and products of their proteolysis

The concentration of total casein and casein fractions was determined spectrophotometrically by absorption in the ultraviolet region of the spectrum at a wavelength of 280 nm. The optical density of casein solutions was measured on a SF-46 spectrophotometer. Absorption coefficients ($D_{1\text{ cm}}^{1\%}$) were used for calculation: for total casein, 8.2; for κ -casein, 9.6; for β -casein, 4.6; for α_{S1} -casein, 10.0 (Farrell et al., 2004).

During determining the proteolysis products of α_{S1} -, β -, and κ -casein substrates, soluble in 12% trichloroacetic acid, their concentration was represented as the value of the optical density at 280 nm. For an objective comparison of the concentrations of different fractions proteolysis products, their optical density values were adjusted to the α_{S1} -casein proteolysis products. At the same time, the value of optical density for β -casein hydrolysates was multiplied by 2.17 and for κ -casein by 1.04.

Isolation of casein from cow milk

Fresh whole milk was skimmed by centrifugation at 4000 g (15 min). The procedure was repeated twice: the first time at 30 °C, and the second time at 4 °C. In skim milk, caseins were precipitated at the isoelectric point. The pH value was adjusted to 4.6 by adding a solution of hydrochloric acid with a concentration of 1 mol/l³. The precipitate was washed with distilled water and dissolved with 1 mol/l³ sodium hydroxide at pH values not higher than 7.7–7.9. The precipitation procedure was repeated twice. Inactivation of milk proteases was carried out by casein incubating in acetic acid (pH 4.0) at a temperature of 4 °C for 5 hours.

Electrophoresis in polyacrylamide gel

Analytical electrophoresis of total casein, homogeneous casein substrates and their proteolysis products was performed in polyacrylamide gel (PAG) plates in an anodic electrophoretic system in the presence of 4.5 M urea (Fox et al., 2015; Tremblay et al., 2003). The details of the analytical electrophoresis method of caseins were described by us earlier (Yukalo et al., 2019).

Preparative electrophoresis of total casein was performed in a Stadler-type apparatus with an enlarged chamber for homogeneous PAG. The electrophoretic system for the isolation of electrophoretically homogeneous fractions of α_{S1} -, β -, and κ -caseins is described in other works (Iukalo, 2014; Yukalo et al., 2018).

Statistical processing

All analyses were performed on duplicate samples, and the experiment was repeated 3 times. Statistical analysis was performed using the program Statistika 10. Differences were considered significant at $P \leq 0.05$. Standard deviations are indicated in the table or shown as error bars in the figures.

Results and discussion

Milk-clotting activity of the CLM coagulant

Milk-clotting activity and moisture content were determined for CLM coagulant of different shelf life time at 4 °C. Clots formed in milk under the action of the preparations were also evaluated. The results of the research are presented in Table 1.

Table 1
Milk-clotting activity of the CLM coagulant at different storage periods

Enzyme preparation	Milk clotting time, min	The milk's clot quality	Milk-clotting activity by Soxhlet, SU	Moisture content, %
Standard rennet	16±1.0	Strong clot	100 000±1000,0	7.2±0.2
CLM coagulant (fresh)	159±11.0	Weak clot	2515±70.0	85±3.0
CLM coagulant (6 months)	69±4.0	Strong clot	5790±120.0	84±2.5
CLM coagulant (12 months)	56±3.0	Strong clot	6937±137.0	84±3.2
CLM coagulant (18 months)	49±3.0	Strong clot	8100±190.,0	82±2.7
CLM coagulant (24 months)	73±4.0	Strong clot	5100±130.0	80±2.5

The milk clotting time and the formation of milk's clot for the fresh CLM coagulant sample are quite long and is about 2.5 h (159 min). It takes place due to the low milk-coagulant activity of the CLM coagulant because of the small amount of enzymes that are extracted from secretory cells of the calf's stomach into the surrounding water environment. During the storage of CLM coagulant, the amount of chymosin and other enzymes increases, as their extraction time lengthened. Therefore, the clotting time is reduced to 69 min after 6 months of CLM coagulant storage, 59 min after 12 months of storage, and 49 min after 18 months of storage. With these samples of CLM coagulant, a strong clot is formed, which in its characteristics is similar to the clot formed by pepsin.

It was established that the milk-clotting activity of the freshly prepared liquid coagulant, when calculated on a dry basis, is close to that of the standard rennet enzyme, as well as the preparation Glek (Iukalo, 2015). This ratio of activity is predictable, taking into account the high water content in CLM coagulant, which varies within the range of 80 to 85% at different periods of its storage. Moreover, in contrast to the preparation Glek, in CLM coagulant the milk-clotting activity increases during storage.

The milk-clotting activity of fresh CLM coagulant is 2515 SU whereas after 6, 12, 18 months of storage are 5790, 6937, 8100, respectively. CLM coagulant exhibits maximum proteolytic activity at 18th month of its storage that is caused by the maximum number of enzymes in the solution, which were extracted from secretory cells of the calf's stomach. During further storage, extraction processes stop, only denaturation processes occur, causing a decrease in the activity of chymosin and other cell's proteolytic enzymes. In particular, the milk-clotting activity in the CLM coagulant after 24 months of storage decreased to 5100 SU. At the same time, the milk's clot becomes a little weaker, although this does not prevent the use of the preparation in the traditional Carpathian cheese's production. The decrease in the milk-clotting activity of CLM coagulant, established after 24 months of storage, requires its use in a larger amount to ensure the necessary quality characteristics of cheese.

During the expedition to Vorokhta, we discovered and researched CLM coagulant with a maximum shelf life of 18 months. According to the testimony of local farmers, liquid coagulants keep their activity for up to three years. It is recommended to use CLM coagulant in the production of Carpathian brynza and budz no earlier than one month after its production. Before this period, the preparations do not provide the formation of a strong clot. Before a clot obtaining, milk is heated to 32-35 °C. Then we add such an amount of CLM coagulant, which ensures the start of milk coagulation during 20-25 min.

Proteolytic properties of CLM coagulant

An important question in the characteristic of a milk coagulant is the establishment of its proteolytic properties (Hyslop, 2003). This primarily concerns its proteolytic activity towards the main casein fractions. To study the specificity of the CLM coagulant proteolytic action, we obtained α_{S1} -, β -, and κ -casein fractions by preparative electrophoresis. As known, the proteolysis of these fractions is of great importance for the process of milk coagulation, as well as the formation of cheeses' organoleptic indicators (Guinee, 2003; Law et al., 2010). In Figure 2 it is shown the PAG plate after preparative fractionation of total casein, as well as the areas from which casein substrates were isolated. The obtained electrophoretic fractions of α_{S1} -, β - and κ -caseins were used to establish the specificity of the CLM coagulant proteolytic action.

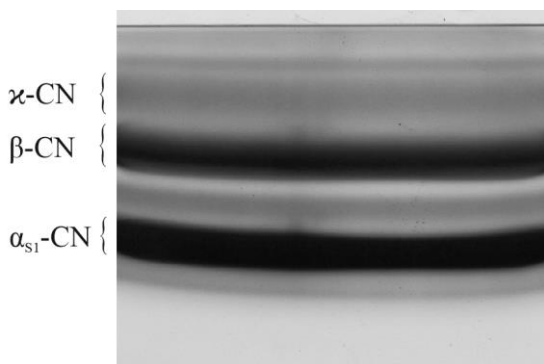


Figure 2. Preparative electrophoresis of cow's milk total casein

The results of proteolysis studies are shown in Figures 3–8. It can be seen that CLM coagulant, on the background of the identical milk-clotting activity, showed at the same time a lower proteolytic activity against α_{S1} - and β -caseins compared to pepsin and close to, but also lower than the standard rennet enzyme.

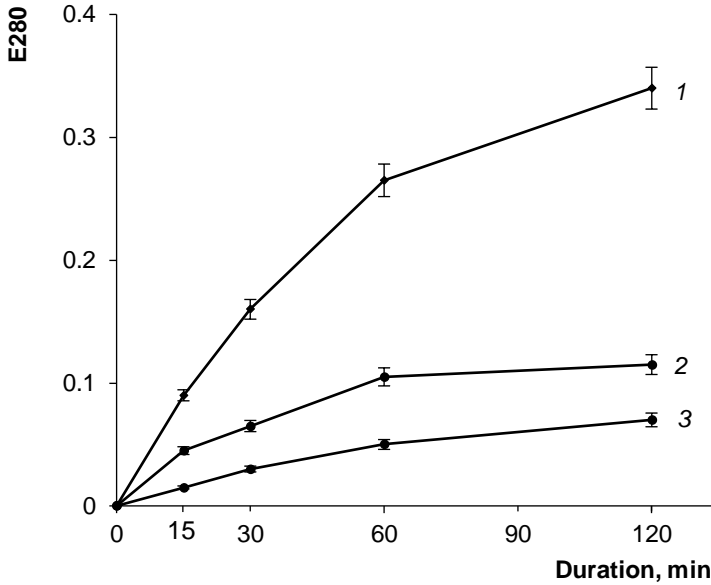


Figure 3. The course of the α_{S1} -casein substrate proteolysis:
1, pepsin; 2, standard rennet enzyme; 3, CLM coagulant

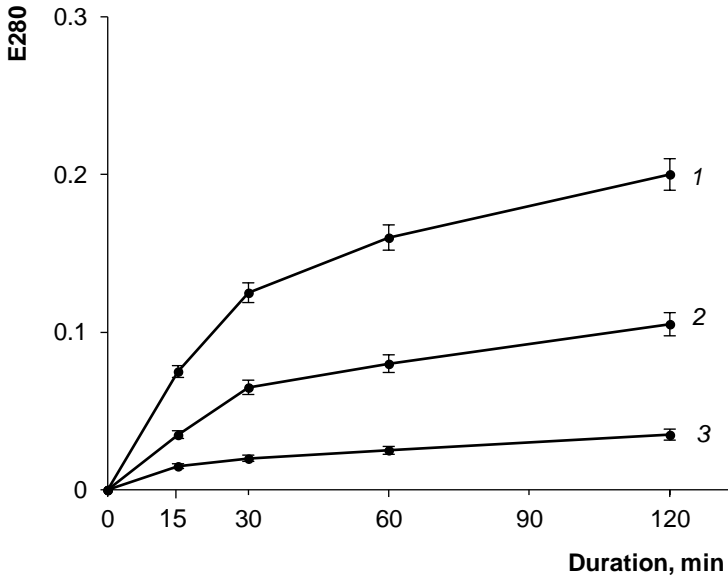


Figure 4. The course of proteolysis of the β -casein substrate:
1, pepsin; 2, standard rennet enzyme; 3, CLM coagulant

Figure 3 shows that the optical density (E_{280}) of cleavage products of the α_{S1} -casein substrate by CLM coagulant (0.05) at the end of proteolysis is 6 times lower compared to pepsin (0.32). It indicates a lower proteolysis products's concentration, caused by the low activity of CLM coagulant proteases in relation to α_{S1} -casein. During the β -casein substrate proteolysis (Figure 4), optical densities (E_{280}) are 0.02 and 0.2, respectively for CLM coagulant and pepsin. Such specificity of proteolytic action in relation to α_{S1} - and β -casein fractions is typical for the natural milk-coagulant enzyme – chymosin (Barbano et al., 1992; Iukalo, 2015). This may be due to the high content of chymosin (EC 3.4.23.4) in the CLM coagulant. The slightly higher proteolytic activity of standard rennet (Figures 3 and 4), compared to CLM coagulant, is caused by the presence in it of two minor proteases: pepsin A (EC 3.4.23.1) and gastricsin (EC 3.4.23.3) in small amounts, in addition to chymosin (Corredig et al., 2016).

These results are confirmed by electrophoretic analysis (Figures 5 and 6).

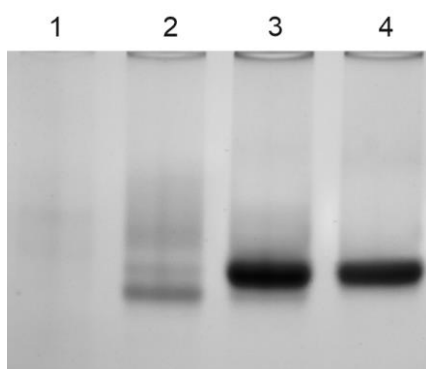


Figure 5. Analytical electrophoresis:

- 1, α_{S1} -casein substrate after proteolysis by pepsin (60 min);
- 2, α_{S1} -casein substrate after proteolysis by standard rennet enzyme (60 min);
- 3, α_{S1} -casein substrate after proteolysis by CLM coagulant;
- 4, α_{S1} -casein substrate

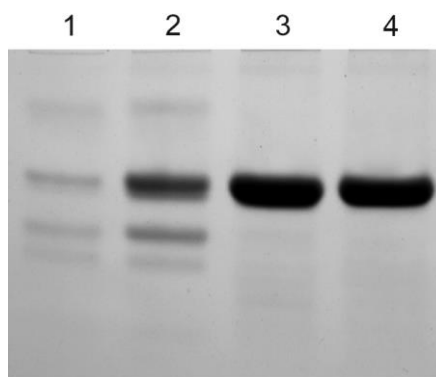


Figure 6. Analytical electrophoresis:

- 1, β -casein substrate after proteolysis by pepsin (60 min);
- 2, β -casein substrate after proteolysis by standard rennet enzyme (60 min);
- 3, β -casein substrate after proteolysis by CLM coagulant;
- 4, β -casein substrate

Figure 5 shows that the α_{S1} -casein substrate remained almost completely unchanged during proteolysis by CLM coagulant enzymes (sample 3), while it was completely cleaved when pepsin was used – absent in sample 1. Similar results are visible in Figure 6: the β -casein substrate in sample 3 (after CLM coagulant proteolysis) is the same as in sample 4, which was not subjected to proteolysis. It apparently indicates a weak enzymatic activity of CLM coagulant in relation to this casein fraction. Similar results are noticeable for standart rennet enzyme (sample 2, Figure 6). Only after proteolysis with pepsin (sample 1, Figure 6) the β -casein substrate was cleaved almost completely.

When analyzing the results of the κ -casein proteolysis, a certain divergence can be noted between the results of determining the concentration of peptides and polypeptides soluble in TCA (Figure 7) and the data of analytical electrophoresis (Figure 8). This is due to the separation's peculiarities of the main products of κ -caseins proteolysis by lactic coagulation enzymes (glycomacropeptide and para- κ -casein) in the anode electrophoresis system (Fox et al., 2015).

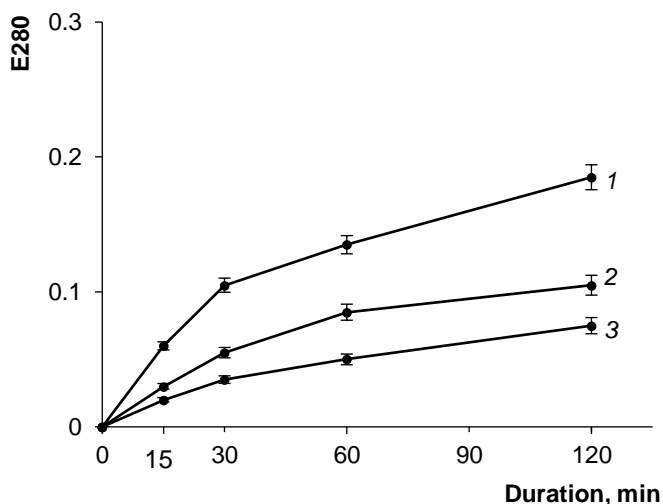


Figure 7. The course of proteolysis of the κ -casein substrate:
1, pepsin; 2, standard rennet enzyme; 3, CLM coagulant

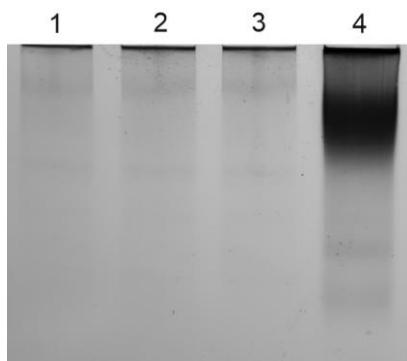


Figure 8. Analytical electrophoresis:
1, κ -casein substrate after proteolysis by pepsin (60 min);
2, κ -casein substrate after proteolysis by standard rennet enzyme (60 min);
3, κ -casein substrate after proteolysis by the CLM coagulant;
4, κ -casein substrate

According to electrophoresis, κ -casein is completely split under the action of all three milk coagulants. This is fully consistent with literature data (Hyslop, 2003). However, the concentration of κ -casein proteolysis products is much lower than in the case of other casein substrates. Obviously, this is related to the features of the primary structure of κ -casein and the specificity of its proteolysis by milk coagulants. In all three cases, para- κ -casein (f 1-105) and glycomacropeptide (f 106-169) are formed. Both of these fragments are absent in the PAG plate. Para- κ -casein has a positive charge and is not included in the PAG of the anode electrophoretic system (Sharma et al., 2021). Glycomacropeptide (GMP) is not fixed in the gel plate. Thus, the cleavage of only one peptide bond (105-106) produces two proteolysis products that are not fixed in the gel plate (Figure 8). On the other hand, during precipitation

with a 12% TCA solution, GMP and low-molecular-weight products of κ -casein proteolysis remain in the solution. At the same time, para- κ -casein is precipitated. It should be noted here that GMP does not contain aromatic amino acid residues in its composition and does not absorb at 280 nm (Fox et al., 2015). Only the breakdown products of para- κ -casein can be absorbed. Such products can be formed to a large extent by the action of pepsin and to a lesser extent by the action of standard rennet, which contains impurities of pepsin. This is also evidenced by the formation of casein bands on electropherograms of α_{S1} - and β -casein proteolysis under the action of pepsin and standard rennet (Figures 3 and 4).

A low level of cleavage of κ -casein (mainly one peptide bond 105-106) is characteristic under the action of purified chymosin, the main component of the standard milk coagulation enzyme (Hyslop, 2003). A similar low level of κ -casein proteolysis is shown by the CLM coagulant.

Conclusion

1. Carpathian liquid milk (CLM) coagulant is characterized by relatively high coagulant activity, which increases during its storage. CLM coagulant exhibits maximum proteolytic activity 8100 SU at 18-th month of its storage. During further storage, CLM coagulant reduces its activity, therefore, in order to ensure the required quality characteristics of cheeses, it is necessary to use the coagulant in a larger amount.
2. To form a strong clot, CLMCP is recommended to be added to milk heated to a temperature of 32-35°C, in an amount that ensures the beginning of coagulation within 20-25 minutes.
3. Analysis of the concentration and composition of proteolysis products of homogeneous α_{S1} -, β - and κ -casein fractions indicates that the main enzyme in the composition of CLM coagulant is chymosin. It is confirmed by the high specificity for the κ -casein cleavage and the lowest activity among the studied milk-clotting preparations in relation to α_{S1} - and β -casein substrates. In further studies, it is important to study in more detail the structure and biological activity of proteolysis products, as well as their influence on the product's taste properties.

Reference

- Barbano D.M., Rasmussen R.R. (1992), Cheese yield performance of fermentation-produced chymosin and other coagulants, *Journal of Dairy Science*, 75(1), pp. 1-12, [https://doi.org/10.3168/jds.S0022-0302\(92\)77731-5](https://doi.org/10.3168/jds.S0022-0302(92)77731-5)
- Blazic M., Pavic K., Zavadlav S., Marcac N. (2017), The impact of traditional cheeses and whey on health, *Croatian Journal of Food Science and Technology*, 9(2), pp.198-203, <https://doi.org/10.17508/CJFST.2017.9.2.11>
- Braichenko O., Hrymych M., Lylo I., Reznichenko V. (2020), *Ukraine: Food and History*, Kyiv.
- Cais-Sokolińska D., Kaczyński Ł. K., Bierzuńska P., Skotarczak E., Dobek A. (2020), Consumer acceptance in context: Texture, melting, and sensory properties of fried ripened curd cheese, *International Journal of Dairy Technology*, 74(1), pp. 225-234, <https://doi.org/10.1111/1471-0307.12747>
- Cetinkaya A., Kaban G.(2021), Some quality properties and volatile compound profile of Ardahan Tel cheese, a traditional cheese in Turkey, *Ukrainian Journal of Food Science*, 9(1), pp. 18-29, <https://doi.org/10.24263/2310-1008-2021-9-1-4>

- Commission regulation (EC) No 642/2007, (2007), *Official Journal of the European Union*, L 150/4.
- Commission Regulation (EC) No 676/2008, (2008), *Official Journal of the European Union*, L 189, pp. 19–20.
- Corredig M., Salvatore E. (2016), Enzymatic coagulation of milk: In: P.L.H. McSweeney and J.A. O'Mahony (Eds.), *Advanced Dairy Chemistry: Proteins: Applied Aspects*, (Fourth edition), Springer, New York, NY, pp 287–307.
- Donnelly C., Kehler M. (2016), *The Oxford Companion to Cheese*, Oxford Companions. Oxford University Press, p. 81.
- Dimitrova, K., Vuchkov, A. (2018), Tulum cheese - cheese making technology and main characteristics, *Journal of Mountain Agriculture on the Balkans*, 21(3), pp.1-26.
- El-Sheikh M., Farrag A., Zaghoul A. (2010), Ricotta cheese from whey protein concentrate, *Journal of American Science*, 6(8), pp. 321–325.
- Farrell H.M., Jimenez-Flores R., Bleck G.T., Brown E.M., Butler J.E., Creamer L.K., Hicks L.C., Hollar C.M., Ng-Kwai-Hang K.F., Swaisgood H.E. (2004), Nomenclature of the proteins of cows' milk sixth revision, *Journal of Dairy Science*, 87(6), pp. 1641–1674, [https://doi.org/10.3168/jds.S0022-0302\(04\)73319-6](https://doi.org/10.3168/jds.S0022-0302(04)73319-6)
- Fox P.F., Uniacke-Lowe T., McSweeney P.L.H., O'Mahony J.A. (2015), *Dairy Chemistry and Biochemistry* (Second edition), Springer, New York.
- Iukalo A.V. (2014), New approach for isolation of individual caseins from cow milk by the preparative electrophoresis, *Advances in Biobological Chemistry*, 4(6), pp. 382–387, <https://doi.org/10.4236/abc.2014.46043>
- Iukalo A.V. (2015), Milk-coagulation and proteolytic activity of «Glek» carpathian enzyme preparation, *Biotechnologia Acta*, 8(2), pp. 91–95, <https://doi.org/10.15407/biotech8.02.091>
- Guinee T.P. (2003), Role of protein in cheese and cheese products: In *Advanced dairy chemistry Volume 1: Proteins (Third edition). Part B*, edited by P.F. Fox and P.L.H. McSweeney, Kluwer academic / Plenum publishers, New York, pp. 1083–1174.
- Hernik J., Walczycka M., Sankowski E., Harris B.J. (2022), *Cultural Heritage—Possibilities for Land-Centered Societal Development*, Springer Nature, Berlin/Heidelberg, , <https://doi.org/10.1007/978-3-030-58092-6>
- Hyslop D.B. (2003), Enzymatic coagulation of milk: In: P.F. Fox and P.L.H. McSweeney (Eds.), *Advanced Dairy Chemistry: Proteins* (Third edition), Part B, Kluwer academic / Plenum publishers, New York, pp. 839–878.
- Kačaniová M., Nagyová L., Štefániková J., Felsöciová S., Godočí-ková L., Haščík P., Horská E., Kunová S. (2020), The characteristic of sheep cheese “Bryndza” from different regions of Slovakia based on microbiological quality. *Potravinárstvo Slovak Journal of Food Sciences*, 14, pp. 69–75, <https://doi.org/10.5219/1239>
- Kamel B., Boubaker K., Attia H. (2013), Implementation of ricotta cheese production process in Tunisia, *International Food Research Journal*, 20(5), pp. 2343–2348.
- Karagozlu C., Kilic S., Akbulut N. (2009), Some characteristics of cimi tulum cheese from producing goat milk, *Bulgarian Journal of Agricultural Science*, 15(4), pp. 292–297.
- Law B. A., Tamime A.Y. (2010), *Technology of cheesemaking (Second Editon)*, Blackwell Publishing Ltd, Oxford, <https://doi.org/10.1002/9781444323740>
- Najgebauer-Lejko D., Domagała J., Walczycka M. (2022), Traditional Cheeses from the Malopolska Region. In: *Cultural Heritage—Possibilities for Land-Centered Societal Development*, Springer Nature, Berlin/Heidelberg, Germany, pp. 171–190.
- Nalepa B., Markiewicz L.H. (2023), Microbiological biodiversity of regional cow, goat and ewe milk cheeses produced in Poland and antibiotic resistance of lactic acid bacteria isolated from them, *Animals*, 13(1), pp.168, <https://doi.org/10.3390/ani13010168>

- Pappa E.C., Samelis J., Kondyli E., Pappas A.C. (2016), Characterisation of Urda whey cheese: Evolution of main biochemical and microbiological parameters during ripening and vacuum packaged cold storage, *International Dairy Journal*, 58, pp. 54–57, <https://doi.org/10.1016/j.idairyj.2015.12.016>
- Rasovic M., Nikolic N., Rasovic R. (2017), Quality of “urda” obtained after production of montenegrin semi-hard cheese, *Food Research*, 1(5), pp. 166–170, <https://doi.org/10.26656/fr.2017.5.107>
- Rybak O. (2016), Milk fat in structure formation of dairy products: a review, *Ukrainian Food Journal*, 5(3), pp. 499–514, <https://doi.org/10.24263/2304-974X-2016-5-3-9>
- Ryzhkova T., Odarchenko A., Silchenko K., Danylenko S., Verbytskyi S., Heida I., Kalashnikova L., Dmytrenko A. (2023), Effect of Herbal Extracts Upon Enhancing the Quality of Low-Fat Cottage Cheese, *Innovative Biosystems and Bioengineering*, 7(2), pp. 22–31, <https://doi.org/10.20535/ibb.2023.7.2.268976>
- Semjon B., Reitznerová A., Poláková Z., Výrostková J., Maľová J., Koréneková B., Lovayová V. (2018), The effect of traditional production methods on microbial, physico-chemical and sensory properties of ‘Slovenská bryndza’ Protected Geographical Indication cheese, *International Journal of Dairy Technology*, 71(3), pp. 709–716, <https://doi.org/10.1111/1471-0307.12522>
- Sharma N., Sharma R., Rajput Y. S., Mann B., Sight R., Gandhi K. (2021), Separation methods for milk proteins on polyacrylamide gel electrophoresis: Critical analysis and options for better resolution, *International Dairy Journal*, 114, 104920, <https://doi.org/10.1016/j.idairyj.2020.104920>
- Slyvka I., Tsisaryk O., Dronyk O., Musiy L. (2018), Strains of lactic acid bacteria isolated from traditional Carpatian cheeses, *Regulatory Mechanisms in Biosystems*, 9(1), pp. 62–68, <https://doi.org/10.15421/021808>
- Teplý M., Mašek J., Havlová J. (1976), *Syrídla živočišná a mikrobiální (Animal and microbial rennets)*, SNTL – Nakladatelství technické literatury, Praha.
- Tremblay L., Laporte M.F., Onil J. U., Dupont D., Paquin P. (2003), Quantitation of proteins in milk and milk products: In: P.F. Fox and P.L.H. McSweeney (Eds.), *Advanced Dairy Chemistry: Proteins* (Third edition), Part A, Kluwer academic / Plenum publishers, New York, pp. 49–138.
- Trichopoulou A., Soukara S., Vasilopoulou E. (2007), Traditional foods: A science and society perspective. *Trends in Food Science & Technology*, 18, pp. 420–427, <https://doi.org/10.1016/j.tifs.2007.03.007>
- Walstra P., Wouters Jan T.M., Geurts T.J. (2006), *Dairy science and technology* (Second edition), CRC Press, NY.
- Weichselbaum E., Benelam B., Soares Costa H. (2009), *Traditional foods in Europe*. Norwich: EuroFIR Project, Available at: https://www.eurofir.org/wp-admin/wp-content/uploads/EuroFIR%20synthesis%20reports/Synthesis%20Report%206_Traditional%20Foods%20in%20Europe.pdf
- Yukalo V.G., Storozh L.A., Datsyshyn K.Y., Krupa O.M. (2018), Electrophoretic systems for the preparative fractionation of proteins-precursors of bioactive peptides from cow milk, *Food Science and Technology*, 12(2), pp. 26–32, <https://doi.org/10.15673/fst.v12i2.932>
- Yukalo V., Krupa O., Storozh L. (2019), Characteristics of proteolytic processes during the isolation of natural casein phosphopeptides, *Ukrainian Food Journal*, 8(1), pp. 61–69, <https://doi.org/10.24263/2304-974X-2019-8-1-7>

Comparative study of lipase preparations for enzymatic degumming of sunflower oil

Tamara Nosenko, Diana Zhupanova

National University of Food Technologies, Kyiv, Ukraine

Abstract

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Corresponding author:

Tamara Nosenko
E-mail:
tamara_nosenko@
ukr.net

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Introduction. Degumming is a crucial stage in the production of refined vegetable oil. Enzymatic degumming has been applied as a mean to improve process efficiency, and currently, many new lipase preparations are available. The present study aimed to assess the efficiency of their application on the degumming process of sunflower oil.

Materials and methods. Enzyme preparations Lecitase® Ultra, Quara® Boost, and Quara Low P were received from Novozyme (Denmark). Phosphorus content in oil ash was detected photometrically. Acid, peroxide, and saponification values were determined by standard methods. Antioxidant properties of the oil were estimated based on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity.

Results and discussion. Enzymatic degumming of sunflower oil results in an increase in oil yield compared with water degumming. Degumming with Quara® Boost preparation results in 98% oil yield, which was 1.5% higher than in the case of water degumming. Using Lecitase® Ultra and Quara Low P increased oil yield by 1 and 0.5%, respectively, compared with water degumming. The phospholipid content decreased from 0.4% in crude oil to 0.2% after water degumming, meanwhile, the application of enzyme preparations Lecitase® Ultra and Quara Low P reduced the content of phospholipids to 0.08% and 0.06%, respectively. The lowest phospholipid content, 0.04%, was in the sunflower oil after degumming with phospholipase C (Quara® Boost), which corresponds to 16 mg of phosphorus per kg of oil. The saponification value of sunflower oil degummed with phospholipase C preparation proved the formation of diacylglycerols in oil, 191.5 mg KOH/g. The highest free fatty acid content in sunflower oil was after degumming with Lecitase® Ultra: the acid value increased from 0.86 mg KOH/g in crude oil to 2.7 mg KOH/g in degumming oil. However, Quara Low P preparation also has phospholipase A1 activity, so, the acid value decreased slightly compared with crude oil. Degumming with Quara® Boost preparation did not affect the free fatty acid content in sunflower oil, and the acid value was even lower than in the oil degummed with water. The peroxide value of sunflower oil was <1 meq O/kg after enzymatic degumming, meanwhile the peroxide value of sunflower oil after water degumming was 2.6 meq O/kg. All oil samples had a similar antioxidant capacity that was 30-36% of scavenged DPPH radical.

Conclusions. The most effective enzyme preparation for sunflower oil degumming was Quara® Boost with phospholipase C activity. Application of Quara® Boost results in the highest oil yield, the lowest content of phospholipids and free fatty acids, low peroxide value, and high antioxidant capacity of oil.

Introduction

Phospholipids of vegetable oil are valuable substances, which can positively impact human health when included in a diet (Xie, 2019). They also have outstanding functionality and could be used as emulsifiers in food systems. Depending on the kind of vegetable oils and the method of their production, they contain 0.3–2.5% phospholipids (O'Brien, 2004). The solvent-extracted oils contain more phospholipids than the pressed oils (Wang and Johnson, 2001). However, the presence of phospholipids negatively affects such stages of oil processing as neutralization, bleaching, deodorization, and hydrogenation (Hamm et al., 2013).

The phospholipids are polar lipids and their solubility is not sufficiently stable and depends on their hydrophilicity. The solubility of a phospholipid molecule in oil decreases as its hydrophilicity increases. In addition, the phospholipids may lose their solubility and form a precipitate in vegetable oils during crude oil storage (Hamm et al., 2013).

While phospholipids are typically considered undesirable in oil processing, they can still be utilized as valuable additives in food or feed production. Thus, modern processing of vegetable oils includes the stage of phospholipid removal, which is also known as oil degumming. Traditionally, phospholipids are removed by water or acid degumming followed by alkaline refining. Only part of the more polar phospholipids is removed by water treatment. Such a group of phospholipids is called hydratable. The other part of the phospholipids could not be removed by water treatment, and therefore, acid degumming is required to remove hydratable and non-hydratable phospholipids from oil. Nonetheless, acid degumming is not a sustainable method of oil processing (Hamm et al., 2013).

The use of enzymes in the food industry is noted as ongoing a developing area in advanced food production technology (Ivanov et al., 2021). The Lurgi Company first proposed enzymatic degumming Aalrust et al., 1992). This method allows converting of non-hydratable phospholipids into hydratable ones increasing oil yield and reducing the application of chemicals, generation of wastes, consumption of energy, and overall costs compared with traditional degumming (Loren et al., 2014) as well as ensures low phospholipid content in the treated oil.

Enzymes such as phospholipase A (PLA), phospholipase C (PLC), and mixtures of phospholipase C and A (PLC/PLA) currently are used for enzymatic degumming (Dijkstra, 2010). Phospholipases of the A type hydrolyze phospholipids producing lysophospholipids and free fatty acids (Heinze et al., 2013). Phospholipase A1 (PLA1) and phospholipase A2 (PLA2) remove the fatty acid from positions 1 and 2 respectively with respect to glycerol (Figure 1, adapted from Guiotto et al., 2015). Phospholipases of the C type hydrolyze phospholipids into diacylglycerol and phosphoric esters (Semproli et al., 2021).

Initially, only two enzyme preparations, such as Lecitase® 10L (porcine pancreas) with PLA2 activity and Lecitase® Novo (microbial lipase produced by fungi *Fusarium oxysporum* and *Thermomyces lanuginose*) with PLA1 activity were used in the industry for oil degumming (Guerrand, 2017). Recently, phospholipase C (Purifine®) and lipid acyl transferase (LysoMax®) with PLA2 activity have also become commercially available. These enzymes have different specificities (Dijkstra, 2010). The Lecitases® and the LysoMax® enzymes catalyze the hydrolysis of all common phospholipids and differ in this respect from the Purifine® enzyme, which is specific for phosphatidyl choline and phosphatidyl ethanolamine. These phospholipids are hydrolyzed to oil-soluble diacylglycerol and water-soluble phosphate esters. Since diacylglycerols remain in the oil during refining, they contribute to the oil yield. The sterol and stanol esters formed as a consequence of the phospholipid hydrolysis catalyzed by the LysoMax® enzyme. In addition, all enzymes result in less oil being emulsified by the gums, which also contributes to an oil yield increase.

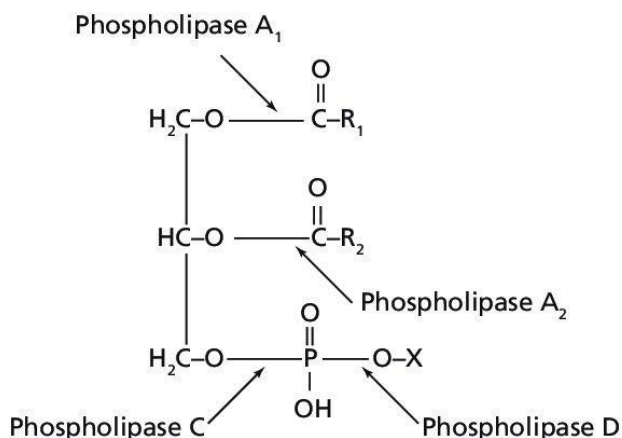


Figure 1. Action of different types of phospholipases (Adapted from Guiotto et al., 2015)

The Lecitase® Ultra enzyme is a microbial lipase (E.C.3.1.1.3) from *Thermomyces lanuginosus/Fusarium oxysporum*, which exhibits phospholipase A1 activity maximally at pH5.0 and can hydrolyze phospholipids as well as triacylglycerols. When the temperature is over 40°C, the phospholipase activity predominates, and the lipase activity is partly suppressed (Yang et al., 2006). It was shown that degumming of rapeseed and soybean oil with Lecitase® Ultra for 5 hours at 50 °C resulted in a phospholipids content of less than 10 mg/kg. Similar results were obtained by Sampaio et al., (2015) during enzymatic degumming of the same oil with a Lecitase Ultra®. The phosphorus content in a degummed oil was less than 10 mg/kg at an enzyme dosage of 30 mg/kg and a treatment time of 10–120 min. While a good degumming efficiency can already be obtained after a relatively short reaction time, it was observed that a longer reaction time, 1–2 hours, is required for the degradation of all phospholipids, which results in an oil yield increase. It was shown that Lecitase Ultra® has no specificity for phospholipids, but the rate of different phospholipids conversion is various. After 60 min, 80% of phosphatidylethanolamine was hydrolyzed to its lysoform, while only 40% of phosphatidylinositol was converted during the same time.

Contrary to common belief, it was suggested that the enzymes were incapable catalyzing the hydrolysis of non-hydratable phosphatides under industrial conditions (Dijkstra, 2010). However, the subsequent study did not confirm this suggestion and indeed demonstrated the decrease of phosphorus content in crude oil to < 10 mg/kg during the enzymatic degumming trial (Yang B. et al., 2006; Yang et al., 2008).

An enzymatic degumming of soybean oil was carried out at a capacity of 400 tons/day by applying microbial phospholipase A1 from *Thermomyces lanuginosus/Fusarium oxysporum* (Yang et al., 2008). The phosphorus content in oil was less than 10 mg/kg when the pH was in the range of 4.8–5.1, and the oil loss was minimal under these conditions. It was shown that content of glycerophospholipids and lysophospholipids in the gums was 45.7 and 54.3%, respectively.

Similar results were obtained for rapeseed oil at the degumming plant (Yang B. et al., 2006). Response surface methodology was used for the optimization of the enzymatic degumming process. Enzyme dosage, temperature, and pH were important determining factors, affecting oil degumming. The optimal set of variables was an enzyme dosage of 39.6

mg/kg, a temperature of 48.3°C, and a pH of 4.9. The phosphorus content could be reduced to 3.1 mg/kg at the optimal levels of the tested factors. At the enzymatic degumming plant, when the pH was 4.6 to 5.1, the corresponding phosphorus content of degummed rapeseed oil could be reduced to less than 10 mg/kg, which met the demands of the physical refining process. In another study, the efficiency of enzymatic degumming was assessed using the same Lecitase®-Ultra with different qualities of crude rice bran oil. The phosphorus content in the oil was reduced to ~10 mg/kg from an initial level of 390 mg/kg after 2 h of incubation at 50°C (Manjula et al., 2011).

A comparison of enzymatic degumming of screw-pressed crude jatropha oil with Lecitase Ultra (phospholipase A1) and LysoMax (phospholipase A2) demonstrated that degumming with phospholipase A2 was less effective (Gofferjé et al., 2014). Phospholipase A1 showed the highest reaction rate at 50 °C, 700 rpm stirring, 3 mL of water per 100 g of oil, and with 75 mg/l of added phospholipase, the pH was adjusted to 5. Laboratory experiments showed that enzymatic degumming of jatropha oil with phospholipase A1 at the adapted parameters enables the phosphorus content to be reduced to a level below 4 mg/l.

For industrial applications enzymatic activity of phospholipase A1 was improved by protein engineering (An et al., 2017). Obtained mutants had higher phospholipase activity and identical optimal pH values with wild-type, while the optimal temperature was decreased to 50°C, and the k_{cat}/K_M was improved. Modified lipases decreased phosphorus content lower than 8.3 mg/kg within 3 h, which was highly improved compared with the wild-type. One mutant decreased phosphorus content to less than 5 mg/kg within 5 h.

Recently, new lipase preparations appeared on the market, particularly Novozyme Quara® Boost (phosphoinositide phospholipase C and phospholipase C) and Novozyme Quara Low P (phospholipase A1). Since every enzyme has a specific affinity for different types of phospholipids, it is necessary to study the peculiarities of oil degumming by each lipase preparation, taking into account the properties of the oil, such as the composition of phospholipids. However, there is currently a lack of information regarding the effectiveness of enzyme preparations in degumming sunflower oil. Therefore, this study aimed to investigate the influence of a new lipase preparation on the degumming of sunflower oil.

Materials and methods

Enzyme preparations and oil sample

Enzyme preparations Lecitase® Ultra, Quara® Boost, and Quara Low P were received from Novozyme (Denmark). The properties of each enzyme are presented in Table 1.

Table 1

Characteristics of enzyme preparations

Enzyme	Characteristics
Lecitase® Ultra	A lipase produced by the genetically modified filamentous fungus <i>Aspergillus oryzae</i> ; has phospholipase A1 activity.
Quara® Boost	A lipase produced by the spore-forming bacterium <i>Bacillus licheniformis</i> ; consists of two types of phospholipase C: phosphoinositide phospholipase C and phospholipase C.
Quara Low P	A lipase produced by ascomycetous fungus <i>Talaromyces leycettanus</i> ; has phospholipase A1 activity.

For enzymatic degumming, 0.5% enzyme solutions were prepared by dispersing the phospholipase in distilled water.

Enzymatic degumming

Crude sunflower oil was purchased from the local market. Crude sunflower oil, 100 g, was placed into a 250-ml conical flask. The oil was heated to about 60 °C followed by the addition of 5% of 0.5% enzyme solution in distilled water. The mixture was thoroughly mixed for 1 min and stirred at 60 °C for 1 h. The enzymatic degumming reaction was followed by enzyme inactivation at 80 °C for 10 min. After that reaction mixture was kept for 30 min at room temperature. The 10 ml of the reaction mixture was put into a graduated centrifuge tube and the gums were separated from the oil by centrifugation at 2000 rpm for 10 min. The control sample was prepared in the same way using 5% water for degumming instead of enzyme solution.

Oil yield analysis

The volume of degummed oil was measured after gums separation and oil yield (X, % of crude oil) was calculated as:

$$X = (b/9.52) \cdot 100,$$

where 9.52 was the volume of the crude oil in 10 ml degumming mixture before centrifugation in ml, and b was the volume of separated oil in ml.

Phosphorus content analysis

The oil samples were ashed for phosphorus content determination according to (Yang, et al., 2006). 0.7 g of MgO and 0.6 to 0.7 g of oil were weighed and heated in the oven at 110 °C for 10 min. After that, the samples were carbonized by heating on the hot plate and then ashed in an electric muffle furnace at 600 °C until a constant mass was achieved.

The phosphorus content of the ash was determined according to American Oil Chemists' Society (AOCS) method Ca 12–55 (1997). Cold ash was transferred to a 100 ml flask, 10 to 12 ml water, and 20 ml 2N H₂SO₄ were added. The solution was heated to dissolve the residue. After that 20 ml molybdenum reagent was added and the solution was heated in boiling water bath for 30 min. The volume of the cold solution was adjusted to 100 ml. Photometric analysis was carried out on photometer KPhK-3 (AS ZOME) at 750 nm.

A standard solution of KH₂PO₄ (concentration 10 µg/ml) was used for the calibration curve obtaining. The calibration solution set was prepared in the same condition as the ash samples. The concentration of KH₂PO₄ in the calibration solution set was adjusted from 0.05 to 2.0 µg/ml.

Phosphorus content (P, % of oil mass) was calculated as follows:

$$P = 0.01 d/m,$$

where d was the phosphorus content in ash solution according to a calibration curve, µg/ml; m was oil mass, g.

The content of phospholipids as stearoiloleil phosphatidylcholine (PC, % of oil mass) was calculated as follows:

$$PC = 25.4 P$$

where P was the phosphorus content, % of oil mass; 25.4 was a coefficient for calculation of the stearoiloleil phosphatidylcholine mass on the base of phosphorus content.

Chemical parameters of oils

The acid value of oil samples was determined by the titrimetric method according to ISO 660:2020.

The oil's peroxide value was determined using the iodometric method according to ISO 3960:2017.

The saponification value of the oil was determined by the titrimetric method according to ISO 3657:2020.

Antioxidant activity

The radical scavenging capacity was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method (Uluata et al., 2012). For the DPPH test, DPPH was dissolved in a small volume of ethyl acetate and diluted with ethyl acetate by adjusting the absorbance to 0.700 ± 0.020 at 520 nm. 100 mg of oil was weighed in a test tube, and 15 ml DPPH' free radical solution was added. The sample was agitated and absorbance was measured at 520 nm against ethyl acetate. After 30 min of incubation in darkness absorbance was measured at 520 nm against ethyl acetate.

The results of the DPPH test were expressed as% of DPPH' free radicals that were scavenged by antioxidants in 100 mg oil (A, %):

$$A = (1 - D_1/D_0) \cdot 100,$$

where D_0 - was the absorbance of the reaction solution at 520 nm before incubation, and D_1 - was the absorbance of the reaction solution at 520 nm after incubation.

Statistical analysis

Each sample was analyzed in triplicate, and the results were reported as mean \pm standard deviation. Differences were considered to be significant at validity $\alpha=0.95$.

Results and discussion

Effect of enzymatic degumming on the sunflower oil yield

The oil yield is a crucial factor for determining the efficiency of the degumming process and overall oil refining. The yield of refined oil determines the profitability of vegetable oil production. The quantity of oil loss with gums is directly correlated with the oil yield, meaning that a higher oil yield results in less oil loss with gums.

According to our findings, the use of water as a degumming agent resulted in the lowest oil yield (Figure 2).

Enzymatic degumming results in higher oil yield compared with water degumming. The highest oil yield was found from the degumming process with the enzyme Quara® Boost (98%), followed by Lecitase® Ultra (97.5%) and Quara Low P (97%).

Our results do not completely agree with the Novozyme company data which had reported about 0.9 and 0.6% higher yield of soybean and rape seed oil, respectively, after degumming with Quara Low P compared to Lecitase® Ultra degumming. Different results of Lecitase® Ultra and Quara Low P action on sunflower oil degumming could be explained by the composition of phospholipids in this oil (Lilbæk et al., 2017).

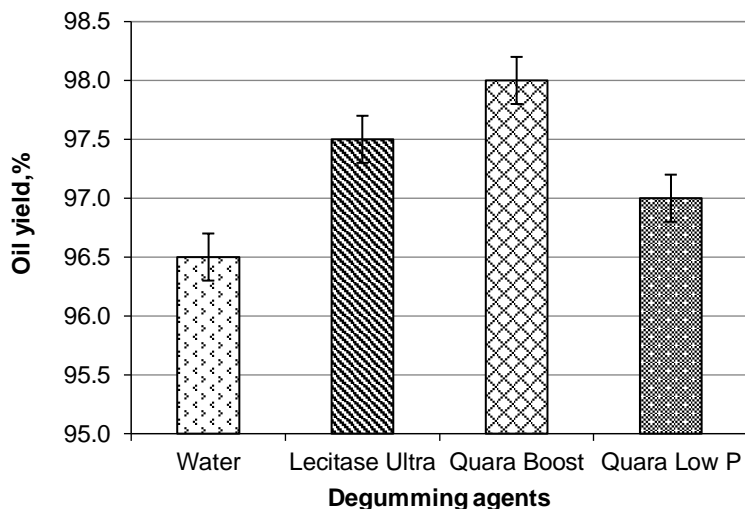


Figure 2. Effect of enzyme degumming on the sunflower oil yield

The increase of oil yield under degumming with phospholipase C (Quara® Boost) is due to diacylglyceride formation and their remaining in the oil. Degumming with phospholipase A1 (Lecitase® Ultra and Quara Low P) results in free fatty acids release. Despite the same enzyme activity, mainly phospholipase A1 activity, the advantages of Quara Low P are its thermal stability (denaturation temperature is around 80 °C) and an optimal low pH range of around 4. In addition, the increase in oil yield was due to a decrease in oil quantity in gums as a result of emulsification for both types of phospholipase.

Effect of enzyme degumming on the content of phospholipids in sunflower oil

The content of phospholipids in degummed oil is an indicator of the efficiency of the degumming capacity. The content of phospholipids in all oil samples decreased as a result of degumming (Figure 3).

Obtained results demonstrated, that about half of the phospholipid content in sunflower oil is hydratable phospholipids. The phospholipids content decreased from 0.4% in crude oil to 0.2% in water-degummed oil. Adding enzyme preparation decreased phospholipids content significantly. The lowest phospholipids content was in the sunflower oil after degumming with phospholipase C (Quara® Boost), that is 0.04% of stearoiloleillectin, which, in turn, corresponds to 16 mg phosphorus /kg of oil. The degumming with Lecitase® Ultra and Quara Low P reduced phospholipids content to 0.08% and 0.06%, respectively.

Previously, even lower phosphorus content was shown in soybean and rape seed oil after Lecitase® Ultra enzyme degumming (Sampaio et al., 2015; Yang B. et al., 2006; Yang et al., 2008). The phosphorus content in these oils was less than 10 mg/kg after Lecitase® Ultra enzymatic degumming. Moreover, the authors (Yang B. et al., 2006) received rape oil with content 3.1 mg phosphorus /kg after optimization of degumming parameters. Noteworthy, the time of enzyme treatment was very different in this research, reaction time varied from 5 hours to 1 hour. According to our results, it was obvious that a duration of 1-2 hours is sufficient for phospholipid content decrease.

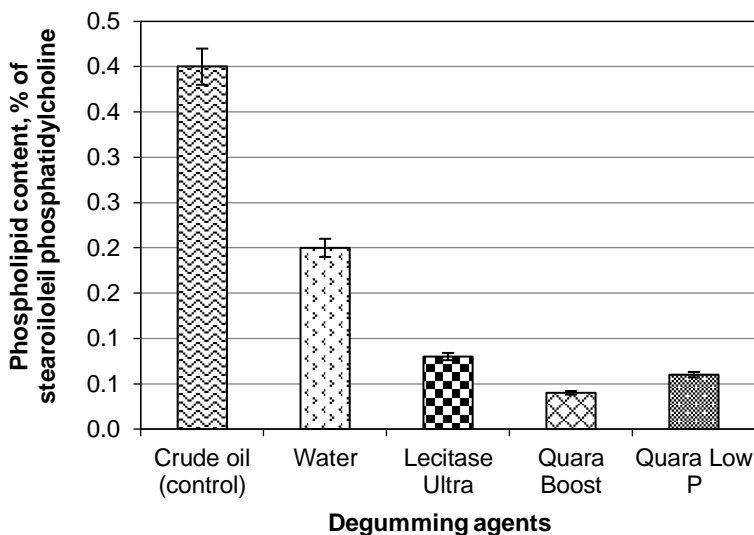


Figure 3. Effect of enzymatic degumming on phospholipids content in sunflower oil

Significant decrease of the phospholipid content in sunflower oil in the presence of phospholipase C is due to production of the hydrophobic diacylglycerols (they remain in oil), and hydrophilic phosphate esters, which are moved to the gums. At the same time, it is possible that not all lysophospholipids, which are forming under the action of phospholipase A1, have high hydrophilicity and are moving to the gum precipitate. Such lysophospholipids contain fatty acid residuals and accordingly have some affinity to triacylglycerol. This can be the reason for higher phospholipids content in sunflower oil after enzymatic degumming with phospholipase A1 preparation.

Higher effectiveness of new phospholipase A1 preparation Quara Low P corresponds to Novozyme data. According to these data phospholipids content in soybean oil was in the range of 3.1 to 4.6 mg/kg phosphorus after enzyme degumming in the presence of Quara Low P (Lilbæk et al., 2017).

The formation of diacylglycerols in oil after degumming with phospholipase C preparation was proved by the decrease in the saponification value of sunflower oil (Table 2). This sample of sunflower oil had the lowest saponification value which is the lowest content of ester bonds. The saponification values of sunflower oil after degumming with phospholipase A1 preparations were lower than the oil sample after water degumming.

Table 2

Saponification value of sunflower oil

Oil sample	Saponification value, mg KOH/g
Crude oil	220.5±3.4
Water degumming	204.1±2.4
Lecitase Ultra degumming	195.8±3.7
Quara Boost degumming	191.5±3.2
Quara Low P degumming	196.6±4.4

Effect of enzyme degumming on the free fatty acids content in the degummed oil

It is known that water degumming can remove acid phosphatides from oil and, as a result, acid value of oil decreases (O'Brien, 2004). According to our data acid value of sunflower oil was reduced after water degumming (Figure 4).

It is obvious that phospholipase A1 enzymatic degumming will increase free fatty acids content in the oil. Indeed, our results revealed that acid value had increased significantly after degumming with Lecitase® Ultra, which means acid value increased from 0.86 mg KOH/g in crude oil to 2.7 mg KOH/g after Lecitase® Ultra degumming. Remarkably, under the action of Quara Low P preparation, which has also phospholipase A1 activity, the acid value decreased slightly compared with crude oil.

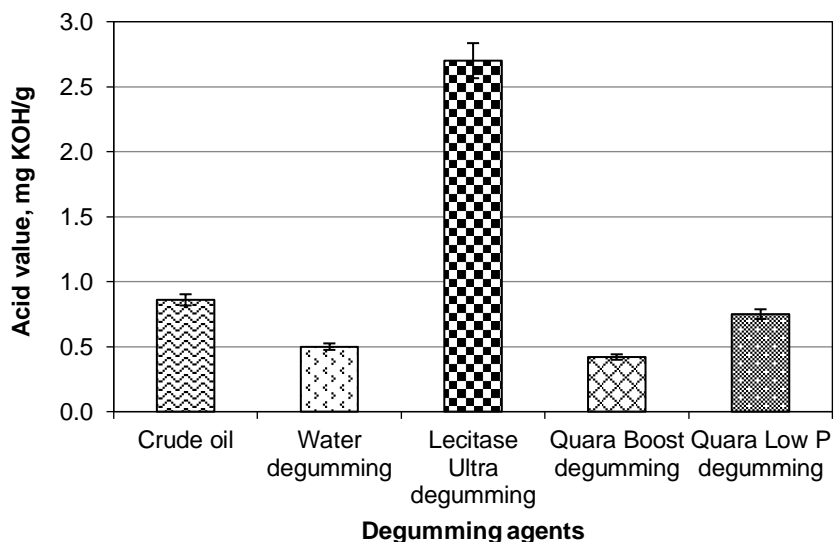


Figure 4. Effect of enzymatic degumming on the acid value of sunflower oil

It could be suggested that such a remarkable increase of oil acidity under Lecitase® Ultra degumming could be due to the activity of this preparation toward triacylglycerol hydrolysis. Although the phospholipid content of two oil samples after phospholipase A1 degumming was very close, triacylglycerol hydrolysis can be responsible for that big acidity difference between these oil samples.

Degumming with Quara® Boost preparation resulted in the lowest free fatty acids content in sunflower oil: the acid value had decreased twice compared with crude oil. We suggest this is due to removing of acid phosphatides from the oil.

Effect of enzymatic degumming on the oxidative stability of the oil

The important property of vegetable oils is their oxidation stability, which depends on many factors such as the composition of fatty acids, the content of antioxidants, and the content of oxides and peroxides (Demidova et al., 2019, Nosenko et al., 2019).

The important indicator of oil oxidation degree and peroxide content is the peroxide value. The peroxide values of sunflower oil after degumming with studied enzyme preparations were significantly lower. The peroxide value of sunflower oil after water degumming was almost two times lower compared with crude oil (Figure 5).

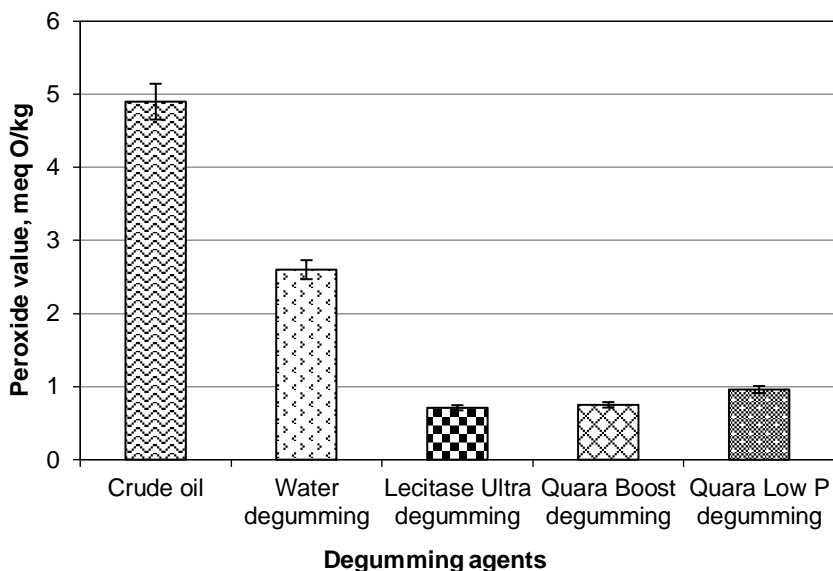


Figure 5. Effect of enzymatic degumming on peroxide value of sunflower oil

Peroxide content had reduced heavily in oil samples due to enzymatic degumming. The peroxide value of enzymatic degummed oil samples was <1 meq O/kg, which means a very low content of peroxides. The peroxide value of water-degummed sunflower oil was significantly higher compared with oil samples after enzymatic degumming.

At the same time, other data about the influence of enzymatic degumming on the oil's oxidative stability had been reported. The authors had shown an increase in the peroxide value of sunflower oil after treatment with phospholipase A1 (Lamas et al. 2014) and a decrease in oxidative stability of rapeseed oil after degumming with phospholipase C (Ye et al., 2016) and soybean oil with phospholipases A1 and C (Jiang et al., 2014).

But it is known that non-hydratable phospholipids contain phosphatidic acids and their salts as well as other salts and complexes with metal ions (O'Brien, 2004). After water degumming oil will contain non-hydratable phospholipids and substantially metal ions such as only hydratable phospholipids are removed. In turn, metal ions, especially Cu^{2+} , Fe^{3+} can promote fatty acid oxidation and increase peroxide content. We suggest, that removing all phospholipids by enzymatic degumming resulted in the higher oxidation stability and low peroxide value of sunflower oil.

Oxidative stability also depends on the antioxidant capacity of oil. The antioxidative properties of sunflower oil samples were 30-36% of scavenged DPPH radical (Figure 6).

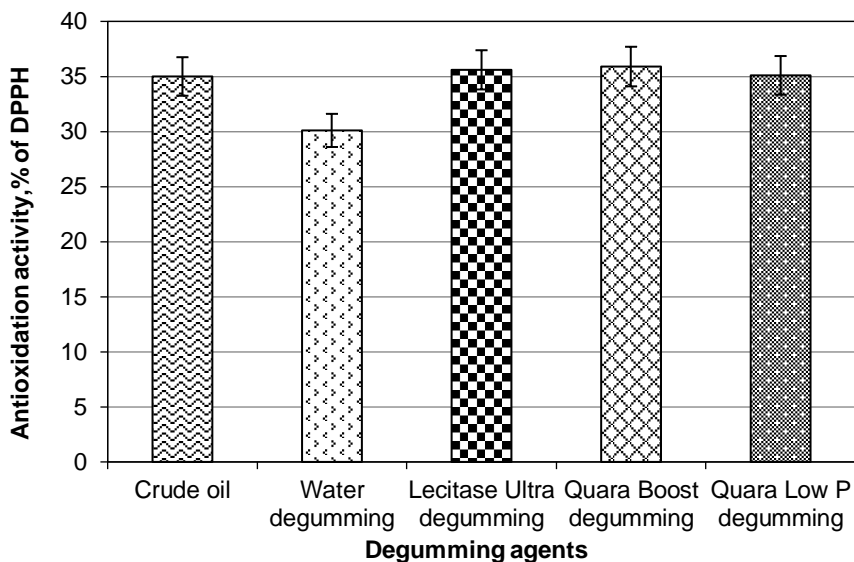


Figure 6. Effect of enzymatic degumming on antioxidant capacity of sunflower oil

The oil sample after Quara® Boost degumming had a slightly higher radical scavenging capacity, which was about 36%. On the contrary, the oil sample after water degumming had the lowest, 30%, radical scavenging capacity. Probably, this radical scavenging capacity decrease was due to the loose of some antioxidants in gums as a result of oil emulsification.

Conclusions

1. Enzymatic degumming is a highly effective tool for removing phospholipids from sunflower oil. The most effective enzyme preparation for degumming sunflower oil is Quara® Boost, which has phospholipase C activity. Quara® Boost degumming results in the highest increase of oil yield, the lowest phospholipid content (16 mg/kg phosphorus), and free fatty acids content, low peroxide value, and high antioxidant capacity of oil.
2. Lecitase® Ultra degumming of sunflower oil resulted in a 1% higher oil yield compared to water degumming, 32 mg phosphorus /kg of oil, the lowest peroxide value, and the high antioxidant capacity of oil. However, this preparation has the disadvantage of significantly increasing the oil acidity. The acid value was increased by three times compared to crude oil.
3. The use of Quara Low P with phospholipase A1 activity for sunflower oil degumming showed lower efficiency with only a 0.5% higher oil yield compared to water degumming. However, the advantages of Quara Low P degumming were a higher degree of phospholipid removal (24 mg phosphorus/kg of oil), a low increase of oil acidity compared to Lecitase® Ultra, high oxidation stability of the oil, as well as thermal stability and low pH optimum of this preparation.

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References

- Aalrust E., Beyer W., Ottofrickestein H., Penk G., Plainer H., Rainer R. (1992), An enzymatic method for reducing the amount of phosphorus-containing components in vegetable and animal oils, European patent 0513709.
- An Q., Wang F., Lan D., Khan F. I., Durrani R., Yang B., Wang Y. (2017), Improving phospholipase activity of PLA₁ by protein engineering and its effects on oil degumming, *European Journal of Lipid Science and Technology*, 119(2), pp. 110-133, <https://doi.org/10.1002/ejlt.201600110>.
- Demidova A., Nosenko T., Shemanska E. (2019), Tocopherol concentrates obtaining from deodorizer distillates of sunflower oil, *Ukrainian Journal of Food Science*, 7(2), pp. 198-206, <https://doi.org/10.24263/2310-1008-2019-7-2-4>.
- Demidova A., Nosenko T., Bahmach V., Shemanska E., Molchenko S. (2021), Study on antioxidants extraction from oak bark and their use for oxidation stability of sunflower oil, *Ukrainian Food Journal*, 10(3), pp.552-563, <https://doi.org/10.24263/2304-974X-2021-10-3-9>.
- Dijkstra A.J. (2010), Enzymatic degumming, *European Journal of Lipid Science and Technology*, 112(11), pp. 1178-1189, <https://doi.org/10.1002/ejlt.201000320>.
- Gofferjé G., Motulewicz J., Stäbler A., Herfellner T., Schweiggert-Weisz U., Flöter E. (2014), Enzymatic degumming of crude jatropha oil: Evaluation of impact factors on the removal of phospholipids, *Journal of American Oil Chemistry Society*, 91(12), pp. 2135-2141, <https://doi.org/10.1007/s11746-014-2559-2>.
- Guerrand D. (2017), Lipases industrial applications: focus on food and agroindustries, *Oilseeds and Fats Crops and Lipids*, 24(4), pp. D403, <https://doi.org/10.1051/ocl/2017031>.
- Guiotto N.E., Tomás M.C., Diehl B.W.K. (2015), Sunflower lecithin, In: *Polar Lipids*, pp. 57-73, <https://doi.org/10.1016/B978-1-63067-044-3.50007-8>.
- Hamm W., Hamilton R. J., Calliau G. (2013), *Edible oil processing*, John Wiley & Sons, Ltd, The Atrium, Southern Gate, Chichester, UK.
- Heinze M., Roos W. (2013), Assay of phospholipase A activity, *Methods of Molecular Biology*, 1009, pp. 241-219, https://doi.org/10.1007/978-1-62703-401-2_22.
- Ivanov V., Shevchenko O., Marynin A., Stabnikov V., Gubenia O., Stabnikova O., Shevchenko A., Gavva O., Saliuk A. (2021), Trends and expected benefits of the breaking edge food technologies in 2021–2030, *Ukrainian Food Journal*, 10(1), pp. 7-36, <https://doi.org/10.24263/2304-974X-2021-10-1-3>.
- ISO 3960:2017, International Organization for Standardisation, Animal and vegetable fats and oils: determination of peroxide value.
- ISO 660:2020, International Organization for Standardisation, Animal and vegetable fats and oils: determination of acid value and acidity.
- ISO3657:2020, International Organization for Standardisation, Animal and vegetable fats and oils: determination of saponification value.
- Jiang X., Chang M., Wang X., Jin Q., Wang X. (2014), A comparative study of phospholipase A₁ and phospholipase C on soybean oil degumming, *Journal of American Oil Chemistry Society*, 91(12), pp. 2125–2134, <https://doi.org/10.1007/s11746-014-2555-6>.

- Lamas D.L., Crapiste G.H., Constenla D.T. (2014), Changes in quality and composition of sunflower oil during the enzymatic degumming process, *LWT – Food Science Technology*, 58(1), pp. 71–76, <https://doi.org/10.1016/j.lwt.2014.02.024>.
- Lilbæk H.M., Nielsen P.M. (2017), Expanding the enzymatic degumming toolbox with a new phospholipase C, *2017 AOCS Annual Meeting: Abstracts of 2017 AOCS Annual Meeting, Processing, April 30– May 3, 2017, Orlando, Florida, USA*, p. 7, <https://www.aocs.org/attend-meetings/archives/2017-aocs-annual-meeting-and-short-courses?SSO=True>.
- Loren C., Dayton G., Galhardo F. (2014), Enzymatic degumming, In: Farr W.E. and Proctor A., eds., *Green Vegetable Oil Processing*, Bunge Global Innovation, White Plains, New York, USA, pp. 107–145.
- Manjula S., Anju Jose A., Soundar Divakar S., Subramanian R. (2011), Degumming rice bran oil using phospholipase-A₁, *European Journal of Lipid Science and Technology*, 113(5), pp. 658–664, <https://doi.org/10.1002/ejlt.201000376>.
- Nosenko T., Koroluk T., Usatuk S., Vovk G., Kostinova T. (2019), Comparative study of walnut and pumpkin seeds oil biological value, *Food Science and Technology*, 13(1), pp. 60–65, <https://doi.org/10.15673/fst.v13i1.1311>.
- O'Brien R.D. (2004), *Fats and oils: formulating and processing for applications*, CRC Press, Boca Raton, USA.
- Official Methods and Recommended Practices of the American Oil Chemists' Society*, AOCS, Champaign (1997), Ca 12–55.
- Sampaio K.A., Zyaykina N., Wozniak B., Tsukamoto J., De Greyt W., Stevens C.V. (2015), Enzymatic degumming: Degumming efficiency versus yield increase, *European Journal of Lipid Science and Technology*, 117(1), pp. 81–86, <https://doi.org/10.1002/ejlt.201400218>.
- Semproli R., Robescu, M. S., Cambò M., Mema K., Bavaro T., Rabuffetti M., Ubiali D., Speranza G. (2021), Chemical and enzymatic approaches to esters of sn -glycero-3-phosphoric acid, *European Journal of Organic Chemistry*, 29, pp. 4027–4037, <https://doi.org/10.1002/ejoc.202100235>
- Uluata S., Ozdemir N. (2012), Antioxidant activities and oxidative stabilities of some unconventional oilseeds, *Journal of American Oil Chemistry Society*, 89(4), pp. 551–559, <https://doi.org/10.1007/s11746-011-1955-0>.
- Wang T., Johnson L.A. (2001), Survey of soybean oil and meal qualities produced by different processes, *Journal of American Oil Chemistry Society*, 78(3), pp. 311–318. <https://doi.org/10.1007/s11746-001-0262-7>.
- Xie M. (2019), Phospholipids, In: *Encyclopedia of Food Chemistry*, Elsevier, pp. 214–217. <https://doi.org/10.1016/B978-0-08-100596-5.21597-7>.
- Yang B., Wang Y., Yang J. (2006), Optimization of the enzymatic degumming process for rapeseed oil, *Journal of American Oil Chemistry Society*, 83(7), pp. 653–658, <https://doi.org/10.1007/s11746-006-1253-4>.
- Yang B., Zhou R., Yang J., Wang Y., Wang W. (2008), Insight into the enzymatic degumming process of soybean oil, *Journal of American Oil Chemistry Society*, 85(5), pp. 421–425, <https://doi.org/10.1007/s11746-008-1225-y>.
- Yang J. G., Wang Y., Yang B., Mainda G., Guo Y. (2006), Degumming of vegetable oil by a new microbial lipase, *Food Technology and Biotechnology*, 44(1), pp. 101–104, <http://www.ftb.com.hr/images/pdfarticles/2006/January-March/44-101>.
- Ye Z., Qiao X., Luo Z., Hu C., Liu L., He D. (2016), Optimization and comparison of water degumming and phospholipase C degumming for rapeseed oil, *CyTA-Journal of Food*, 14(4), pp. 604–612, <https://doi.org/10.1080/19476337.2016.1182218>.

Analysis of the milk raw materials used in the production of Dorna Swiss cheese in different seasons

Necula Doru^{1,2}, Tamas-Krumpe Octavia¹, Feneşan Daria¹,
Mădălina Ungureanu-Iuga^{2,3}, Ognean Laurenţiu¹

1 – University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Romania

2 – "Costin C. Kirişescu" National Institute of Economic Researches (INCE), Romanian Academy, Vatra Dornei, Romania

3 – Stefan cel Mare University of Suceava, Suceava, Romania

Abstract

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Corresponding author:

Mădălina
Ungureanu-Iuga
E-mail:
madalina.iuga@
ce-mont.ro

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Introduction. The objective of this work consists of the analysis of the chemical and hygienic-sanitary properties of the milk used in the production of Dorna Swiss cheese in two seasons, along with the documentation of the technological particularities.

Materials and methods. The milk samples used in Swiss cheese manufacturing were analyzed in two seasons (summer and winter) for the chemical composition, pH, and hygienic-sanitary parameters. Correlations and Principal Component Analysis were employed to study the relationships between variables.

Results and discussion. The main particularity in the processing of Dorna Swiss cheese can be summarized as the coagulation in copper tanks of a mixture of unpasteurized milk (70%) with slow pasteurized milk at 65 °C (30%). The chemical composition and the hygienic-sanitary indices of the milk raw material are crucial for obtaining a high-quality Swiss cheese product. The evolution of the main chemical properties of milk raw material revealed important variations in the summer and winter seasons, regarding the fat, total protein, and casein content. The total somatic count values were higher in the summer season than in winter (201.75×10^3 vs. 78.45×10^3 cells/mL). The total bacteria count also presented low values, with very tight oscillations in the summer and winter seasons (81.70×10^3 vs. 87.65×10^3 cfu/mL), which were lower compared to those presented in the European standards. The fat content in summer ranged from 3.32 to 4.67% and in winter from 3.86 to 3.25%. Significant differences ($p < 0.05$) regarding the protein and casein contents were observed between seasons (3.15% and 2.55%, respectively, in summer vs. 3.32% and 2.68%, respectively, in winter). The lactose content was higher in the summer (4.62%) compared to the winter period (4.25%). The total dry matter and non-fat dry matter of milk registered lower values in summer compared to winter (11.98% and 8.54%, respectively vs. 12.86% and 8.95%, respectively). The milk samples showed lower pH values in the summer (6.37) compared to winter (6.60), the difference being significant at $p < 0.05$. According to the results, significant correlations ($p < 0.05$) were obtained between the chemical characteristics of milk and season. The overall analysis of the main chemical compounds and sanitary indices of milk raw material revealed small individual variations, which eliminates any risk of affecting the producer, processor, or consumer.

Conclusion. The evolution of hygienic-sanitary parameters of milk showed that they are in agreement with the current standards. The particularities of the Dorna Swiss cheese production are a mix of milk raw material quality and the use of copper tanks, along with slow partial pasteurization which contributes to the unique final product characteristics.

Introduction

Emmental cheese is known throughout the world as "Swiss-type" cheese, a name found in the United States, Canada, New Zealand, and Australia (Thierry et al., 2010). The Codex Alimentarius (CODEX STAN 269-1967) of the United States defines Emmental cheese as a mature cheese of hard consistency in the form of wheels, weighing 40 kg or more, with an elastic texture and with holes ("eyes") having sizes varying from those of a cherry to of a walnut (FAO, 2007). Emmental cheese production dates back to the 13th century, having been obtained for the first time in the valley of the Tal mountain, on the river Emme, in the Swiss canton of Bern. Currently, "Swiss" type cheese is internationally recognized (Fröhlich-Wyder and Bachmann, 2004) and is frequently considered a traditional product in many European countries, such as France, Germany, Austria, Ireland, and Finland (Bisig et al., 2010). In Romania, this variety of cheese is mainly represented by Dorna Swiss, produced in the mountainous area of Dorna, an area where Pinzgau cattle and a great diversity of pastures and hayfields still exist. This product also contains biologically active compounds originating from soils rich in deposits of manganese, iron, and copper which pass through the soil-plant-animal chain into milk and dairy products and provide the high nutritional value of Emmental cheese.

Swiss cheese is an extremely valuable assortment of matured cheese, obtained from cow's milk, which is presented in the form of a hard paste, with a sweet, pleasant taste and specific aroma, with a very long shelf life (Necula and Babii, 2012). The production of a high-quality Swiss cheese depends on the quality of the milk, the climatic conditions of the area, the professional training and experience of the processor and the staff involved, as well as some specific spaces, equipment, and installations. The quality of the fodder is also essential, with the best milk coming from cows raised in mountain areas located at over 800 m altitude, where the basic fodder is grass, grazed during the summer, or administered as hay during the stable period. This constitutes a very good feed, aromatic, easily digestible, and rich in protein, mineral salts, and vitamins, which ensures superior quality of the milk obtained (Leniuc, 2015). It is also important that fresh milk gets processed as quickly as possible and is not allowed to be transported over long distances, which can lead to its depreciation and, as a consequence, a decrease in the quality of the final product (Leniuc, 2015).

Dorna geographic area has a tradition in the processing of cheeses, especially Swiss cheese. Historical documents attest to the fact that in 1827, in Vatra Dornei, a Swiss industrialist, Otto Stacker, established the "Alpina" cheese factory equipped with high-performance machinery for that period, which is presented in an article in the local Vatra Dornei newspaper, since 1936. It had the capacity to process 10,000 L of milk/day, in different assortments of cheeses, including 3 types of Emmentaler, 3 types of Holland, Petit-Gryere, Blockfromage, Trappist, Gervais, and Camembert, intended for the domestic market and for export (the Dorna monitor August 23, 2017). We also mention that in 1937 a developer established the "Nufărul" cheese factory in Vatra Dornei, which processed Emmentaler as the main product. In 1948, the Enterprise for the Collection and Industrialization of Milk was established, being founded with the equipment from the Alpina and Nufarul factories and developed through national investment funds.

Among the basic conditions necessary to obtain a high-quality Swiss cheese, it should be mentioned that the cows' feed must not contain fermented fodder, and the maturation period of the cheeses must be at least 90 days. The rules of good practice must also be respected: the distance and time of transport as well as the storage time should fall within a maximum of 24 hours, in the case of the Swiss cheese. An element of safety is also the

veterinary sanitary control carried out by the Romanian Veterinary Sanitary and Food Safety Agency (ANSVSA), which analyzes from a microbiological point of view the water, milk and cheese samples every month. The analysis of the samples consists in evaluating the presence and count of *Escherichia coli*, coliform bacteria, *Clostridium perfringens*, and intestinal enterococci. In the case of milk, compliance with the current European norms regarding total somatic cell count and total bacteria count is evaluated. The examinations of the cheese are focused on establishing the degree of contamination regarding the load with coagulase-positive staphylococci.

Nowadays, mountain agriculture in the Dorna area is reduced compared to the communist period (1947–1989), with the main producers being small farms or familial households (Jujea, 2023). However, there are two certified producers in the Dorna area trying to resurrect the Swiss cheese production tradition. Their main problem in the manufacturing process is the quality of milk raw material. To our knowledge, there are no papers presenting the processing technology of Dorna Swiss cheese and the quality parameters of the milk raw material used.

Considering the above-mentioned facts, the *aim* of research was to document the technological process of Dorna Swiss cheese production and to evaluate the chemical and microbiological characteristics of the milk raw material.

Materials and methods

Materials

The research was carried out between June 2021 and April 2022 on dairy products obtained in a factory authorized for the production of Swiss cheese and Raclette in the Dorna geographic area. The research consisted of conducting documentation and investigations on the compositional and hygiene-sanitary indices of the milk raw material, as well as the technological procedures for processing an assortment of Dorna Swiss cheese, specific to this commercial unit.

Description of the studied factory

The factory was sanitary and veterinary authorized (based on law 84/1998) and registered in the Trade Register (under the I.I. Țăranu Cristian brand), being regulated to use the Călimani "Mountain Product" logo. In the Dorna area, there is a cooperative called "Cașca Șarul Dornei", which gathers milk from most farmers to deliver it to the major processors in the country. From this, the processors first select the milk necessary for the production of the Swiss cheese. At the reception of the milk, an expeditious summary analysis is made of each can, with 70% alcohol to detect acidified milk and with R-mastitest reagent to detect milk provided by cows with mastitis. The milk that meets the standards at the first preliminary examination is submitted to the analysis with the Eco Milk device, for the evaluation of some compositional parameters (fat and total protein) and physical parameters (density, cryoscopic point, and acidity), and the antibiotic content with the Biokom device. In the end, the milk samples with increased acidity remain at the cooperative to be directed to the processing of other products, and the ones with antibiotic content are returned to the farmers.

Methods

Technology documentation

The technological process of Dorna Swiss cheese was analyzed by observing the production steps and by underlying the particularities.

Milk characteristics

Samples of milk raw material ($n = 20$) were collected, from cans selected from the Cooperative intended for Swiss processing, which were investigated at the Foundation's Milk Control Laboratory in Cluj Napoca. The tests were carried out on milk samples collected in 2 seasons (summer, 2021 and winter, 2021-2022), using equipments such as: Milkoscan (model 7 RM, FOSS Analytical, Denmark) for the determination of physico-chemical parameters, Fossomatic (FOSS Analytical, Denmark) for the determination of total somatic cells count and BactoScan FC (model 73710, FOSS Analytical, Denmark) for the determination of total bacteria count. Thus, in the case of the investigated raw milk samples, the content of fat, total protein, lactose, non-fat dry matter, total dry matter, respectively the pH values, total somatic cells count, $\text{cells/mL} \times 10^3$, and total bacteria count colony formed units, $(\text{cfu})/\text{mL} \times 10^3$, were determined.

The principle of the Fossomatic 7DC method for the total somatic cells is to color the cells with a fluorescent dye Dye DC, after which the cells are counted automatically (Ilea, 2019a). BactoScan FC device colors the milk bacteria with a fluorescent dye, reduces and disperses the milk components, so that the bacteria can be counted (Ilea, 2019b).

Milkoscan is a high capacity, fully automatic, mid-range infrared FTIR (Fourier Transformation Infrared Spectroscopy) analyzer and is used to determine the parameters of milk samples. The determined parameters are: fat, protein, casein, lactose, urea, dry matter, pH (Ilea, 2019c).

Statistics

The statistical analysis of the obtained data was performed by using XLSTAT software for Excel 2022 version (Addinsoft, New York, NY, USA). The variation coefficient (VC) was calculated using Equation 1 and the standard error (SE) was calculated using Equation 2.

$$VC(\%) = \frac{s}{|\bar{x}|} \cdot 100 \quad (1)$$

where s is the standard deviation and \bar{x} is the mean value of the data.

$$SE = \frac{s}{\sqrt{n}} \quad (2)$$

where s is the standard deviation and n is the number of samples.

The differences among samples considered significant at $p < 0.05$ were underlined by means of the *Student t* test, and the relationships between variables were evidenced by Principal Component Analysis (PCA) with varimax rotation.

Results and discussion

Documentation regarding the technological process of Dorna Swiss cheese

The Swiss cheese factory processes 600–1000 L of milk daily, reaching approximately 14.000 L of milk per month. From a quantity of approximately 600 L of milk, 420 L is used to obtain a piece of Swiss (approximately 35–40 kg), and the remaining 180 L to obtain 4 pieces of Raclette (of 4–5 kg). By processing 1000 L of milk, 840 L is used to obtain two pieces of Swiss cheese, and the rest to obtain Raclette. The processing is traditionally done in a Copper tank (Figure 2A, B) with a capacity of 430 L, on a hob heated with wood (Figure 2C) going through the stages described below (Figures 1 and 2). The processing steps of the technological flow implemented by the factory to obtain the Dorna Swiss, are presented in Figure 1. In Figure 2, some images that capture details from the steps implemented in the processing procedure of this assortment of cheese are shown.

Processing steps for Dorna Swiss cheese production:

- Slow pasteurization of 30% of the milk quantity at 60 °C for 35 min (Figure 2C, D);
- Addition of 70% raw milk, manual mixing for normalization at 30 °C (Figure 2E);
- Proceeding with the normalization, with the extraction of the cream, so that the fat content reaches 3.2%;
- Addition of thermophilic culture of propionic bacteria and mesothermophilic culture of lactic bacteria, continuing manual homogenization (Figure 2F);
- Addition of the rennet, coagulation taking place in 30–40 minutes (Figure 2G);
- Cutting the curd by going through the steps of mixing with the sieve (from the bottom of the tank to the surface to homogenize the temperature of the entire curd) (Figure 2H), cutting it into prismatic columns (Figure 2I, J), perpendicular cutting to form cubes, shredding with the harp until it reaches the size of rice grain;
- Manual homogenization for dehydration and drying of the curd grains, for 20–30 minutes, depending on the consistency (Figure 2J);
- Stop homogenization and remove 30% of the whey (Figure 2K), following a new heating, also called scalding (Figure 2L), in which the temperature must reach 54 °C. During all this time, it is continuously homogenized, the tank is removed from the hob and the manual stirring continues, with short breaks (40–60 minutes), to dry and dehydrate the curd grains. At the same time, a manual test of the grains is done by squeezing them by hand, and if the grains stick together, the homogenization continues for complete dehydration, followed by a new evaluation, where if the grains break into pieces, it means that the process is over (Figure 2M);
- Sedimentation and removal of the remaining whey, until it reaches a few centimeters above the formed curd (Figure 2N);
- Removing the curd, and placing it into the press, where a rounded piece of approximately 35–42 kg is formed (Figure 2O, P, Q, R);
- Turning the curd for 6–7 hours (about 7–8 times), at first more often and then less often, with the time increasing with the rise of pressing force, so that the last turning is done in the evening at the end of the program and the curd should remain in the press until the next morning;
- The curd is removed from the press the next day and the edges that have extravasated after pressing are cleaned, giving them a conical shape to avoid pinching and contamination;
- Brining, at a temperature of 16 °C, for 3 days, in 26% brine;

- Whipping and placing on the shelf in the first chamber for pre-fermentation, about 14–21 days, at 14 – 160 °C and 85% humidity, during which the curd is turned every day (Figure 2S);
- Continuing maturation for about 6 weeks, in the 2nd room, at 23–25 °C and 85% humidity;
- Airing and washing the curd pieces, with brine, 2–3 times a week, during maturation in the first and second rooms, to prevent the appearance of mold;
- Drying again, after placing the pieces of cheese on the shelf, during this time the shelves are sanitized for maturing;
- Storage of the cheese, at 10–12 °C and 80–85% humidity, for 30 days in the 3rd room, where the humidity is kept, to maintain the rind moistly and favor the release of excess gases, thus perfecting the quality of the Swiss cheese (Figure 2T);
- The total maturation time is 90 days, after which the Swiss cheese can be consumed, (Figure 2U), and if it remains in storage beyond this period, its sensory qualities increase.

The last images (Figures 2T and U) show the detailed appearance of the Dorna Swiss, with 90 days of fermentation, which is presented in the form of cylindrical pieces, with a slightly grainy side surface, with a diameter of 65–68 cm and a height between 16–17 cm.

Chemical composition and hygienic-sanitary characteristics of the milk raw material

The evolution of the chemical composition and hygienic-sanitary characteristics of the milk raw material fell within the Romanian and European standards SR2418/2008 and Romanian Government decision no. 954/2005 for milk physicochemical and microbiological characteristics (Tables 1 and 3). The comparative evolution of the compositional parameters of raw milk in the summer-winter period showed that their average and individual values presented more or less important variations.

According to the data presented in Tables 1, 3 and 5, the fat reached similar values (3.86%) in the two seasons, but with different individual oscillations (3.32–4.67% in summer and 3.25–4.63% in winter). The total protein content values were lower in summer (3.15%; variation between 2.74–3.50%) than in winter (3.32%; variation between 2.91–4.19%). Total casein values were also lower in summer (2.55%, varying between 2.23 and 2.80%) than in winter (2.68%, varying between 2.29 and 3.41%), and in the case of lactose, the decreases from the summer season were even more important (4.62%; 4.00–4.91% in summer and 4.90%; 4.25–5.67% in winter). Summer milk presented higher values compared to winter in terms of non-fat dry matter (8.95%; 7.75–10.76% in winter, and 8.54%; 7.32–8.68% in summer) and total dry matter (12.86; 11.81–15.48% in winter and 12.35%; 11.19–13.54% in summer). The pH was characterized by significant differences between seasons (6.32 in summer, 6.60 in winter). The evolution of total somatic cell count and total bacteria count, hygienic-sanitary indices with a major impact in monitoring udder health and implicitly milk, revealed low average and individual values in line with the Romanian and European standards. The standard SR2418/2008 mentions that the minimum content of fat and protein in milk must be 3.2%, while the non-fat dry mater must be at least 8.5%. The Romanian Government decision no. 954/2005 for the approval of the specific rules of hygiene for food of animal origin specifies that the total bacteria count for milk intended for the manufacture of products obtained from raw milk through a process that does not involve heat treatment must be lower that 500×10^3 cfu/mL, while the total somatic cells must be below 400×10^3 cells/mL.

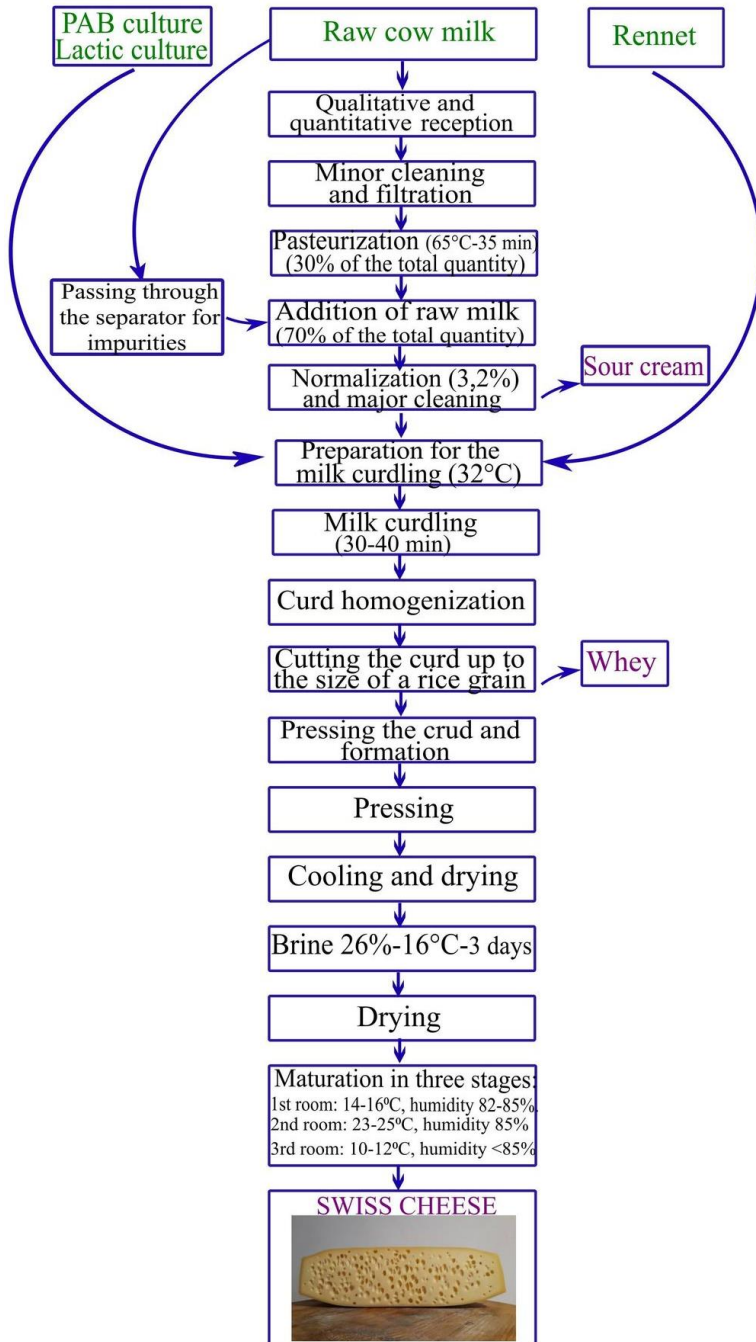


Figure 1. Representation of the technological processing flow of Dorna Swiss cheese



Figure 2. Representative steps from the technological processing flow, materials, and equipments used in the manufacturing of Dorna Swiss cheese



i



j



k



l



m



n



o



p

Continue of Figure 2.
Representative steps from the technological processing flow, materials, and equipments used
in the manufacturing of Dorna Swiss cheese



q



r



s



t



u

Continue of Figure 2.

Representative steps from the technological processing flow, materials, and equipments used in the manufacturing of Dorna Swiss cheese

- a, copper tank;
- b, milk for pasteurization (30% of the total quantity);
- c, fireplace heated with wood;
- d, pasteurization at 60°C;
- e, addition of 70% unpasteurized raw milk;
- f, addition of propionic and lactic acid bacteria culture;
- g, coagulation of milk;
- h, mix of the curd;
- i, cutting the curd;
- j, manual homogenization;
- k, elimination of 30% of the whey;
- l, scalding at 54°C;
- m, annual testing;
- n, elimination of the remaining whey;
- o, curd removing;
- p, removal of the curd by using a pulley;
- q, placing the curd in the press;
- r, pressing;
- s, ripening room;
- t, storage room;
- u, swiss cheese final product

Table 1
Physico-chemical and hygienic-sanitary parameters of milk in the summer season

Sample	Fat (%)	Protein (%)	Casein (%)	Lactose (%)	Non-fat dry matter (%)	Total dry matter (%)	pH	Somatic cells count (cells/mL $\times 10^3$)	Total bacteria count (cfu/mL $\times 10^3$)
1	3.32	3.45	2.71	4.43	8.60	12.00	6.57	169	77
2	3.52	2.86	2.41	4.85	8.52	12.06	6.51	17	100
3	3.92	3.08	2.50	4.61	8.44	12.44	6.56	185	82
4	3.58	2.92	2.47	4.89	8.58	12.12	6.51	56	80
5	3.72	2.74	2.23	4.00	7.32	11.19	6.43	144	91
6	3.93	3.09	2.60	4.61	8.70	11.36	6.52	162	31
7	3.93	3.3	2.62	4.61	8.67	11.75	6.58	222	70
8	3.71	3.27	2.62	4.64	8.68	12.47	6.56	330	45
9	3.84	3.15	2.49	4.43	8.35	12.28	6.35	339	101
10	4.08	3.2	2.58	4.56	8.54	12.70	6.57	333	96
11	4.44	3.08	2.53	4.74	8.62	13.14	6.62	399	95
12	3.75	3.01	2.42	4.32	8.03	11.90	6.49	379	100
13	4.44	3.10	2.54	4.77	8.62	12.04	6.08	69	73
14	3.64	3.01	2.43	4.55	8.33	12.05	6.61	307	84
15	3.69	3.17	2.58	4.87	8.85	12.56	6.67	357	68
16	4.5	3.4	2.70	4.70	8.92	13.39	6.70	46	71
17	3.5	3.13	2.51	4.35	8.17	11.75	6.57	248	69
18	4.6	3.5	2.80	4.80	8.95	13.40	6.24	28	99
19	4.67	3.16	2.60	4.83	8.82	13.54	6.65	187	61
20	4.2	3.33	2.67	4.91	9.04	12.99	6.67	58	76
VC (%)	0.10	0.06	0.05	0.05	0.04	0.05	0.02	0.64	0.24
SE	0.09	0.04	0.03	0.05	0.09	0.15	0.03	28.80	4.19

VC – variation coefficient, SE – standard error of the mean.

Pearson correlation coefficients between variables in the summer season are presented in Table 2. Significant positive correlations ($p < 0.05$) were observed between casein and protein content ($r = 0.95$), between protein and non-fat dry matter ($r = 0.70$) and total dry matter ($r = 0.55$). All the chemical parameters studied were positively correlated ($0.55 < r < 0.72$, $p < 0.05$) with the total dry matter content and with the non-fat dry matter ($0.46 < r < 0.87$, $p < 0.05$). The casein content was positively correlated ($r = 0.51$, $p < 0.05$) with the lactose content of milk in the summer season.

Table 2

Pearson correlations coefficients between variables in the summer season

Variables	Fat	Protein	Casein	Lactose	Non-fat dry matter	Total dry matter	pH	Somatic cells count	Total bacteria count
Fat	1								
Protein	0.36	1							
Casein	0.44	0.95	1						
Lactose	0.41	0.30	0.51	1					
Non-fat dry matter	0.46	0.70	0.85	0.87	1				
Total dry matter	0.72	0.55	0.60	0.61	0.67	1			
pH	-0.17	0.10	0.10	0.12	0.20	0.23	1		
Somatic cells count	-0.21	-0.09	-0.21	-0.33	-0.24	-0.07	0.27	1	
Total bacteria count	0.00	-0.20	-0.32	-0.18	-0.33	0.13	-0.26	0.05	1

Values in bold are different from 0 with a significance level alpha = 0.05

The first two principal components explained 62.88% of the total data variance, of which 46.42% was attributed to the first component (PC1) and 16.46% to the second one (PC2), as can be depicted in Figure 3.

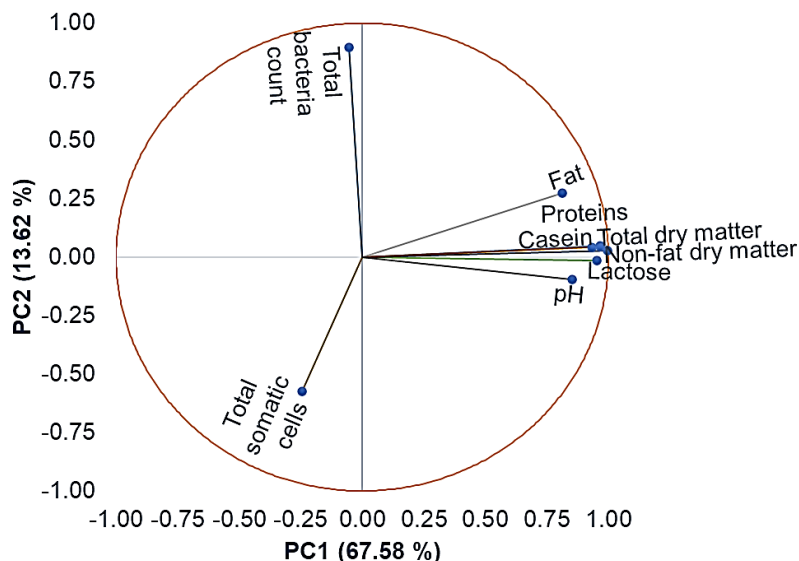


Figure 3. Principal component analysis variables plot for the summer season

The first component (PC1) was associated with the chemical components of milk – protein, casein, non-fat dry matter, total dry matter, lactose, and fat, while the second one (PC2) was associated with the microbiological and pH characteristics – total bacteria count, somatic cells count, and pH. It can be seen that an opposition occurs between the total bacteria count and pH (Figure 3).

The data registered in Tables 1, 3, and 5 show that during the summer season, the total somatic cell count presented average values of 201.75×10^3 cells/mL, with oscillations between 17×10^3 and 399×10^3 cells/mL, which exceeded the values recorded in the winter season, when the average levels were 78.45×10^3 cells/mL, with oscillations between 70×10^3 and 320×10^3 cells/mL. The total bacteria count also highlighted low and very close average levels in the two seasons, with oscillations between 31×10^3 and 101×10^3 cfu/mL in the summer period, and with oscillations between 36×10^3 and 101×10^3 cfu/mL during the winter period.

Table 3
Physico-chemical and hygienic-sanitary parameters of milk in the winter season

Sample	Fat (%)	Protein (%)	Casein (%)	Lactose (%)	Non-fat dry matter (%)	Total dry matter (%)	pH	Somatic cell count (cells/mL $\times 10^3$)	Total bacteria count (germs/mL $\times 10^3$)
1	3.93	3.3	2.62	4.61	8.67	11.75	6.57	113	95
2	3.28	2.91	2.29	4.25	7.75	11.18	6.52	216	93
3	3.93	3.30	2.62	4.61	8.67	11.75	6.58	139	99
4	3.25	3.06	2.44	4.57	8.27	11.67	6.56	165	101
5	4.15	3.70	2.98	5.04	9.52	13.81	6.58	125	100
6	3.95	3.13	2.54	4.85	8.70	12.82	6.59	85	100
7	3.98	3.10	2.57	4.88	8.60	12.78	6.57	103	87
8	4.07	3.08	2.50	4.76	8.56	12.80	6.56	320	100
9	3.54	3.05	2.42	4.47	8.14	11.81	6.56	299	43
10	3.85	3.48	2.83	5.26	9.55	13.51	6.66	114	75
11	3.94	3.62	2.94	5.36	9.79	13.85	6.66	163	36
12	4.10	3.64	2.91	4.90	9.32	13.70	6.60	112	100
13	3.60	2.97	2.40	4.69	8.32	11.85	6.54	164	92
14	3.45	3.17	2.57	4.82	8.68	12.27	6.59	106	64
15	3.87	3.49	2.84	5.3	9.61	13.61	6.66	159	100
16	4.05	3.43	2.77	4.95	9.13	13.33	6.59	101	100
17	4.04	3.53	2.86	5.04	9.35	13.55	6.59	78	70
18	3.84	3.09	2.53	4.99	8.83	12.82	6.63	111	100
19	4.63	4.19	3.41	5.67	10.76	15.48	6.68	189	100
20	3.84	3.09	2.53	4.99	8.83	12.82	6.63	116	98
VC (%)	0.08	0.10	0.10	0.07	0.08	0.08	0.01	0.44	0.23
SE	0.07	0.07	0.06	0.07	0.15	0.23	0.01	14.61	4.42

VC – variation coefficient, SE – standard error of the mean.

Significant positive correlations were obtained between the physico-chemical composition and hygienic-sanitary indices of the milk in the winter season (Table 4). Thus, the fat content was correlated with all the other chemical compounds content and pH ($0.54 < r < 0.85, p < 0.05$), similar to the protein content ($0.68 < r < 0.99, p < 0.05$) which showed strong correlations with the other variables, to the casein ($0.80 < r < 0.97, p < 0.05$), to the lactose content ($0.71 < r < 0.95, p < 0.05$), to the non-fat dry matter ($0.79 < r < 0.79, p < 0.05$) and to the total dry matter ($0.78 < r < 0.95, p < 0.05$). The pH variable was positively correlated with all the other studied characteristics ($0.54 < r < 0.91, p < 0.05$).

Table 4
Pearson correlations coefficients between variables in the winter season

Variables	Fat	Protein	Casein	Lactose	Non-fat dry matter	Total dry matter	pH	Somatic cells count	Total bacteria count
Fat	1								
Protein	0.78	1							
Casein	0.80	0.99	1						
Lactose	0.71	0.79	0.86	1					
Non-fat dry matter	0.79	0.94	0.97	0.95	1				
Total dry matter	0.82	0.88	0.92	0.93	0.95	1			
pH	0.54	0.68	0.72	0.91	0.85	0.78	1		
Somatic cells count	-0.17	-0.20	-0.23	-0.27	-0.26	-0.20	-0.26	1	
Total bacteria count	0.23	-0.01	-0.01	-0.06	-0.02	0.02	-0.11	-0.19	1

Values in bold are different from 0 with a significance level $\alpha = 0.05$

The plot of the Principal component analysis (PCA) for the variables in the winter season is shown in Figure 4. The first two components explained 81.20% of the data variation, with 67.58% being explained by the first one (PC1) and 13.62% by the second one (PC2). The physicochemical characteristics (fat, protein, casein, total dry matter, non-fat dry matter, lactose and pH) were associated with the PC1, while the hygienic-sanitary properties (total bacteria count, total somatic cells count) were associated with PC2.

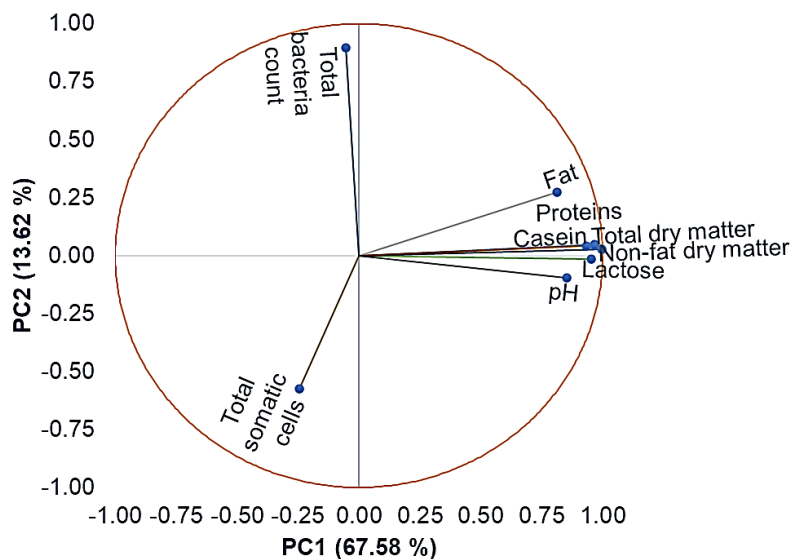


Figure 4. Principal component analysis variables plot for the winter season

Discussion

The set of positive results obtained in the physicochemical and hygienic-sanitary examinations of the milk intended for the Dorna Swiss cheese (Table 5), reveals an important concern of the farmers for its quality, of the processor for its suitability for the specific processing and of the consumer for food safety.

In recent decades, the globalization of food markets has taken a large scale and diversification, thus favoring the industry of milk and dairy products. Thus, the circulation of food products has greatly increased, at the same time intensifying the exchanges produced between the most distant regions of the globe (Silanikove et al., 2015). This globalization also represents a great challenge for processors, who must increase the quality and safety of the products sold, respectively satisfy the growing demands of consumers (Someşan et al., 2016; Svederberg, and Wendin, 2011). Current cheese production technologies ensure a wide variety of assortments, which respond to the needs and preferences of consumers. These considerations explain the classification of dairy products into: conventional products, obtained according to conventional recipes and technologies; traditional products, processed in a certain geographical area, based on traditional recipes; and ecological products, obtained from raw materials generated by ecological agriculture (De Pasquale et al., 2014; Ercolini et al., 2003). For all these products, a common characteristic is the quality of milk as a raw material, which must reach a high degree of compliance with regard to the compositional and sanitary parameters.

The analysis of the data obtained during the investigation of the seasonal evolution of the physicochemical parameters of milk, the raw material for Swiss cheese processing, revealed a slightly lower concentration of some milk parameters in the summer season compared to the winter. Lower levels of total dry matter (12.35 vs. 12.86%), total protein (3.15 vs. 3.32%), lactose (4.62 vs. 4.90%), and non-fat dry matter (8.54 vs. 8.95%) were

obtained in summer compared to winter. The changes in hygienic and sanitary parameters values were marked by important increases in total somatic cell count in the summer season (201.75×10^3 cells/mL), compared to those recorded in the winter season (78.45×10^3 cells/mL) and very close levels values of total bacteria count (81.7 and 87.65×10^3 cfu/mL, respectively). Similar results were reported by other researchers in the field (Cziszter et al., 2012; Dobranić et al., 2008).

Table 5
Average values and variations of the physicochemical and hygienic-sanitary characteristics of the milk used in the processing of Dorna Swiss cheese

Parameter	Summer		Winter	
	Mean value	Variations	Mean value	Variations
Fat (%)	3.95±0.40 ^a	3.32–4.67	3.86±0.32 ^a	3.25–4.63
Protein (%)	3.15±0.19 ^b	2.74–3.50	3.32±0.32 ^a	2.91–4.19
Casein (%)	2.55±0.13 ^a	2.23–2.80	2.68±0.26 ^a	2.29–3.41
Lactose (%)	4.62±0.23 ^b	4.00–4.91	4.90±0.33 ^a	4.25–5.67
Non-fat dry matter (%)	8.54±0.38 ^b	7.32–9.04	8.95±0.69 ^a	7.75–10.76
Total dry matter (%)	11.98±0.67 ^b	11.19–13.54	12.86±1.03 ^a	11.18–15.48
pH	6.37±0.15 ^b	6.08–6.70	6.60±0.04 ^a	6.52–6.68
Somatic cells count (cells/mL×10 ³)	201.75±12.87 ^a	17–399	78.45±65.34 ^b	78–320
Total bacteria count (cfu/mL×10 ³)	81.7±18.75 ^a	31–101	87.65±19.77 ^b	36–101

^{a,b} – different letters in the same row indicate significant differences between means ($p < 0.05$).

Soil-plant-animal transfer of nutrients depends on the quality of the soil and its rich mineralization, and the great floristic diversity of pastures and mountain meadows will lead to a milk rich in biologically active substances (terpenes, carotenoids, lactic bacteria, propionic and compounds high-quality volatiles) (Kilcawley et al., 2018; O'Callaghan, et al., 2016; Necula, et al., 2021; Nozière et al. 2006). The propionic bacteria existing in raw milk play an essential role in the production of Emmental cheese, because the fermentation of propionic acid during maturation gives a specific flavor to this type of cheese and causes the formation of holes in a controlled manner (Fröhlich-Wyder et al., 2017).

Among the producers of Dorna Swiss cheese, the studied factory is currently the only one that processes Swiss cheese in copper tanks and vats, similar to those used to obtain the traditional Swiss Emmental. According to the literature, the copper in these tanks has the role of inhibiting the uncontrolled generation of lactic and propionic acid, thus slowing down the explosive propionic fermentation. It also inhibits the sporulated germination of *C. Tyrbutyricum* and implicitly the late swelling of the curd during maturation (Rodriguez and Alatossava 2010; Rodriguez et al 2011). The copper in these tanks forms complexes with the sulfurous compounds resulting from amino acid catabolism, having a positive effect on the production of cheese flavor (Sieber, et al. 2006). Another particularity is the formation of the well-known holes that appear during maturation when propionic bacteria consume lactic acid and release CO₂, and the bubbles formed give holes (Chen et al., 2009; Fröhlich-Wyder et al., 2017; Ji et al., 2004; Rehn et al., 2011).

From an organoleptic point of view, the quality of the Swiss cheese in terms of color, fermentation holes aspect, consistency, smell, and taste is appreciated (Necula and Babii, 2012). The curd must be smooth, elastic, thick (about 1 cm), golden – yellow to reddish – yellow in color, and slightly greasy in fully matured cheese. Golden – yellow paste, lighter in winter and more intense in summer, with a smooth, glossy inner surface and fermentation meshes, 1–2 cm, evenly distributed. The consistency is elastic, fine, not crumbly. The smell and taste are slightly sweet, and the aroma is similar to green walnut core. Salt also influences the organoleptic properties of this cheese assortment, contributing to ensuring the specific flavor and its preservation (Düsterhöft et al., 2017; Guinee and Fox, 2017). Strict compliance with the temperature and humidity conditions in the maturation and storage areas has a major role in perfecting the biologically active and sensory qualities of the Emmental type cheese.

Conclusions

Dorna Swiss cheese is part of the large brand of "Emmental" type cheeses, or as is also called "Swiss type" cheese. This type of cheese is made in Romania in Dorna geographic area. All traditional Emmental hard cheeses made from raw milk enjoy an excellent food safety record from a veterinary health point of view as those made from fully pasteurized milk, due to the fact that pathogens are annihilated by the initial culture of propionic bacteria, the long period of curd scalding at 54°C and the long maturation period of three months. The main characteristic of Swiss cheese is given by its unique texture, taste, aroma, and the well-known image of the holes that make it different from any other type of cheese.

The factory studied is the only one in the Dorna area that still uses the traditional Swiss processing procedure, in copper tanks, on a hob heated with wood, and from milk obtained predominantly from Pinzgau cows, raised in mountain conditions.

The evolution of the compositional parameters of raw milk in the summer-winter period, revealed no significant differences ($p < 0.05$) in fat content, lower values in summer than in winter for the total protein and casein, and significant decreases in lactose in the summer season. Higher values of non-fat dry matter and total dry matter of milk were obtained in winter compared to the summer season.

The evolution of the hygienic-sanitary indices revealed the low values, in agreement with the Romanina and European standards. The total somatic cell count was slightly higher in the summer season compared to the winter, while the total bacteria count showed low and very close levels in the two seasons.

References

- Berdagué J.L., Grappin R. Chaillet B. Clément J. F. Duboz, G. Pidoux P. (1990), Caractérisation de l'emmental français" grand-cru". II. Analyses sensorielles, *Le Lait*, 70(2), pp. 133–145.
- Bisig W., Fröhlich-Wyder M. T., Jakob E., Wechsler D. (2010), Comparison between Emmentaler PDO and generic emmental cheese production in Europe, *Australian Journal of Dairy Technology*, 65(3), p. 206.
- Chen G., Kocaoglu-Vurma N.A., Harper W.J., Rodriguez-Saona L.E. (2009), Application of infrared microspectroscopy and multivariate analysis for monitoring the effect of adjunct cultures during Swiss cheese ripening, *Journal of Dairy Science*, 92(8), pp. 3575–3584, <https://doi.org/10.3168/jds.2008-1939>

- Cziszter L.T., Acatincăi S., Neciu F.C., Neamț R.I., Ilie D.E., Costin L.I., Gavojdian D., Tripon I. (2012), The influence of the season on the quantity, quality, and hygiene of cow's milk, *Scientific papers: Animal Science and Biotechnologies*, 45(2), pp. 305–312.
- De Noni I., Battelli G. (2008), Terpenes and fatty acid profiles of milk fat and “Bitto” cheese as affected by transhumance of cows on different mountain pastures, *Food Chemistry*, 109, pp. 299–309, <https://doi.org/10.1016/j.foodchem.2007.12.033>
- De Pasquale I., Di Cagno R., Buchin S., De Angelis M., Gobbetti M. (2014), Microbial ecology dynamics reveal a succession in the core microbiota involved in the ripening of pasta filata Caciocavallo Pugliese cheese, *Applied and Environmental Microbiology*, 80(19), pp. 6243–6255, <https://doi.org/10.1128%2FAEM.02097-14>
- Dobranić V., Njari B., Samardžija M., Mioković B., Resanović R. (2008), Influence of season on the chemical composition and somatic cell count of bulk cow's milk, *Veterinarski arhiv*, 78, pp. 235–242.
- Düsterhöft E. M., Engels W., Huppertz T. (2017), *Dutch-Type Cheeses*, In: *Global Cheesemaking Technology: Cheese Quality and Characteristics*, Wiley, pp. 326–335, <https://doi.org/10.1002/9781119046165.ch5>
- Ercolini D., Hill P.J., Dodd C.E. (2003), Bacterial community structure and location in Stilton cheese, *Applied and Environmental Microbiology*, 69(6), pp. 3540–3548, <https://doi.org/10.1128%2FAEM.69.6.3540-3548.2003>
- Fröhlich-Wyder M.T., Bisig W., Guggisberg D., Jakob E., Turgay M. Wechsler D. (2017), Cheeses With Propionic Acid Fermentation. In: P. McSweeney, P. Fox, P. Cotter, D. Everett (Eds.), *Cheese: Chemistry, Physics and Microbiology*, 4th ed., Academic Press, London, pp. 889–910, <https://doi.org/10.1016/B978-0-12-417012-4.00035-1>
- Fröhlich-Wyder M.T., Bachmann H.P. (2004), Cheeses with propionic acid fermentation. In: P. McSweeney, P. Fox, P. Cotter, D. Everett (Eds.), *Cheese: Chemistry, Physics and Microbiology*, vol. 2, 3rd ed, Elsevier, London, pp. 141–156, [https://doi.org/10.1016/S1874-558X\(04\)80042-3](https://doi.org/10.1016/S1874-558X(04)80042-3)
- FAO (2007), *Codex standard for Emmental*, CODEX STAN 269-1967.
- Grappin R., Beuvier E., Bouton Y., Pochet S. (1999), Advances in the biochemistry and microbiology of Swiss-type cheese, *Lait*, 79, pp. 3–22.
- Guinee T.P., Fox P.F. (2017), Salt in cheese: physical, chemical and biological aspects, In: P. McSweeney, P. Fox, P. Cotter, D. Everett (Eds.), *Cheese: Chemistry, Physics and Microbiology*, Academic Press, London, pp. 317–375, <https://doi.org/10.1016/B978-0-12-417012-4.00013-2>
- Ilea A. (2019a), *Determination of the number of differential somatic cells in milk. Specific procedure*, Foundation for Milk Quality Control, p. 5.
- Ilea A. (2019b), *Specific procedure for establishing the number of colony forming units*, Foundation for Milk Quality Control, p. 5.
- Ilea A. (2019c), *Specific procedure for the determination of the fat content*: Foundation for Milk Quality Control, pp. 5–8.
- Ji T., Alvarez V.B., Harper W.J. (2004), Influence of starter culture ratios and warm room treatment on free fatty acid and amino acid in Swiss cheese, *Journal of Dairy Science*, 87(7), pp. 1986–1992, [https://doi.org/10.3168/jds.S0022-0302\(04\)70015-6](https://doi.org/10.3168/jds.S0022-0302(04)70015-6)
- Jujea V., Stoilov-Linu V., Boboc M., Popa I., Nedelea A., Crăciun N., Negrea B. M. (2023), Are there any land use dynamics in the Upper Bistrița Basin, Eastern Carpathians, Romania, in the period 1990–2021?, *Diversity*, 15(9), pp. 980, <https://doi.org/10.3390/d15090980>
- Kilcawley K.N., Faulkner H., Clarke H.J., O'Sullivan M.G., Kerry J.P. (2018), Factors influencing the flavour of bovine milk and cheese from grass based versus non-grass based milk production systems, *Foods*, 7(3), pp. 37, <https://doi.org/10.3390/foods7030037>

- Kongo J.M., Malcata F.X., (2016), Cheese: Processing and Sensory Properties. In: Caballero B., Finglas P.M., Toldrá F. eds., *Encyclopedia of Food and Health*, Elsevier, London, pp. 748-754, <https://doi.org/10.1016/B978-0-12-384947-2.00136-7>
- Leniuc V. (2015), *Comparative study Emmental-Switzerland and Switzerland-Romania*, Available at: https://www.academia.edu/11825161/Studiu_comparativ_emmental,Elvetia_si_svaiter_Vatra_Dornei_Romania
- Mietton B., Chablain I., (2018), Du lait au fromage: les fondamentaux technologiques, In: Gillis J.C., Ayerbe A., *Le fromage*, Lavoisier Tec & Doc, Paris, pp. 321–359.
- Necula V., Babii M. (2012), *Sensory analysis of food and food products*, Transilvania University of Braşov Publisher.
- Necula D., Ilea A., Coman I., Tamas-Krumpe O., Feneşan D., Ognean L. (2021), Characteristics of the composition and bioactive properties of mountain milk used for Emmental cheese making-review, *Scientific Papers: Series D, Animal Science-The International Session of Scientific Communications of the Faculty of Animal Science*, 64(1).
- Nozière P., Graulet B., Lucas A., Martin B., Grolier P., Doreau M. (2006), Carotenoids for ruminants: From forages to dairy products, *Animal Feed Science and Technology*, 131(3-4), pp. 418-450, <https://doi.org/10.1016/j.anifeedsci.2006.06.018>
- O'Callaghan T. F., Hennessy D., McAuliffe S., Kilcawley K. N., O'Donovan M., Dillon P., Stanton C. (2016), Effect of pasture versus indoor feeding systems on raw milk composition and quality over an entire lactation, *Journal of Dairy Science*, 99(12), pp. 9424–9440, <https://doi.org/10.3168/jds.2016-10985>
- Ojala T., Laine P.K., Ahlroos T., Tanskanen J., Pitkänen S., Salusjärvi T., Kankainen M., Tynkkynen S., Paulin L., Auvinen P. (2017), Functional genomics provides insights into the role of *Propionibacterium freudenreichii* ssp. *shermanii* JS in cheese ripening, *International Journal of Food Microbiology*, 241, pp. 39–48, <https://doi.org/10.1016/j.ijfoodmicro.2016.09.022>
- Rehn U., Vogensen F. K., Persson S. E., Saedén K. H., Nilsson B. F., Ardö Y. (2011), Influence of microflora on texture and contents of amino acids, organic acids, and volatiles in semi-hard cheese made with DL-starter and propionibacteria, *Journal of Dairy Science*, 94(3), pp. 1098–1111, <https://doi.org/10.3168/jds.2010-3146>
- Rodriguez L.M., Alatosava T. (2010), Effects of copper on germination, growth and sporulation of *Clostridium tyrobutyricum*, *Food Microbiology*, 27(3), pp. 434–437, <https://doi.org/10.1016/j.fm.2010.01.003>
- Rodriguez L.M., Ritvanen T., Joutsjoki V., Rekonen J., Alatosava T. (2011), The role of copper in the manufacture of Finnish Emmental cheese, *Journal of Dairy Science*, 94, pp. 4831–4842, <https://doi.org/10.3168/jds.2011-4536>
- Romanian Government (2005), Decision no. 954 of August 18, 2005 regarding the approval of the specific rules of hygiene for food of animal origin, *Official Monitor*, 805.
- Rybak O. (2016), Milk fat in structure formation of dairy products: a review, *Ukrainian Food Journal*, 5(3), pp. 499–514, <https://doi.org/10.24263/2304-974X-2016-5-3-9>
- Sieber R., Rehberger B., Schaller F., Gallmann P. (2006), Technological aspects of copper in milk products and health implications of copper, *ALP Science (Switzerland)*, 493, pp. 3–16.
- Silanikove N., Leitner G., Merin U. (2015), The interrelationships between lactose intolerance and the modern dairy industry: global perspectives in evolutionary and historical backgrounds. *Nutrients*, 7(9), pp. 7312–7331, <https://doi.org/10.3390/nu7095340>
- Someşan R., Ognean L., Trăncă S., Vlasiu A., Hărţan R. (2013), Actualities regarding obtaining ecological dairy products, *Bulletin of the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Veterinary Medicine*, 70(1).

- SR2418/2008, *Whole Raw Milk. Quality Requirements*, ASRO, Available at: <https://magazin.asro.ro/ro/standard/162775>
- Svederberg E., Wendin K. (2011), Swedish consumers' cognitive approaches to nutrition claims and health claims, *Food & Nutrition Research*, 55(1), pp. 5929, <https://doi.org/10.3402/fnr.v55i0.5929>
- Thierry A., Berthier F., Gagnaire V., Kerjean J. R., Lopez C., Noël Y. (2010), Eye formation and Swiss-type cheeses, in: Law B.A., Tamime A.Y. eds., *Technology of cheesemaking*, 2nd ed., Wiley Blackwell, Oxford, pp. 360–383.
- Visioli F., Strata A. (2014), Milk, dairy products, and their functional effects in humans: a narrative review of recent evidence, *Advances in Nutrition*, 5(2), pp. 131–143, <https://doi.org/10.3945/2Fan.113.005025>

Determination of food additive zinc-cobalt(II) phosphate form resistant to high temperatures

Nadiia Antraptseva¹, Olena Podobii², Galyna Bila²

1 – National University of Life and Environmental Sciences of Ukraine, Ukraine

2 – National University of Food Technologies, Kyiv, Ukraine

Abstract

Keywords:

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Introduction. The aim of the research was to study the process of dehydration of zinc-cobalt(II) phosphates during heat treatment.

Materials and methods. Zinc-cobalt(II) phosphate tetrahydrates were used as the main research objects. The content of ingredients in the composition of phosphates was as follows, % mass.: Zn, 41.8–23.5; Co, 2.3–12.8; P, 13.6–13.8; H₂O, 16.1–16.3. Spectral methods were used to study the process and products of dehydration. Infrared absorption spectra were recorded at 20 °C and –190 °C, as well as in the process of heating phosphates. X-ray phase analysis was performed.

Results and discussion. In the structure of zinc-cobalt(II) phosphates there are two types of crystallographically non-identical water molecules, the OH groups of which form a rigid system of hydrogen bonds of different strength and directionality (from 29.35–30.48 kJ·mol⁻¹ in the OH···OPO₃ group to 12.48–13.31 kJ·mol⁻¹ in the OH···H₂O group). The energy of H-bonds, cation-water bonds and the asymmetry of water molecules increase with an increase in the cobalt content from 2.3 to 12.8 % mass., which is associated with a stronger polarizing effect of cobalt on water molecules that make up the nearest coordination environment of the cation. Zinc-cobalt(II) phosphates are thermally stable up to 85–90 °C. Upon further increase of temperature, their dehydration occurs with the pairwise removal of four water molecules and the formation of one hydrated form as an intermediate product – zinc-cobalt phosphate dihydrate. The temperature regimes of the process of dehydration of phosphates of different cationic composition correlate with the energy state of water molecules and are maximal in phosphate containing 12.8 % mass. cobalt. It is stable during heat treatment up to 90 °C. In the interval from 90 to 125 °C two water molecules are removed with the formation of zinc-cobalt (II) phosphate dihydrate. This compound is stable in the temperature interval from 125 to 250 °C. A further increase in temperature is accompanied by the release of the last two moles of water and the formation of completely dihydrated zinc-cobalt(II) phosphate. Temperature intervals of thermal stability of zinc-cobalt(II) phosphates and products of their dehydration are by 15–20 °C higher than phosphates with a higher cobalt content.

Conclusions. Zinc-cobalt(II) phosphate, containing the maximum amount of cobalt, is the most thermostable form. Zinc-cobalt(II) phosphate dihydrate formed as a result of the release of two water molecules at 90–125 °C is the only stable product.

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Corresponding author:

Olena Podobii
E-mail:
o.podobii@
gmail.com

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Introduction

Production of food with an increased content of microelements is an effective way to compensate their deficiency in the human diet (Damodaran et al., 2012; Stabnikova et al., 2021). The one of the most common methods to obtain food products enriched with microelements is their supplementing with individual salts or their mixtures (Damodaran et al., 2012). The use of phosphates of zinc, cobalt, manganese, magnesium, iron, calcium, and potassium to enhance food nutritional quality is known (Miller, 2010, 2017; Zimmermann et al., 2011). Phosphates are more often used to improve the quality meat and chicken products, as well as seafood (Bach, et al., 2011).

It was shown that the presence of phosphates in the meat products stabilized the pH value (Xu et al., 2021), increased the moisture-holding capacity, reduced the loss of moisture during the preparation, and improved the taste of the finished product (Wang, 2009). An improvement of the sensory properties of the finished product was also found in the case of adding 0.2% phosphate to chicken sausage (Stabnikova et al., 2022). A reduction of phosphate content leads to increasing cooking loss, and a deterioration of sensory properties of the finished meat products (Pinton et al., 2019).

Most technological processes of food preparation include heat treatment. Under heating the properties of treated material change, namely the moisture content and composition, not only of the raw materials, but also of additives that were incorporated in food product to enhance its nutrition value (Shao et al., 2016). To preserve the useful properties of biologically active additives, knowledge of the temperature ranges of their stability, the composition and properties of the products formed during their heat treatment is necessary.

Thermal properties of hydrated phosphates are determined by the energy state of water molecules included in their crystal lattice (Antraptseva et al., 2020). Therefore, the correct choice of the heat treatment mode of raw materials with the addition of phosphates of trace elements is possible only taking into account the process of their dehydration (Tiwari et al., 2015).

Knowledge about the processes occurring with phosphates of microelements used as additives during thermal processing of food, as well as information about the products formed from them under heating is extremely limited (Bila et al., 2016). As for zinc-cobalt phosphates, it is known that during heating they gradually lose water of crystallization with the formation of partially dihydrated phosphates (Bach et al., 2015). The formation of other hydrate forms and rehydration processes, which change the entire dehydration process, have not yet been studied.

The purpose of this work is to investigate the process and products of dehydration of food additive zinc-cobalt(II) phosphates during heat treatment.

Materials and methods

Zinc-cobalt(II) phosphates of the general formula $Zn_{3-x}Co_x(PO_4)_2 \cdot 4H_2O$ ($0 < x \leq 1.00$), in particular, $Zn_2Co(PO_4)_2 \cdot 4H_2O$, phosphate of the composition $Zn_{2.5}Co_{0.5}(PO_4)_2 \cdot 4H_2O$ and $Zn_3(PO_4)_2 \cdot 4H_2O$ was used as research objects. The content of ingredients in their composition varies within the range, % mass.: Zn, 41.8–23.5; Co, 2.3–12.8; P, 13.6–13.8; H_2O , 16.1–16.3.

Zinc hydroxocarbonate, ACS reagent; cobalt hydroxocarbonate, ACS reagent; phosphoric acid, ACS reagent; magnesium sulfate $MgSO_4 \cdot 7H_2O$, purified grade; manganese(II) sulfate $MnSO_4 \cdot 5H_2O$, purified grade; cobalt(II) sulfate $CoSO_4 \cdot 7H_2O$, purified

grade; zinc sulfate $ZnSO_4 \cdot 7H_2O$, purified grade, were used as initial substances for synthesis zinc-cobalt(II) phosphates.

Synthesis Zinc-cobalt(II) phosphates

The synthesis of zinc phosphate tetrahydrate and zinc-cobalt(II) phosphates was carried out by the interaction of a mechanical mixture of hydroxocarbonates of zinc and cobalt(II) with a solution of phosphoric acid, using recommendations of (Antraptseva et al., 2022). For this, a 45–87% H_3PO_4 solution was added to a reaction vessel thermostated at 40–70 °C containing distilled water until the pH was within 2.9–3.1. A homogenized mixture of zinc hydroxocarbonates with a zinc oxide ZnO content of 77.25 % mass. and cobalt with a CoO content of 68.32 % mass. was added to the resulting solution in parallel with constant stirring (ratio $K = Zn/Co = 50.0–2.0$, atomic) and a 45–87% H_3PO_4 solution maintaining pH 2.9–3.1. The precipitate was filtered, washed with water, dried at 40 °C and analysed. The characteristics of the received phosphates are given in the Table 1.

Table 1
Characteristics of the obtained zinc-cobalt(II) phosphates
(55% H_3PO_4 , 70 °C)

K = Zn/Co, atomic	Composition of phosphates, % mass.				Chemical composition	Phase composition (according to the results of X-ray and IR spectroscopy)
	Zn	Co	P	H ₂ O		
10.00	42.61	-	13.52	15.70	$Zn_3(PO_4)_2 \cdot 4H_2O^*$	$Zn_3(PO_4)_2 \cdot 4H_2O$
9.00	30.73	4.62	13.72	16.19	$Zn_{2.63}Co_{0.37}(PO_4)_2 \cdot 4H_2O$	Phase composition $Zn_{3-x}Co_x(PO_4)_2 \cdot 4H_2O$ ($0 < x \leq 1.00$) structures $Zn_3(PO_4)_2 \cdot 4H_2O$
4.00	28.29	7.54	13.76	16.20	$Zn_{2.41}Co_{0.59}(PO_4)_2 \cdot 4H_2O$	
2.33	24.49	11.65	13.75	16.32	$Zn_{2.10}Co_{0.90}(PO_4)_2 \cdot 4H_2O$	
2.00	23.46	12.84	13.78	16.30	$Zn_{2.00}Co_{1.00}(PO_4)_2 \cdot 4H_2O$	

Note: * zinc phosphate obtained under these conditions contains Zn, P, H₂O, the content of which corresponds to the known calculated values for $Zn_3(PO_4)_2 \cdot 4H_2O$ (Antraptseva et al., 2022).

Chemical analysis

Phosphorus content was determined similarly (Antraptseva et al., 2022) by the weight quinolinemolybdate method (error 0.2 % relative). The total content of Zn^{2+} and Co^{2+} – cations by complexometric titration (back titration, trilon B, standard zinc sulfate solution, eriochrome black T indicator, ammonia buffer solution with pH 10.0) analysis error 3 % relative (Scoog et al., 1992). The content of Co^{2+} was by the spectrophotometric method (Spectrophotometer SF–46, light absorption wavelength $\lambda = 510$ nm) in the form of a complex with surfactant (1-(2-pyridylazo)-resorcinol). The pH of the solution is 6.5–9.0 (citrate-ammonia buffer solution), the optimal concentration of cobalt ions – 0.1–4.0 $\mu g \cdot ml^{-1}$, the optimal concentration of surfactant is 60 $\mu g \cdot ml^{-1}$, the error is 0.5% relative (Antraptseva et al., 2022). The zinc content was calculated as the difference between the sum of cations and the content of Co^{2+} . Water content was according to the loss of mass of samples when heated to 800 °C, determination error 1% relative (Derivatograph Q-1500 D).

Physico-chemical analysis

Spectral methods. IR absorption spectra were recorded at 20 °C and –190 °C in the frequency range 400–4000 cm^{-1} , as well as in the process of heating phosphates (Spectrometers Nexus – Nicolet 470 with Fourier transform and software Omnic and Specord 75 JR).

X-ray phase analysis. X-ray diffraction patterns were recorded using a DRON-4-M X-ray diffractometer in continuous shooting mode using Cu K_{α} , Fe K_{α} radiation. The speed of movement of the counter was 1 $\text{degree}\cdot\text{min}^{-1}$. In order to reduce the systematic error, an internal standard was introduced – NaCl.

X-ray structural analysis was performed by full-profile analysis according to the Rietveld method using the FullProf Suite software package. For this, we used an array of diffraction data obtained from radiographs taken in the range of angles from 15 to 140° (2 θ). The scanning step was 0.05°.

The thermal properties were studied in the temperature range of 25–900 °C under the conditions of dynamic and quasi-isothermal (labyrinth crucible, heating rate 3.0 $\text{degree}\cdot\text{min}^{-1}$.) heating regimes (Derivatograph Q-1500 D).

Study of the process and products of dehydration of zinc-cobalt(II) phosphates

Spectral methods as are IR and CR spectroscopy, which are recognized as the most widespread and informative for assessing the state of OH groups and their functional connection in the crystal lattice of crystal hydrates, were chosen as the main research methods (Nakamoto, 2009).

Spectral studies were performed using two different IR spectroscopic techniques. According to the first of them, IR spectra of tetrahydrates and products of their partial and complete dehydration were recorded at 20 °C and –190 °C in the range of 400–4000 cm^{-1} on Specord-75 IR and Nexus-470 spectrometers with Fourier transform and software Omnic. The samples were prepared by pressing a fixed amount (0.05 % mass.) into a KBr matrix as in article (Koleva et al., 2019). The absorption spectra in the region corresponding to the oscillations of the water of crystallization (1400–4000 cm^{-1}) were obtained using a suspension of phosphates in butyl alcohol applied to a neutral fluorite substrate. Vaseline oil was added to the suspension to improve band resolution and reduce the overall absorption background.

The according to the second method, recording of IR spectra was used during the heating of the samples. Measurements of zinc-cobalt(II) tetrahydrate phosphates were ground with butyl alcohol, the suspension was applied to a neutral fluorite substrate. After drying the sample, the substrate was placed in the oven, which was in the cuvette compartment of the spectrophotometer, and heated. Dehydration of hydrated phosphate was assessed by changes in the intensity of absorption bands of valence vibrations of water molecules during heating.

Changes in the intensity of the $\nu(\text{OH})$ absorption bands during heating were recorded in two ways. First, the spectrum was recorded at a constant frequency corresponding to the maximum of the absorption band being studied, while fixing the heating temperature. After each sharp drop in intensity, which was registered by the deviation of the curve from horizontality and the appearance of steps of different heights, the spectrum was recorded in the entire absorption range of $\nu(\text{OH})$ – 3700–3000 cm^{-1} at a temperature corresponding to the step under consideration. If in the spectrum of the original tetrahydrate, the valence vibrations of the OH-groups of water molecules were characterized by several maxima, then the changes in the intensity of each of them were studied separately.

The use of this technique of spectroscopic study of the dehydration of crystal hydrates made it possible to trace the sequence of water removal and the formation of all intermediate hydrate forms, including those unstable at room temperature.

Results and discussion

State of water molecules and phosphate anion in the structure of zinc-cobalt(II) phosphates

The state of water molecules in the structure of zinc-cobalt(II) phosphates

Analysis of the IR spectra of zinc-cobalt(II) phosphates of composition $\text{Zn}_2\text{Co}(\text{PO}_4)_2 \cdot 4\text{H}_2\text{O}$, $\text{Zn}_{2.5}\text{Co}_{0.5}(\text{PO}_4)_2 \cdot 4\text{H}_2\text{O}$, the phosphate-matrix, $\text{Zn}_3(\text{PO}_4)_2 \cdot 4\text{H}_2\text{O}$, and their deuterioanalogues was writing at 20 °C and –190 °C (Figure 1) shows that at room temperature in the region of valence vibrations of OH groups of water molecules ($3000\text{--}3600\text{ cm}^{-1}$) (Nakamoto, 2009), two absorption bands are observed (the values of their maxima are given in Table 2) and one – in the region of deformation vibrations ($1550\text{--}1750\text{ cm}^{-1}$).

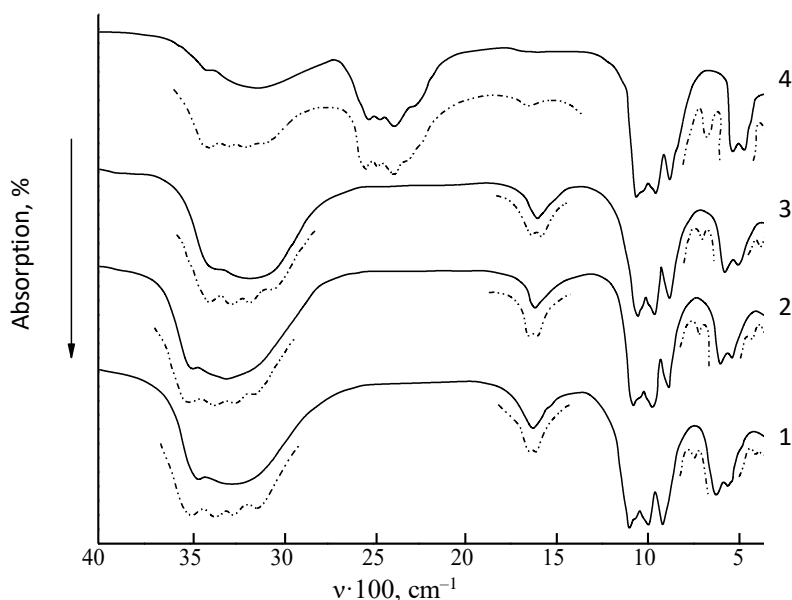


Figure 1. IR absorption spectra of $\text{Zn}_3(\text{PO}_4)_2 \cdot 4\text{H}_2\text{O}$ (1), $\text{Zn}_{2.5}\text{Co}_{0.5}(\text{PO}_4)_2 \cdot 4\text{H}_2\text{O}$ (2), $\text{Zn}_2\text{Co}(\text{PO}_4)_2 \cdot 4\text{H}_2\text{O}$ (3), $\text{Zn}_2\text{Co}(\text{PO}_4)_2 \cdot 4\text{D}_2\text{O}$ (4), recorded at 25 °C and –190 °C (marked with a dotted line)

At the temperature of liquid nitrogen, four maxima are clearly registered in the range of valence oscillations, and two in the area of strain oscillations, which indicate the presence of non-equivalent types of water molecules in their structure (Antraptseva et al., 2020).

The study of the IR spectrum of the deuterioanalogue of the composition $\text{Zn}_2\text{Co}(\text{PO}_4)_2 \cdot 4\text{D}_2\text{O}$ made it possible to reliably identify the absorption bands due to the

presence of librational vibrations of water molecules, which indicate their rigid attachment to both Oxygen and Hydrogen (Bernardino et al., 2022). The nature of the configurations of the $\nu(\text{OH})$ absorption bands also indicates the presence of hydrogen bonds in the structure of zinc-cobalt phosphates (Figure 1, Table 2).

Table 2
Characteristics of H-bonds in the structure of zinc-cobalt(II) phosphates

Composition of phosphates	Wave numbers of absorption band maxima $\nu(\text{OH}), \text{cm}^{-1}$		Characteristics of H-bonds		
			E, $\text{kJ}\cdot\text{mol}^{-1}$		R O...O, nm ($\text{PO}_4\text{O}\cdots\text{O}(\text{H}_2\text{O})$)
			OH...OPO ₃	OH...H ₂ O	
Zn _{2.80} Co _{0.20} (PO ₄) ₂ ·4H ₂ O	3264	3518	29.35	12.48	0.274
Zn _{2.60} Co _{0.40} (PO ₄) ₂ ·4H ₂ O	3260	3515	29.41	12.51	0.274
Zn _{2.50} Co _{0.50} (PO ₄) ₂ ·4H ₂ O	3254	3510	29.50	12.60	0.274
Zn _{2.30} Co _{0.70} (PO ₄) ₂ ·4H ₂ O	3247	3504	30.34	12.94	0.274
Zn _{2.20} Co _{0.80} (PO ₄) ₂ ·4H ₂ O	3244	3503	30.36	12.96	0.274
Zn _{2.00} Co _{1.00} (PO ₄) ₂ ·4H ₂ O	3240	3500	30.48	13.31	0.274

The simultaneous presence of narrow high-frequency and wide low-frequency absorption bands in the spectra of zinc-cobalt phosphates indicates the different loading of each OH-group of water molecules and their participation in the formation of H-bonds of different strength and direction. This is consistent with the results of a study of IR spectra of cobalt phosphates (Koleva et al., 2019).

A comparative analysis of the IR spectra of phosphates with different contents of zinc and cobalt(II) shows that the frequencies of valence vibrations of OH-groups of water molecules depend on the ratio of zinc and cobalt in the octahedral of the phosphate crystal structure. With an increase in the content of cobalt(II) in the coordination polyhedron, there is a natural shift of the maxima of the $\nu(\text{OH})$ absorption bands to the low-frequency region, both in the $\text{M}^{2+} - \text{OH}_2\cdots\text{OPO}_3$ group and in the $\text{M}^{2+} - \text{OH}_2\cdots\text{OH}_2$ group. It is especially clearly registered in the IR spectra of samples deposited on CaF₂ (Figure 2 a).

For Zn₃(PO₄)₂·4H₂O, the maximum of the long-wave band corresponds to the frequency of 3280 cm^{-1} ; for phosphates, in which half of the octahedral are filled with cobalt (Zn_{2.5}Co_{0.5}(PO₄)₂·4H₂O), a broad maximum of 3180–3300 cm^{-1} is noted; replacing zinc with cobalt in all octahedral positions (Zn₂Co(PO₄)₂·4H₂O) leads to a shift of $\nu(\text{OH})$ to a frequency of 3150 cm^{-1} . A similar effect is also mentioned in the study (Petersen et al., 2022; Wu et al., 2015).

The energy of hydrogen bonds, estimated according to (Bartl, 2009) according to this shift, increases when going from Zn₃(PO₄)₂·4H₂O to Zn₂Co(PO₄)₂·4H₂O. At the same time, the difference in the charge of OH-groups in the same water molecule also increases (Table 2). This is consistent with work (Grabowski, 2016) and is explained by the stronger polarizing effect of cobalt on water molecules that make up the nearest coordination environment of the cation (Petersen et al., 2022).

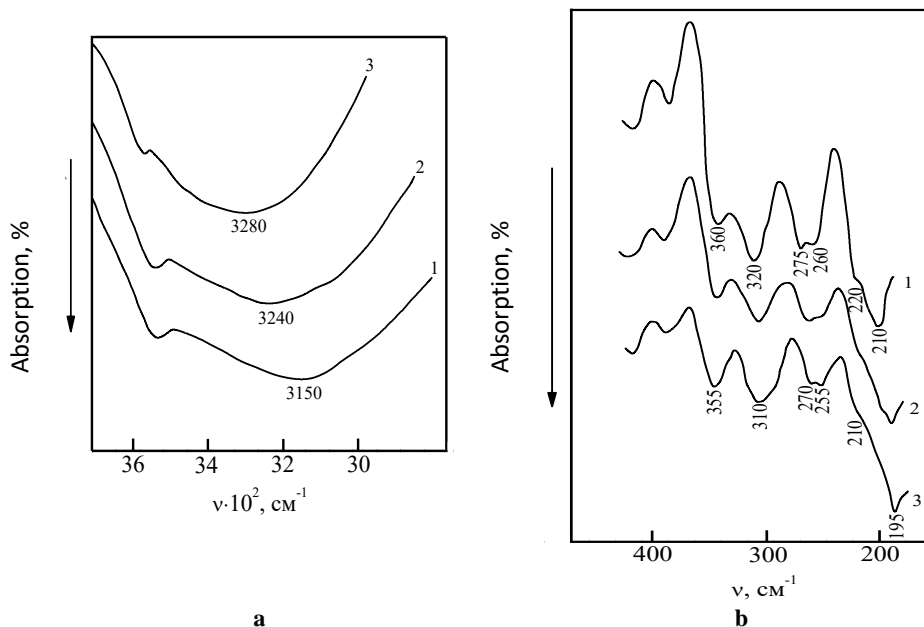


Figure 2. IR spectra of $\text{Zn}_2\text{Co}(\text{PO}_4)_2 \cdot 4\text{H}_2\text{O}$ (1), $\text{Zn}_{2.5}\text{Co}_{0.5}(\text{PO}_4)_2 \cdot 4\text{H}_2\text{O}$ (2) and $\text{Zn}_3(\text{PO}_4)_2 \cdot 4\text{H}_2\text{O}$: a – in the $\nu(\text{OH})$ region, b – in the low-frequency spectral regions (samples were applied to CaF_2).

3150 cm^{-1} , 3240 cm^{-1} , 3280 cm^{-1} – the maximums of the long-wave band $\nu(\text{OH})$.

Fluctuations of the cationic sublattice

In addition to the shift in the frequencies of the valence vibrations of the OH groups of water molecules in the IR spectra of zinc-cobalt phosphates of the composition $\text{Zn}_{3-x}\text{Co}_x(\text{PO}_4)_2 \cdot 4\text{H}_2\text{O}$ in the low-frequency region (350–180 cm^{-1}), a shift of the absorption bands is recorded, which, according to (Bernardino et al., 2022), characterize the oscillations of the cationic sublattice (Figure 2b). When going from $\text{Zn}_3(\text{PO}_4)_2 \cdot 4\text{H}_2\text{O}$ to $\text{Zn}_2\text{Co}(\text{PO}_4)_2 \cdot 4\text{H}_2\text{O}$, a shift in absorption frequencies is observed from 310, 270, 255, 210, 195 cm^{-1} to 320, 275, 260, 225, 210 cm^{-1} , respectively, which agrees with the decrease in atomic mass phosphates, which is 458.11 for $\text{Zn}_3(\text{PO}_4)_2 \cdot 4\text{H}_2\text{O}$ and 451.69 for $\text{Zn}_2\text{Co}(\text{PO}_4)_2 \cdot 4\text{H}_2\text{O}$.

State of the phosphate anion in the structure of zinc-cobalt(II) phosphates

In the region of skeletal vibrations (400–1200 cm^{-1}) in the IR spectra of $\text{Zn}_{3-x}\text{Co}_x(\text{PO}_4)_2 \cdot 4\text{H}_2\text{O}$ phosphates, valence (800–1200 cm^{-1}) and deformation (500–650 cm^{-1}) vibrations of the PO_4 anion are well separated (Bartl, 2020). The effect of the nature of the cation on the internal vibrations of the phosphate tetrahedron is practically not revealed, since, according to the structural data (Whitaker, 1995), only two oxygen vertices of each octahedron are common with PO_4 tetrahedral. Phosphorus atoms and all its coordinating oxygen atoms are in general positions, that is, the local symmetry of the anion $\text{PO}_4 - \text{C}_1$. The correlation scheme constructed for the PO_4 anion taking into account the D_{2h}^{16} factor group ($\text{Zn}_{3-x}\text{Co}_x(\text{PO}_4)_2 \cdot 4\text{H}_2\text{O}$ crystallizes in the space group D_{2h}^{16} or P_{nma}) looks like this (Figure 3):

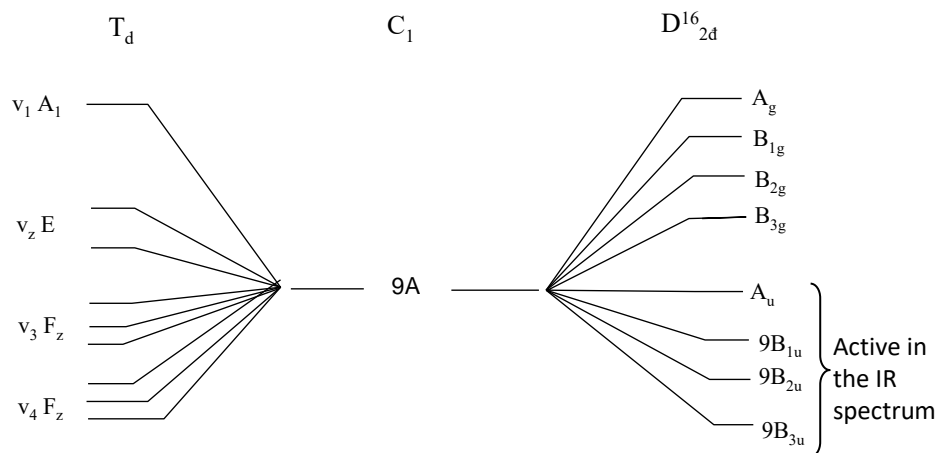


Figure 3. Correlation scheme for PO_4 anion (factor group D_{2h}^{16})

Based on this scheme, the number of frequencies that correspond to the internal vibrations of the PO_4 anion should be equal to 27. At room temperature, it was possible to register 9 bands, at $-190\text{ }^\circ\text{C} - 13$; of them, 5 are in the ν_3 bands (out of 9 calculated), 2 are in ν_1 (out of 3 calculated), 2 are in ν_4 (out of 9 calculated), and 4 are in the ν_2 band (out of 6 calculated) (Figure 1). The discrepancy between the calculated and experimental number of bands for the vibrations of the PO_4 tetrahedron is explained by the coincidence of some vibration frequencies of different bonds (Bartl, 2020).

Quite significant splitting of the main absorption bands of the phosphate anion, the appearance of fully symmetric oscillation $\nu_1(A_1) - 940, 930\text{ cm}^{-1}$ and $\nu_2(E) - 500$ and 420 cm^{-1} , usually inactive in IR spectra (Koleva et al., 2018), indicates significant distortions of the tetrahedron in the lattice zinc-cobalt phosphates. This is confirmed by the data of X-ray structural analysis and the interaction of anions with each other due to the presence of hydrogen bonds (Petersen et al., 2022).

Study of process and products of dehydration of zinc-cobalt phosphates during heating

Process and products of dehydration of zinc phosphate tetrahydrate

The heating of $\text{Zn}_3(\text{PO}_4)_2 \cdot 4\text{H}_2\text{O}$ is accompanied a decrease in the intensity of the band of valence vibrations of OH-groups of water molecules (3250 cm^{-1}). That occurs in three stages corresponding to the temperature ranges: $65-80$, $85-115$, and $210-250\text{ }^\circ\text{C}$ (Figure 4 b, curve 1). In the spectrum obtained at $85\text{ }^\circ\text{C}$, the intensity of the band with a maximum at 3540 cm^{-1} decreases, and the frequency of the main absorption band (3250 cm^{-1}) shifts to 3390 cm^{-1} in the high-frequency region of the spectrum (Figure 4 a, II).

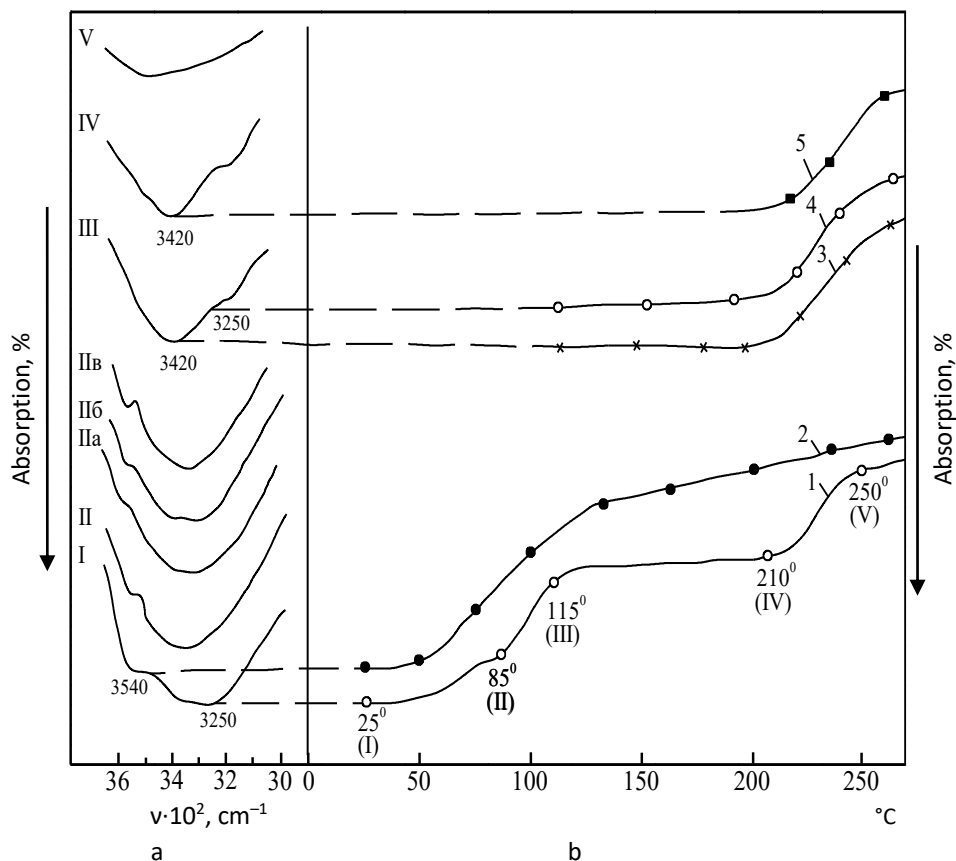


Figure 4. IR spectra of $Zn_3(PO_4)_2 \cdot 4H_2O$ and its dehydration products:
a – $\nu(OH)$ bands of water molecules recorded at 25(I), 85(II), 115(III), 210(IV) and 250 °C (V);
II a – $\nu(OH)$ of the sample (25 °C) cooled to 85 °C,
II b and II c – $\nu(OH)$ 1 and 2 hours after cooling;
b – change in intensity of bands $\nu(OH)$ 3250 (1), 3450 (2), 3240 (3), 3250 (4), 3420 cm^{-1} (5)

After cooling the sample to room temperature, the maximum of the main absorption band $\nu(OH)$ broadens and again shifts towards low frequencies (3280–3380 cm^{-1}). From the side of the high-frequency wing of the absorption curve, a shoulder at 3540 cm^{-1} is clearly visible, which becomes even more evident in the IR spectrum recorded 1 and 2 hours after the sample has cooled to room temperature (Figure 4a, IIa, IIb, IIc). The main absorption band of 3280–3380 cm^{-1} narrows, and a maximum of 3320 cm^{-1} is more clearly registered. Similar changes in the spectrum of heated to 85 °C and cooled $Zn_3(PO_4)_2 \cdot 4H_2O$ indicate, according to (Anushya et al., 2021), that water removal does not occur in the range of 65–80 °C. This correlates with the results of the study (Bach et al., 2015), in which it is determined that zinc phosphate is stable when heated to 90–100 °C (depending on the heating conditions).

The second stage of a fairly sharp drop in absorption intensity begins at 90 °C (Figure 4 b). The spectrum of $Zn_3(PO_4)_2 \cdot 4H_2O$, recorded at 115 °C, the temperature corresponding

to the beginning of the section of almost constant intensity $\nu(\text{OH})$, differs sharply from the spectrum of the original tetrahydrate in the configuration of the absorption band as a whole (Figure 4, curve I, 3). It is characterized by a maximum at 3420 cm^{-1} and a broad shoulder at 3250 cm^{-1} on the low-frequency side of the spectrum. Its identification, made according to known spectroscopic data (Nakamoto, 2009), showed that the product of partial dehydration of $\text{Zn}_3(\text{PO}_4)_2 \cdot 4\text{H}_2\text{O}$ is its dihydrate (Anushya et al., 2021).

Therefore, in the range of $90\text{--}115\text{ }^\circ\text{C}$, two molecules of the least tightly bound water are removed. The formed dihydrate is stable in a wide range of temperatures ($115\text{--}210\text{ }^\circ\text{C}$), as evidenced by the IR spectra recorded under these conditions (Figure 4, curves 3, 4, III, IV). This is also mentioned in the work (Bach et al., 2015).

Removal of water from the dihydrate begins at $210\text{ }^\circ\text{C}$ and ends at $250\text{ }^\circ\text{C}$. On the curve reflecting changes in the absorption intensity of peaks 3420 and 3250 cm^{-1} , this process corresponds to one step in the range of $210\text{--}250\text{ }^\circ\text{C}$ (Figure 4 b, curves 3-5). The IR spectrum recorded in the entire range of $\nu(\text{OH})$ at $250\text{ }^\circ\text{C}$ indicates that heating $\text{Zn}_3(\text{PO}_4)_2 \cdot 4\text{H}_2\text{O}$ to $250\text{ }^\circ\text{C}$ leads to its almost complete dehydration. This correlates with the results of thermoanalytical studies (Bila et al., 2016), according to which during the heat treatment of zinc phosphate tetrahydrate in the range of $260\text{--}280\text{ }^\circ\text{C}$, anhydrous zinc phosphate is formed.

Therefore, the process of dehydration of $\text{Zn}_3(\text{PO}_4)_2 \cdot 4\text{H}_2\text{O}$ occurs in two stages with the formation of one stable hydrated form as an intermediate product is the dihydrate of the composition $\text{Zn}_3(\text{PO}_4)_2 \cdot 2\text{H}_2\text{O}$.

Process and products of dehydration of zinc-cobalt phosphates

Dehydration of phosphate of composition $\text{Zn}_2\text{Co}(\text{PO}_4)_2 \cdot 4\text{H}_2\text{O}$, in the crystal lattice of which all octahedral positions are occupied by cobalt, in general, occurs analogously to thermal dehydration of $\text{Zn}_3(\text{PO}_4)_2 \cdot 4\text{H}_2\text{O}$ (Figure 5). However, in the course of the curve, which registers the change in the intensities of the maxima of the absorption bands $\nu(\text{OH})$ 3150 and 3530 cm^{-1} during the heating process, cancellations are observed.

The intensity of both bands present in the $\nu(\text{OH})$ region in the IR spectrum of $\text{Zn}_2\text{Co}(\text{PO}_4)_2 \cdot 4\text{H}_2\text{O}$ at $90\text{ }^\circ\text{C}$ begins to decrease almost simultaneously and ends at $125\text{ }^\circ\text{C}$. A sharp change in absorption intensity occurs in one step, which is equal in magnitude to the first two steps, which are registered in the case of $\text{Zn}_3(\text{PO}_4)_2 \cdot 4\text{H}_2\text{O}$ dehydration.

The spectrum of $\text{Zn}_2\text{Co}(\text{PO}_4)_2 \cdot 4\text{H}_2\text{O}$, recorded in the $\nu(\text{OH})$ region at $125\text{ }^\circ\text{C}$, is characterized by a maximum at 3400 cm^{-1} and a shoulder at 3180 cm^{-1} (Figure 5 a, II). It is similar to the spectrum of $\text{Zn}_2\text{Co}(\text{PO}_4)_2 \cdot 2\text{H}_2\text{O}$ dihydrate obtained under thermography conditions and recorded at room temperature (Anushya et al., 2021). That is, in the interval of $90\text{--}125\text{ }^\circ\text{C}$, two water molecules are removed with the formation of a dihydrate of the composition $\text{Zn}_2\text{Co}(\text{PO}_4)_2 \cdot 2\text{H}_2\text{O}$, which corresponds to one degree on the curve of change in absorption intensity $\nu(\text{OH})$.

The same process during the dehydration of $\text{Zn}_3(\text{PO}_4)_2 \cdot 4\text{H}_2\text{O}$ is registered by two degrees of decrease in absorption intensity, the first of which is associated with the breaking of H-bonds in the structure preceding the removal of water (Bernardino et al., 2022).

The absence of such a degree when $\text{Zn}_2\text{Co}(\text{PO}_4)_2 \cdot 4\text{H}_2\text{O}$ is heated is explained by the greater strength of H-bonds realized by OH-groups of water molecules in its structure. The energies of these bonds in $\text{Zn}_2\text{Co}(\text{PO}_4)_2 \cdot 4\text{H}_2\text{O}$ are most likely close to the energy of bonds between water molecules and the cation (Bartl, 2020). That is why, during the removal of water from $\text{Zn}_2\text{Co}(\text{PO}_4)_2 \cdot 4\text{H}_2\text{O}$ tetrahydrate, there are no differences in the breaking energy of hydrogen bonds of OH groups and Me-OH₂ bonds.

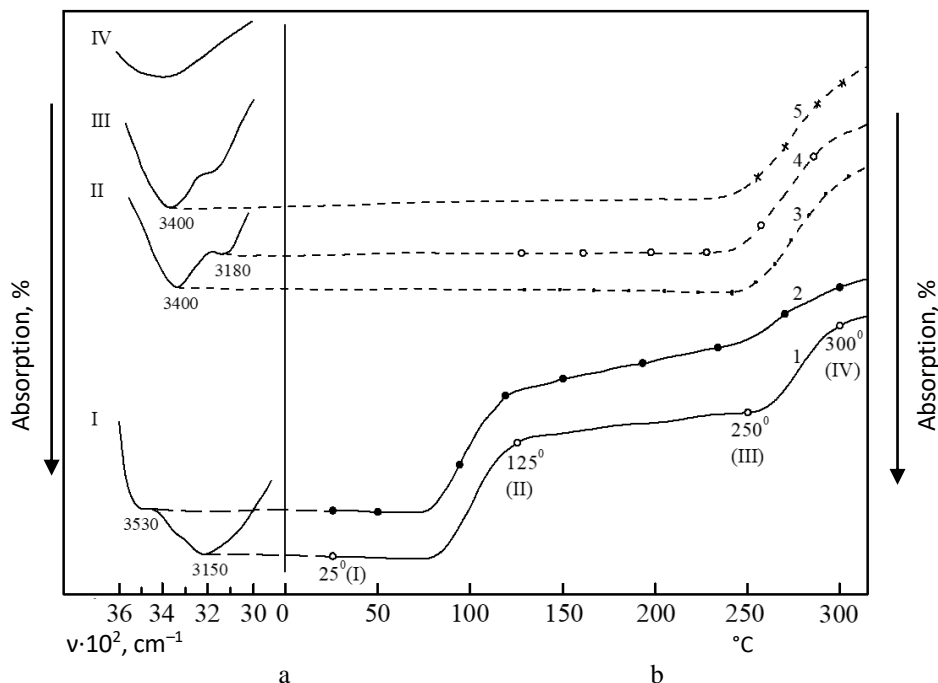


Figure 5. IR spectra of $Zn_2Co(PO_4)_2 \cdot 4H_2O$ and its dehydration products:
a – $\nu(OH)$ bands recorded at 25(I), 125(II), 250(III) and 300 °C (IV);
b – change in intensity of band maxima $\nu(OH)$ 3150 (1), 3532 (2), 3400 (3), 3180 (4), 3400 cm^{-1} (5)

The formed $Zn_2Co(PO_4)_2 \cdot 2H_2O$ dihydrate is stable in a wide range temperature (125–250 °C), as evidenced by the spectra taken at these temperatures (Figure 5 a, II, III).

Further heating of $Zn_2Co(PO_4)_2 \cdot 4H_2O$ (above as 250 °C) causes the appearance of a second, less sharp decrease in the intensity of the absorption bands in the range of 250–300 °C (Figure 5 a, curves 3, 4). The $\nu(OH)$ bands in the IR spectrum of the sample recorded at 300 °C are practically absent. That is, the removal of two water energy state of water molecules differs insignificantly (Bartl, 2020).

Influence of the nature of the cation on the process and products of dehydration of zinc-cobalt phosphates

In full compliance with the above, dehydration of $Zn_{2.5}Co_{0.5}(PO_4)_2 \cdot 4H_2O$ occurs, a tetrahydrate whose coordination polyhedral in the unit cell contain equal amounts of zinc and cobalt. The nature of the curve of changes in the intensities of the main absorption bands in the $\nu(OH)$ region during its heating contains elements characteristic of the dehydration of both $Zn_3(PO_4)_2 \cdot 4H_2O$ and $Zn_2Co(PO_4)_2 \cdot 4H_2O$. A similar effect was noted in work (Yagofarov et al., 2023)

The first stage of water removal is characterized by two degrees of decrease in the absorption intensity of $\nu(OH)$ that are less clear than for $Zn_3(PO_4)_2 \cdot 4H_2O$. One of them

characterizes the rupture of hydrogen bonds in the $Zn_{2.5}Co_{0.5}(PO_4)_2 \cdot 4H_2O$ structure (75-90 °C) and is analogous to the dehydration of $Zn_3(PO_4)_2 \cdot 4H_2O$. The second stage is associated with the breaking of Me-OH₂ bonds and the removal of two water molecules (Bartl, 2020). The IR spectrum of the sample obtained at 120 °C and 220 °C is similar to the spectra of dihydrates obtained in the work (Anushya et al., 2021).

The second stage of water removal is registered on the curve of intensity drop $\nu(OH)$ by one step. The spectrum of the sample heated to 260 °C corresponds to the completely dehydrated product. It is similar to the IR spectrum of the known anhydrous zinc phosphate (Bach et al., 2015) except for the temperature regimes of formation.

The effect of the nature of the cation is manifested in the stronger polarization effect of cobalt(II) on coordinatively bound water (Antraptseva et al., 2020). As a result, in tetrahydrates, the breaking energy of H-bonds realized by OH-groups of water molecules and M-OH₂ bonds becomes comparable.

The thermal stability of phosphates correlates with the energy state of water molecules and is maximum in phosphates of the composition $Zn_2Co(PO_4)_2 \cdot 4H_2O$ and products of its partial and complete dehydration. The temperature ranges of their formation and thermal stability are 15–20 °C higher than those of phosphates with a lower cobalt content.

So, it was shown that the dehydration of zinc-cobalt phosphate tetrahydrates of the composition $Zn_{3-x}Co_x(PO_4)_2 \cdot 4H_2O$ ($0 \leq x \leq 1.0$) occurs in two stages with the formation of one hydrate form as an intermediate product is a stable dihydrate of the composition $Zn_{3-x}Co_x(PO_4)_2 \cdot 2H_2O$.

Conclusions

1. In the structure of the zinc-cobalt(II) phosphates of the composition $Zn_{3-x}Co_x(PO_4)_2 \cdot 2H_2O$ ($0 < x \leq 1.00$) there are two types of crystallographically non-identical water molecules, the OH-groups of which form a rigid system of hydrogen bonds connections of different strength and direction.
2. The energy of H-bonds, $M^{II}-O(OH_2)$ and P-OH bonds and the asymmetry of water molecules increase with increasing cobalt content in zinc-cobalt phosphates.
3. Dehydration of zinc-cobalt phosphates occurs in two stages with the pairwise removal of water molecules and the formation as an intermediate product of one hydrated form – a stable dihydrate of the composition $Zn_{3-x}Co_x(PO_4)_2 \cdot 2H_2O$.
4. Dehydration temperature regimes correlate with the energy state of water molecules and are maximal in phosphate of the composition $Zn_2Co(PO_4)_2 \cdot 4H_2O$ and products of its partial and complete dehydration. The temperature intervals of their formation and thermal stability are 15–20 °C higher than those of phosphates with a lower cobalt content.

References

- Acton A.Q. (2013), *Phosphates – advances in research and application*, Scholarly Editions, Atlanta, Georgia.
- Antraptseva N., Solod N., Kochkodan O., Kravchenko O. (2022), Co-precipitation of cations of zinc and divalent metals from phosphoric acid solutions, *Functional Materials*, 29(4), pp. 597–604, <https://doi.org/10.15407/fm29.04.597>

- Antraptseva N., Solod N., Kravchenko O. (2020), Influence of crystal hydrate water on the process and products of heat treatment of magnesium-manganese(II) of dihydrogen phosphates, *Functional Materials*, 27(4), pp. 820–826, <https://doi.org/10.15407/fm27.04.820>
- Anushya G., Freeda T.H. (2021), Thermal parameters of undoped and medicines doped calcium hydrogen phosphate dihydrate and magnesium ammonium phosphate hexahydrate crystals, *Journal of Thermal Analysis and Calorimetry*, 146(5), pp. 1983–1989, <https://doi.org/10.1007/s10973-021-10638-0>.
- Bach S., Celinski V.R., Dietzsch M., Panthofer M., Bienert R., Emmerling F., Schmedt auf der Gunne J., Tremel W. (2015), Thermally highly stable amorphous zinc phosphate intermediates during the formation of zinc phosphate hydrate, *Journal of the American Chemical Society*, 137, pp. 2285–2294, <https://doi.org/10.1021/ja5103663>
- Bartl H. (2020), Water of crystallization and its hydrogen-bonded crosslinking in vivianite $\text{Fe}_3(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}$; a neutron diffraction investigation, *Journal of Analytical Chemistry*, 333, pp. 401–403, <https://doi.org/10.1007/BF00572335>
- Bernardino K., Ribeiro Mauro C.C. (2022), Hydrogen-bonding and symmetry breaking in the protic ionic liquid 1-ethylimidazolium nitrate, *Vibrational Spectroscopy*, 120, pp. 103358, <https://doi.org/10.1016/j.vibspec.2022.103358>
- Bila G.N., Antraptseva N.M., Povshuk V.V. (2016), Study of the composition of heat treatment products of manganese(II)-zinc dihydrogen phosphates, *Scientific Works, University of Food Technologies*, 63, pp. 142–147, <https://doi.org/10.30888/2663-5712.2022-13-01-014>
- Damodaran S., Parkin K. L. (2017), *Fennema's Food Chemistry*, CRC Press, Taylor & Francis Group, <https://doi.org/10.1201/9781315372914>
- Goemaere O., Glorieux S., Govaert M., Steen L., Fraeye I. (2021), Phosphate elimination in emulsified meat products: Impact of protein-based ingredients on quality characteristics, *Foods*, 10(4), pp. 882, <https://doi.org/10.3390/foods10040882>
- Grabowski S. (2016), *Hydrogen bonding – new insights*, Springer.
- Jurado J.F., Vargas-Hernández C., Vargas R.A. (2012), Raman and structural studies on the high-temperature regime of the $\text{KH}_2\text{PO}_4\text{-NH}_4\text{H}_2\text{PO}_4$ system, *Revista Mexicana de Física*, 58(5), pp. 411–416, <http://www.redalyc.org/articulo.oa?id=57025089005>
- Koleva V., Stefov V. (2018), Phosphate ion vibrations in dihydrogen phosphate salts of the type $\text{M}(\text{H}_2\text{PO}_4)_2 \cdot 2\text{H}_2\text{O}$ (M = Mg, Mn, Co, Ni, Zn, Cd): spectra-structure correlations, *Vibrational Spectroscopy*, 64, pp. 89–100, <https://doi.org/10.1016/j.vibspec.2018.11.004>
- Koleva V., Stefov V., Cahil A., Najdoski M., Šoptrajanov B., Engelen B., Lutz H.D. (2019), Infrared and Raman studies of manganese dihydrogen phosphate dihydrate, $\text{Mn}(\text{H}_2\text{PO}_4)_2 \cdot 2\text{H}_2\text{O}$. I: Region of the vibrations of the phosphate ions and external modes of the water molecules, *Journal of Molecular Structure*, 917(2-3), pp. 117–124, <https://doi.org/10.1016/j.molstruc.2019.09.001>
- Miller D.D. (2010), Food nanotechnology: New leverage against iron deficiency, *Nature Nanotechnology*, 5(5), pp. 318–319, <https://doi.org/10.1038/nnano.2010.91>
- Miller D. (2017), Minerals, In: Damodaran S., Parkin K.L. (Eds.), *Fennema's Food Chemistry*, Boca Raton, CRC Press, <https://doi.org/10.1201/9781315372914>
- Nakamoto K. (2009), Infrared and Raman spectra of inorganic and coordination compounds, Part B. Applications in coordination, organometallic, and bioinorganic chemistry, John Wiley & Sons, Inc, <https://doi.org/10.1002/9780470405888>

- Long N.H.B.S., Gál R., Buňka F. (2011), Use of phosphates in meat products, *African Journal of Biotechnology*, 10(86), pp. 19874-19882, <https://doi.org/10.5897/AJBX11.023> ISSN 1684-5315
- Petersen H., Stegmann N., Fischer M., Zibrowius B., Radev I., Philippi W., Schmidt W., Weidenthaler C. (2022), Crystal structures of two titanium phosphate-based proton conductors: Ab initio structure solution and materials properties, *Inorganic Chemistry*, 61(5), pp. 2379–2390, <https://doi.org/10.1021/acs.inorgchem.1c02613>
- Pinton M.B., Correa L.P., Facchi M.M.X., Heck R.T., Leães Y.S.V., Cichoski A.J., Lorenzo J.M., Dos Santos M., Pollonio M.A.R., Campagnol P.C.B. (2019), Ultrasound: A new approach to reduce phosphate content of meat emulsions, *Meat Science*, 152, pp. 88–95, <https://doi.org/10.1016/j.meatsci.2019.02.010>
- Scoog D.A., West D.M., Holler F.J. (1992), *Fundamentals of Analytical Chemistry*, Sanders college publ., Boston.
- Shao J.H., Deng Y.M., Jia N., Li R.R., Cao J.X., Liu D.Y., Li J.R. (2016), Low-field NMR determination of water distribution in meat batters with NaCl and polyphosphate addition, *Food Chemistry*, 200, pp. 308–314, <https://doi.org/10.1016/j.foodchem.2016.01.013>
- Stabnikova O., Danylenko S., Kryzhaska T., Shang F., Zhenhua D. (2022), Effects of different phosphate content on the quality of wheat bran chicken sausage, *Ukrainian Food Journal*, 11(4), pp.588–600, <https://doi.org/10.24263/2304-974X-2022-11-4-8>
- Stabnikova O., Marinin A., Stabnikov V. (2021), Main trends in application of novel natural additives for food production, *Ukrainian Food Journal*, 10(3), pp. 524–551, <https://doi.org/10.24263/2304-974X-2021-10-3-8>
- Tiwari A., Raj B. (2015), Reactions and mechanisms in thermal analysis of advanced materials, Scrivener Publishing LLC, 592 p., <https://doi.org/10.1002/9781119117711>.
- Trojan M., Brandova D. (2010), Study of thermal dehydration of $\text{Co}_{1/2}\text{Mg}_{1/2}(\text{H}_2\text{PO}_4)_2 \cdot 3\text{H}_2\text{O}$, *Thermochimica Acta*, 159, pp. 1–12, <https://doi.org/10.1016/j.tca.2010.10.003>
- Wang P., Xu X., Zhou G. (2009), Effects of meat and phosphate level on water-holding capacity and texture of emulsion-type sausage during storage, *Agricultural Sciences in China*, 8(12), pp. 1475–1481, [https://doi.org/10.1016/s1671-2927\(08\)60361-2](https://doi.org/10.1016/s1671-2927(08)60361-2)
- Whitaker A. (1995), The crystal structure of hopeite, *Acta Crystallographica*, 31(8), pp. 2026–2035, <https://doi.org/10.1107/S0567740878008286>
- Wu W. Y., Liang X. Q., Li Y. Z. (2015), $\text{CoZn}_2(\text{PO}_4)_3 \cdot 4\text{H}_2\text{O}$, a cobalt-doped modifications of hopéite, *Acta Crystallographica*, E 61, pp. 105–107, <https://doi.org/10.1107/S1600536805014078>
- Xu L., Lv Y., Su Y., Chang C., Gu L., Yang Y., Li J. (2021), Enhancing gelling properties of high internal phase emulsion-filled chicken gels: Effect of droplet fractions and salts, *Food Chemistry*, 367, 130663, <https://doi.org/10.1016/j.foodchem.2021.130663>
- Yagofarov M. I., Sokolov A.A., Iya S. Balakhontsev I.S., Nizamov I.I., Solomonov B.N. (2023), Thermochemistry of fusion, solution and hydrogen bonding in benzamide, *N-methylbenzamide*, and acetanilide, *Thermochimica Acta*, 728, pp. 179579-179586, <https://doi.org/10.1016/j.tca.2023.179579>
- Zimmermann M.B., Hilty F.M. (2011), Nanocompounds of iron and zinc: Their potential in nutrition, *Nanoscale*, 3(6), pp. 2390–2398, <https://doi.org/10.1039/c0nr00858c>

Utilization of plant processing wastes for enrichment of bakery and confectionery products

Olena Stabnikova¹, Anastasiia Shevchenko¹,
Viktor Stabnikov¹, Octavio Paredes-López²

1 – National University of Food Technologies, Kyiv, Ukraine

2 – National Polytechnic Institute, Guanajuato, Mexico

Abstract

Keywords:

Processing waste
Cereal
Fruit
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Bakery
Confectionery

Introduction. In this mini-review some technologies proposed by Ukrainian scientists for utilization of plant processing waste to enrich bakery and confectionery products are present.

Material and methods. The use of cereal, vegetable, fruit and oil processing wastes in the manufacturing of bakery and confectionery products was proposed. The effects of partial replacement of wheat flour with plant additives on technological parameters and sensory characteristics of food products are described.

Results and discussion. Partial replacement of wheat flour with plant additives allowed to increase nutritional value of food products. Thus, replacement of wheat flour with 5–20% of pumpkin seed flour resulted in an increase of protein content by 13.9–55.5% and fiber content by 12.07–48.7% in bread in comparison with control one without additives. Replacement of wheat flour, 5–15%, with pumpkin cellulose increased the content of protein 1.1–1.4 times and dietary fiber in 1.4–2.2 times in comparison with control bread. Bread supplemented with oat bran, 5–15% instead of wheat flour had higher by 19.5–52.2% score of lysine than bread without plant additives. Waste from grape processing containing protein, lipids, fiber, minerals and polyphenols, could be successfully used in preparation of flour-based confectionery products. Grape seed powder could serve as a substitute of cocoa powder in confectionery coatings technologies. Grape seed cake powder and grape skin powder being used for partial replacement of wheat flour in biscuits enriched the product with dietary fiber, polyphenolic compounds, minerals and vitamins. Addition of flour from extruded sunflower seed kernels in preparation of gingerbread allows to enrich it with valuable nutrients and improve its technological characteristics.

Conclusions. Wastes from the processing of plant materials contain valuable substances and can be used in the preparation of functional products. It is essential to maintain and preferentially increase the high quality of products; thus, it is necessary to replace wheat flour with a plant additive in amounts not exceeding 10%.

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Corresponding author:

Olena Stabnikova
E-mail:
stabstab6@
gmail.com

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Introduction

Processing of plant materials generates a large amount of waste with a high content of valuable substances, which with common improper deposition causes nowadays pollution of the environment. Meanwhile, these wastes can be a source of useful compounds such as proteins, dietary fiber, lipids, starch, minerals, vitamins and antioxidants. The Food and Agriculture Organization of the United Nations declares that reducing food waste is an important way for increasing efficiency of the food system and for contributing to environmental sustainability (FAO, 2019).

In Ukraine, the agricultural sector is quite developed and there is a wide range of plant products, the processing of which generates a significant amount of waste. This mini-review aims to describe some technologies that are being developed in Ukraine for the use of plant processing waste in food production to protect the environment from potential pollution by minimizing the waste itself, and by increasing the nutritional value of food products, supplemented with additives based on these wastes; this strategy may be useful in other regions of the world as well.

These technologies are presented in more detail in the books of the series Food Biotechnology and Engineering published by CRC Press, Taylor & Francis Group in 2022 and 2023 (Figure 1).

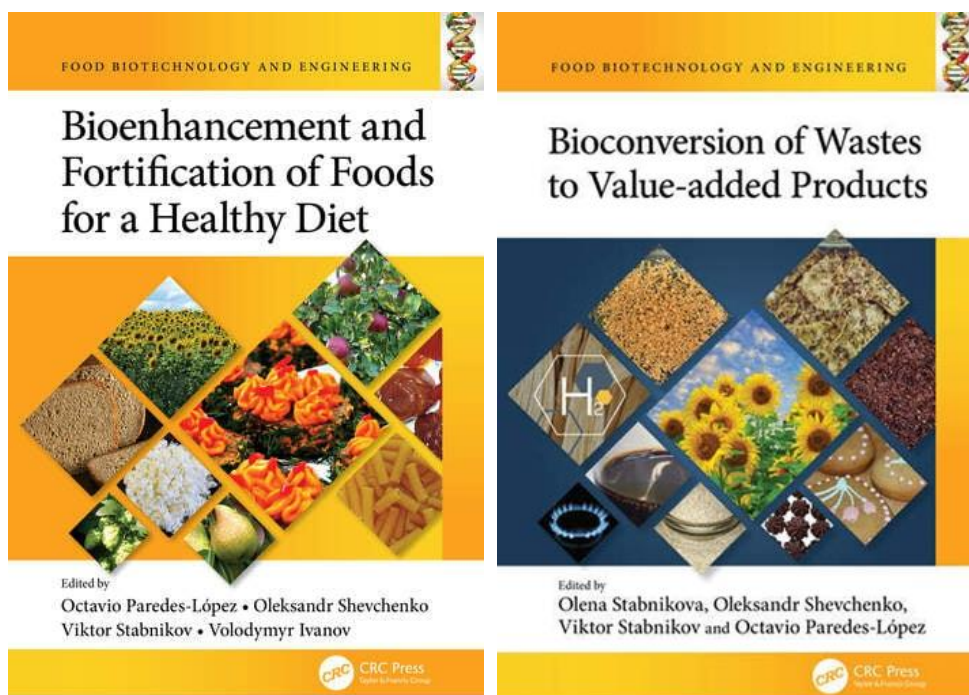


Figure 1. Books “Bioenhancement and fortification of foods for a healthy diet” (<https://doi.org/10.1201/9781003225287>) and “Bioconversion of wastes to value-added products” (<https://doi.org/10.1201/9781003329671>).

Utilization of plant processing wastes in bakery products

Ukraine ranks third in the world in growing pumpkins with 1 346 160 tons of production per year (Atlas Big, 2023). Processing of pumpkin (*Cucurbita pepo*) generates a large amount of waste including seeds and peels. Pumpkin seeds could serve as a source of proteins, carotenoids, minerals, dietary fiber, omega 3 and omega 6 fatty acids, and polyphenols, and all these substances turn into seed flour, which is characterized by high biochemical, nutritional, and functional properties (Stabnikova et al, 2021; Vinayashre et al., 2021). Pumpkin seed flour contains 3.8 times more protein and 3.5 times more fiber than wheat flour (Figure 2, Table 1).

Replacement of wheat flour with 5–20% of pumpkin seed flour resulted in an improvement of nutritional value of final bakery products due to increase of protein content by 13.9–55.5% and fiber content by 12.07–48.7% as compared with control bread without additive (Shevchenko et al., 2022). However, as the size of particles in pumpkin seed flour is much larger than wheat ones, the change of the structural and mechanical properties of dough and bread prepared from the flour mixture is predictable. Pumpkin seed flour has 1.5-fold higher water absorption capacity than wheat flour, and the viscosity of the dough prepared with replacement of 5–20% of wheat flour with pumpkin seed flour increased by 3.8–15.3%, respectively. The gas forming capacity of dough with pumpkin seed flour added to replace wheat flour from 5 to 20% decreased by 1.9–7.4%, which can be explained by the formation of protein complexes of pumpkin seed flour with wheat flour starch, which reduces its availability to amylolysis and resulted in a decrease of fermentation activity of yeast and decrease of the amount of formed sugars by 7.6–16.2% depending on the amounts of pumpkin seed flour. Finished product had crumbs with slightly increased acidity, specific volume decreased by 3.6–38.4% whereas porosity decreased by 1.4–4.1%; changes likely due to swelling of pumpkin fiber (Pereira et al., 2018). However, the bread did not change its shape. Sensorial analysis showed improvement of taste and smell of bread, which acquired a pleasant pumpkin hue. The bread crumb was elastic and well fluffed. Authors recommended 10% replacement of wheat flour with pumpkin seed flour, as this percentage does not cause any technological changes but provides an increase in the nutritional value and sensory properties acceptable to consumers of bread (Table 2).

Pumpkin cellulose is obtained after oil extraction from pumpkin seed flour (Atuonwu and Akobundu, 2010). The defatted mass is ground, sieved, and dried. The final product is a greenish flake with an average size of 0.67 mm, which contains 4.0 times more protein and 9.1 times more dietary fiber than wheat flour (Figure 1). The content of amino acid lysine in pumpkin cellulose is 1.35 g/100 g, meanwhile in wheat flour it is only 0.23 g/100 g (Shevchenko et al., 2023). Replacement of wheat flour, 5–15%, with pumpkin cellulose allowed to increase the content of protein 1.1–1.4 times and dietary fiber in 1.4–2.2 times in comparison with control bread prepared from wheat flour only (Table 2). A portion of 277 g of wheat bread represents a recommended daily intake, for the Ukrainian population (Stabnikova et al., 2023), 29% of the need for dietary fiber, but bread with 15% replacement of wheat flour with pumpkin cellulose increases this percentage to 65. Meanwhile, bakery products enriched with fibers are useful in the diet for people with diseases of gastrointestinal tract, especially irritable bowel syndrome (Loponen and Gänzle, 2018). Replacement of 5–15% of wheat flour with pumpkin cellulose reduced gas forming capacity of dough by 10.5–12.5% and the specific volume of bread by 7.7–41.0%, porosity and shape stability were also negatively affected, and at the end caused the greenish shade of the crumb and crust of the bread as well as pumpkin taste and aroma. So, to produce bread with high quality, replacement of wheat flour with pumpkin cellulose should not exceed 7%.

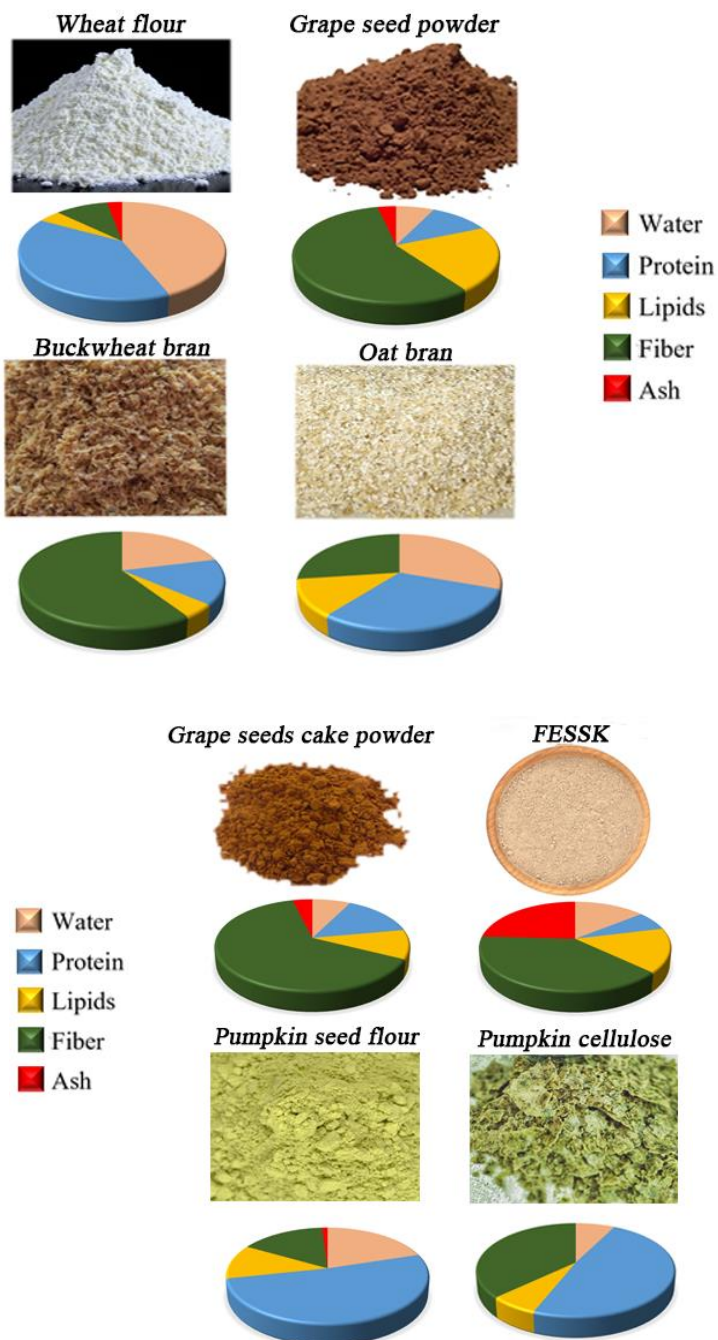


Figure 2. Chemical composition of plant processing wastes, g/100 g (wet basis)
 (Grevtseva et al., 2023; Shevchenko, 2023; Shevchenko et al., 2022; Tsykhanovska et al., 2023).

Table 1

Chemical composition of plant processing wastes, g/100 g (wet basis)

Component	High grade wheat flour	Bran		Pumpkin		Grape powder		FESSK*
		buckwheat	oat	seed flour	cellulose	seed	cake	
Water	11.5-14.3	15.0±0.1	17.3±0.1	15.6±0.1	6.4±0.1	6.0±0.4	6.0±0.4	4.7±0.2
Protein	10.3±0.1	10.8±0.1	17.0±0.1	40.0±0.3	42.0±0.2	9.5±0.5	11.80±0.5	2.3±2.1
Lipids	1.1±0.1	3.2±0.05	7.0±0.1	9.0±0.1	6.0±0.2	18.1±0.9	8.26±0.3	5.3±0.3
Fiber	2.6-3.5	42.1±0.1	15.4±0.1	12.2±0.1	32.0±0.1	47.1±2.2	51.3±2.5	12.9±0.7
Ash	0.75	N.D.**	N.D.	0.8±0.0		2.9±0.1	3.10±0.1	8.0±0.4

*FESSK is flour from extruded sunflower seed kernels, **N.D., not determined.

Table 2

Production of food products using plant processing wastes

Food product	Plant waste	Amount and technological stage, effects	Reference
Bakery products			
Wheat bread	Pumpkin seed flour	Replacement of wheat flour, 10%, increased protein content by 27.8%, fiber content by 12.5% and improved taste and aroma	Shevchenko et al., 2022
	Pumpkin cellulose from defatted pumpkin seeds	Replacement of wheat flour, 7%, increased protein content by 20.7%, fiber content by 57.0% and allowed to obtain high quality bread	Shevchenko et al., 2023
	Oat bran, waste from oat milling	Replacement of wheat flour, 7%, increased protein content by 4.4%, fiber content by 23.8% and allowed to obtain high quality bread	
Bakery product with fructose	Buckwheat bran, waste from buck wheat milling	Addition in the amount of 7.3% to the wheat flour increased fiber content by 14.0% and allowed to obtain high quality bread	Shevchenko, 2022
Flour confectionary			
Cookies, gingerbread, and waffles	Grape seed powder from grape pomace, waste of winemaking	Replacement of cocoa powder, 20%, in coating helps better preservation of test and aroma, an increase of shelf life from 8 to 12 months	Grevtseva et al., 2023
Butter biscuits	Grape skin powder, waste of winemaking	Replacement of wheat flour, 16%, increased content of dietary fiber in 81.7, vitamin PP in 2.4, magnesium by 3.4, iron by 4.2 times and allowed to better shape preservation	
Gingerbread	Flour from defatted (in an extruder) sunflower seed kernels	Replacement of wheat flour, 10%, reduced the time of formation of gingerbread dough by 1.8 min, heat treatment losses by 1.15 times, increased protein content by 84.3%, decrease carbohydrate content by 11.6%	Tsykhanovska et al., 2023

Wastes from the cereal processing industry are often underestimated, but they contain valuable chemical compounds, in particular a high content of proteins and dietary fibers (Ivanov et al., 2021; Safaripour et al., 2021). Cereal bran, a main by-product from grain milling, has often been used as animal feed, but more often is discharged. For example, wheat bran consists from 14.5 to 25% of wheat (*Triticum aestivum* L.) grain, but only 10% of it is used in bakery and in breakfast cereals (Safaripour et al., 2021). One of the reasons that such wastes still remains underutilized is the lack of easy-to-use techniques for processing and application.

Oat bran, which is obtained as waste after processing oat (*Avena sativa* L.) into flour, has a creamy color, and consists of small particles with an average size of 0.44 mm (Figure 1). It contains 1.6 times more protein and 4.4 times more dietary fiber than wheat flour, and a significant amount of the amino acid lysine, 0.76 g/100 g (Shevchenko et al., 2023). The replacement of wheat flour with oat bran, 5–15%, allowed to increase the amino acid score of lysine in bread by 19.5–52.2%.

A portion of 277 g of bread with 15% replacement of wheat flour with oat bran ensured consumption of 44.4% of the daily need for dietary fiber. However, a high amount of soluble dietary fibers and β -glucan in the composition of oat bran may lead to an increase of effective viscosity of aqueous solutions. The infrared spectra of the dough at a wavelength of 2100 nm showed that the presence of the dietary fiber of oat bran delays the development of the gluten network, and the structure of the protein matrix of the dough with bran will be less stable and more weakened than that of the control sample (Shevchenko and Litvynchuk, 2022). This is in agreement with Wang et al. (2017) results, who showed that an excess of oat bran caused a decrease of processing characteristics of dough and reduced its stability.

In the same time, replacement of 5–15% of wheat flour by oat bran led to a decrease in gas forming capacity of dough compared to the control by 1.8–10.2%, and a decrease of specific volume of bread by 0.0–25%, and porosity by 4–14%. Meanwhile, sensorial properties of bread assessed on a scale of 0 to 100, with 100 being the best quality, showed that bread with wheat flour replacement by 5 and 7% with oat bran had 97.4 and 96.2 points, which is almost the same as for control, 97.2 points. These observations are consistent with those obtained by Astiz et al. (2023), who used oat bran as supplement to wheat flour up to 25% of weight and produced breads of good technological quality.

Buckwheat bran, a byproduct of buckwheat (*Fagopyrum esculentum*) milling, shows brown particles with an average size of 0.53 mm (Figure 1). The content of fiber in buckwheat bran is 16 times higher than that in wheat flour. Addition of buckwheat bran to wheat flour, in quantities of 7.3, 10.9 and 14.6% provides 20, 30 and 40% of the recommended daily amount for dietary fiber by consuming 277 g of bread (Shevchenko et al., 2022).

Introduction of buckwheat bran in the recipe of wheat bread resulted in an increase of total gas formation during fermentation from 1276 to 1334 cm³ of CO₂/100 g of dough in comparison with 1192 cm³ of CO₂/100 g of dough in the control, which indicates an intensification of the fermentation activity of yeast.

Meanwhile, addition of buckwheat bran led to change of structural and mechanical properties of dough, which augmented its water absorption capacity, duration of formation, springiness and extensibility, and decreased elasticity and stability. Interestingly, at the dosage of buckwheat bran of 7%, related to weight of wheat flour, all parameters were almost the same as for control. Thus, addition of buckwheat bran in an amount of 7% to wheat flour allowed to increase nutritional value of bread while keeping its high quality.

Utilization of plant processing wastes in confectionery products

Grapes (*Vitis vinifera*) are one of the world's largest fruit crops with production of 73.5 million tons in 2021 (Statista, 2023a). Approximately 75% of produced grapes is used in wine-making, and 25 – 30% of this amount is waste (Beres et al., 2017). This waste called grape or wine pomace includes seeds, skins, pulp residues and stalks (Antonić et al., 2020). Waste from wine production is used as fertilizer or animal feed, but very often it is left in open fields causing environmental pollution (Dwyer et al., 2014). Meanwhile, grape pomace has valuable chemical composition and in recent years there is a lot of research considering its application as an ingredient in the food production (García-Lomillo et al., 2017).

Flour-based products, the most popular and the most frequently consumed ones among the confectionery, have high energy value due to containing of wheat flour, sugar, and fat, meanwhile their nutritional value is low (Oručević Žuljević and Akagić, 2021). Thus, these products are needed to be enriched with ingredients that have high biological value. In Ukraine 124.2×10³ tons of grapes were used for production of wine in 2019 generating 3.7 ×10³ tons of seeds, and 12.1×10³ tons of skins (Osipova et al., 2021). Application of grape seeds and skin, containing protein, lipids, fiber, minerals and polyphenols, in preparation of flour-based confectionery products, were proposed (Grevtseva et al., 2023). Three value-added products from grape seeds and skin were produced. Grape seeds and skin were separated, then dried at temperature of 60 °C and then grinding to obtain grape seed powder (particles with size 20–30 μm), and grape skin powder (particles with size 30–50 μm). A third byproduct, grape seed cake powder, was obtained from grape seed powder by cold pressing to produce grape oil followed by grinding to particles with a 20–30 μm size.

Grape seed powder was proposed as a substitute of cocoa powder, which is traditionally used in confectionery coatings technologies. Confectionery coating is made from sugar, fat, cocoa powder, emulsifiers (lecithin), stabilizers, flavoring substances (vanillin), and powder milk, which largely determines the appearance of the flour confectionery products. Altogether, coating reduces the rate of fat oxidation, could improve the product flavor and taste, and enhances product nutritional value. It was shown that chemical compositions of grape seed powders are richer with polyphenols, dietary fiber, minerals, and vitamins than cocoa powder. Thus, grape seed and grape seed cake powders contained total phenols 1.8 and 2.0 times, fiber 1.1 and 1.2, and vitamin PP 1.5 times higher than cocoa powder, respectively. Amounts of B-group vitamins (thiamine, riboflavin, and pyridoxine) were also higher in grape powders. In addition, they contain vitamin B12 (cobalamin), which is completely absent in cocoa powder. It has been shown that the replacement of 20% cocoa powder with grape seed powder increases the stability of the finished product during storage by suppressing fat oxidation and microbial activity due to the increased content of polyphenols in the coating. Cookies, gingerbread and waffles with coating with grape seed powders crumbled less and were characterized by better preservation of taste and aroma, as well as an increased shelf life due to inhibition of oxidation and hydrolysis of fats.

Grape seed and grape skin powders were used in preparation of butter biscuits, which have as main components wheat flour, butter, and sugar; thus, they contain a lot of fat and carbohydrates and few bioactive compounds. Partial replacement of wheat flour with grape seed cake powder, 20%, or grape skin powder, 16%, enriches the product with dietary fiber, polyphenolic compounds, minerals and vitamins (Table 2) (Grevtseva et al., 2023). Plant additives were added during the emulsification step with oil as antioxidants. Addition of plant supplements did not change physicochemical and sensorial properties except the colors, which changed from golden to chocolate in case of grape seed cake powder, and chocolate with a purple tint in case of grape skin powder applications. Meanwhile, the content of the

dietary fiber with the addition of grape seed powder increases from 0.3 to 31.2%, and grape skin powder from 0.3 to 24.5%. Incorporation of grape powders in dough ensured its texture; strengthening resulted in increased stability during the formation of biscuits and a better preservation of their shape.

Ukraine is the world's leading exporter of sunflower (*Helianthus annuus*) oil, which produced 4.08 million tons of sunflower oil in 2022/2023 (Statista, 2023b). After extracting oil from sunflower seed kernels in an extruder, the remaining cake is grinded to obtain a fraction with a particle size of 350–400 microns, used as animal feed, and a fraction with a particle size of 90–110 microns can be used in flour confectionery (Tsykhanovska et al., 2023). This fraction, flour from extruded sunflower seed kernels (FESSK), has low moisture content, 4.7%, which will protect it from rapid mold development during storage. It contains 4 times more protein, 5 times more fat and fiber than wheat flour. The content of unsaturated acids in FESSK fat is $84.78 \pm 4.18\%$ from total fatty acids, and the main acids are linoleic, C18:2(ω -6), 65.05%, and oleic, C18:1(ω -9), 19.32%. FESSK could be used in flour confectionery for partial replacement of wheat flour. High soluble protein content, 32.31 g/100 g of FESSK, ensures its emulsions properties and helps in foam stabilization. Thus, replacing 10% of wheat flour with FESSK in the recipe of traditional gingerbread resulted in improving technological parameters, for instance by increasing viscosity and improving emulsification and gelation of the four systems. The amount of bound water in gingerbread with FESSK increased by 20% which allows improving wetting by 20%, chewing by 11%, crumbling by 33% and prolonging the shelf life of the final product. Incorporation of FESSK in wheat flour resulted in activation of biochemical processes that leads to decrease density, increase porosity, and improvement of texture of the gingerbread. Altogether with higher quality properties, gingerbread enriched with FESSK had higher contents of protein, minerals and fiber (Table 2). Thus, the use of secondary products from the sunflower oilseed industry in preparation of flour confectionery allowed to convert waste to value-added products increasing their nutritional and sensory characteristics and it is in line with an eco-friendly strategy for organic waste minimization.

Conclusions

Food waste is a big global challenge and its reduction is a way to a better preservation of food resources, protection of the natural environment, and at the same time increasing the productivity of the food system. Meanwhile, plant processing waste, which is usually disposed of or partially used for animal feeding, contains valuable substances that can be involved in preparation of different food products, particularly in bakery and confectionery. This strategy is especially relevant for regions of the world with developed agriculture systems.

It was shown that different wastes from cereal, fruit, and vegetable processing such as oat and buckwheat brans, pumpkin flour, and pumpkin cellulose could be successfully used in production of bakery goods by increasing their nutritional value and functionality; meanwhile waste from winery and oilseed could find their application in confectionary. It is essential to maintain and preferentially increase the high quality of products; thus, based in different previous studies, it is necessary to replace wheat flour with a plant additive in amounts not exceeding 10%.

In brief, future research should involve the importance of increasing the level of plant wastes while producing new food products of high quality and high level of acceptability.

Reference

- Antonić B., Jančíková S., Dordević D., Tremlová B. (2020), Grape pomace valorization: A systematic review and meta-analysis, *Foods*, 9(11), 1627, <https://doi.org/10.3390/foods9111627>
- Atlas Big. (2023), World pumpkin production by country, <https://www.atlasbig.com/en-us/countries-pumpkin-production>
- Atuonwu A.C., Akobundu E.N.T. (2010), Nutritional and sensory quality of cookies supplemented with defatted pumpkin (*Cucurbita pepo*) seed flour, *Pakistan Journal of Nutrition*, 9, 672-677, <https://doi.org/10.3923/pjn.2010.672.677>
- Astiz V., Guardianelli L.M., Salinas M.V., Brites C., Puppo M.C. (2023), High β -glucans oats for healthy wheat breads: Physicochemical properties of dough and breads, *Foods*, 12(1), 170, <https://doi.org/10.3390/foods12010170>
- Beres C., Costa G.N.S., Cabezudo I., da Silva-James N.K., Tels A.S.C., Cruz A.P.G., Mellinger-Silva C., Tonon R.V., Cabral L.M.C., Freitas, S.P. (2017). Towards integral utilization of grape pomace from winemaking process: A review, *Waste Management*, 68, pp. 581–594, <https://doi.org/10.1016/j.wasman.2017.07.017>
- Bibra M., Samanta D., Sharma N.K., Singh G., Johnson G.R., Sani R.K. (2023), Food waste to bioethanol: opportunities and challenges. *Fermentation*, 9, 8, <https://doi.org/10.3390/fermentation9010008>
- Dwyer K., Hosseinian F., Rod M. (2014), The market potential of grape waste alternatives, *Journal of Food Research*, 3, 91, <https://doi.org/10.5539/jfr.v3n2p91>.
- FAO. (2019), The state of food and agriculture. Moving forward on food loss and waste reduction, Rome, Italy, Accessed May 25, 2023, <https://www.fao.org/3/ca6030en/ca6030en.pdf>
- García-Lomillo J., González-SanJosé M.L. (2017), Applications of wine pomace in the food industry: Approaches and functions, *Comprehensive Reviews in Food Science and Food Safety*, 16, pp. 3–22, <https://doi.org/10.1111/1541-4337.12238>
- Grevtseva N., Gorodyska O., Brykova T., Gubsky S. (2023), The use of wine waste as a source of biologically active substances in confectionery technologies. In O. Stabnikova, O. Shevchenko, V. Stabnikov, O. Paredes-López (Eds.), *Bioconversion of Waste to Value-Added Products*, CRC Press, Boca Raton, London, pp. 69-111, <https://doi.org/10.1201/9781003225287-3>
- Ivanov V., Shevchenko O., Marynin A., Stabnikov V., Gubenia O., Stabnikova O., Shevchenko A., Gavva O., Saliuk A. (2021), Trends and expected benefits of the breaking edge food technologies in 2021–2030, *Ukrainian Food Journal*, 10, pp. 7–36, <https://doi.org/10.24263/2304-974X-2021-10-1-3>
- Loponen J., Gänzle M.G. (2018). Use of sourdough in low FODMAP baking, *Foods*, 7(7), 96, <https://doi.org/10.3390/foods7070096>
- Oručević Žuljević S., Akagić A. (2021). Flour-based confectionery as functional food. In M.S. Arshad, M.H. Ahmad (Eds.), *Functional Foods - Phytochemicals and Health Promoting Potential*, IntechOpen, <https://www.intechopen.com/chapters/75487>.
- Osipova L., Radionova O., Khodakov A., Tkachenko L., Abramova T. (2021), The current state and trends of processing secondary raw materials of winemaking in Ukraine, *Food Science and Technology*, 15(2), pp. 50–60, <https://doi.org/10.15673/fst.v15i2.2031>.
- Pereira A., Krumreicha F., Ramos D., Krolow A., Santos R., Gularte M. (2018), Physicochemical characterization, carotenoid content and protein digestibility of

- pumpkin access flours for food application, *Food Science and Technology (Campinas)*, 40, pp. 691–698, <https://doi.org/10.1590/fst.38819>
- Safaripour M., Hossain K.G., Ulven C.A., Pourhashem G. (2021), Environmental impact tradeoff considerations for wheat bran-based biocomposites, *Science of the Total Environment*, 781, 146588, <https://doi.org/10.1016/j.scitotenv.2021.146588>
- Shevchenko A., Drobot V., Galenko O. (2022), Use of pumpkin seed flour in preparation of bakery products, *Ukrainian Food Journal*, 11(1), pp. 90-101, <https://doi.org/10.24263/2304-974X-2022-11-1-10>
- Shevchenko A., Litvynchuk S. (2022), Protein substances of oat bran and their influence on conformational transformations in dough and bread from wheat flour, *Ukrainian Journal of Food Science*, 10(2), pp. 161-170, <https://doi.org/10.24263/2310-1008-2022-10-2-6>
- Shevchenko A. (2022), Artichoke powder and buckwheat bran in diabetic bakery products In: O. Paredes-Lopez, V. Stabnikov, O. Shevchenko, V. Ivanov (Eds.), *Bioenhancement and Fortification of Foods for a Healthy Diet*, CRC Press, Boca Raton, USA, pp. 115-134, <https://doi.org/10.1201/9781003225287-8>
- Shevchenko A., Fursik O., Drobot V., Shevchenko O. (2023), The use of wastes from the flour mills and vegetable processing for the enrichment of food products. In: O. Stabnikova, O. Shevchenko, V. Stabnikov, O. Paredes-López (Eds.), *Bioconversion of Waste to Value-Added Products*, CRC Press, Boca Raton, London, pp. 2-24, <https://doi.org/10.1201/9781003329671-1>
- Stabnikova O., Antoniuk M., Stabnikov V., Arsen'eva L. (2019). Ukrainian dietary bread with selenium-enriched malt, *Plant Foods for Human Nutrition*, 74(2), pp. 157–163, <https://doi.org/10.1007/s11130-019-00731-z>.
- Stabnikova O., Marinin A., Stabnikov V. (2021), Main trends in application of novel natural additives for food production, *Ukrainian Food Journal*, 10(3), pp. 524–551, <https://doi.org/10.24263/2304-974X-2021-10-3-8>.
- Statista (2023a), Global fruit production in 2021, by selected variety, Accessed on May 2, 2023, <https://www.statista.com/statistics/264001/worldwide-production-of-fruit-by-variety>
- Statista (2023b), Production volume of sunflower oil in Ukraine from marketing year 2019/2020 to 2022/2023, Accessed on May 2, 2023, <https://www.statista.com/statistics/1299764/sunflower-oil-production-volume-ukraine>
- Tsykhanovska, I., Yevlash, V., Tovma, L., Adamczyk, G., Alexandrov, A., Lazarieva, T., Blahyi, O. (2023). Flour from sunflower seed kernels in the production of flour confectionery. In: O. Stabnikova, O. Shevchenko, V. Stabnikov, & O. Paredes-López (Eds.), *Bioconversion of Waste to Value-Added Products*, CRC Press, Boca Raton, London, pp. 129-167, <https://doi.org/10.1201/9781003329671-5>.
- Vinayashre S., Prasanna V. (2021), Biochemical, nutritional and functional properties of protein isolate and fractions from pumpkin (*Cucurbita moschata* var. Kashi Harit) seeds, *Food Chemistry*, 340, 128177, <https://doi.org/10.1016/j.foodchem.2020.128177>
- Wang L., Ye F., Li S., Wei F., Chen J., Zhao G. (2017), Wheat flour enriched with oat β -glucan: A study of hydration, rheological and fermentation properties of dough, *Journal of Cereal Science*, 75, pp. 143–150, <https://doi.org/10.1016/j.jcs.2017.03.004>

Анотації

Харчові технології

Фізико-хімічні та реологічні властивості м'ясних паштетів з використанням суспензій кукурудзяного крохмалю, виготовлених на електрохімічно активованій воді

Андрій Маринін, Владислав Шпак, Василь Пасічний,
Роман Святненко, Євгенія Шубіна

Національний університет харчових технологій, Київ, Україна

Вступ. Метою дослідження було визначення впливу електрохімічно активованої води на реологічні характеристики суспензій кукурудзяного крохмалю та паштетів з їх вмістом.

Матеріали і методи. Суспензії кукурудзяного крохмалю готували на артезіанській воді, яку активували електрохімічною обробкою на діафрагмовому електролізері. Фізико-хімічні показники активованої води визначали за допомогою фотометра Palintest 7500. Морфологічні дослідження крохмальних гранул проводили методом сканувальної електронної мікроскопії, а гранулометричний склад визначали методом лазерної дифракції. Реологічні властивості крохмальних суспензій і паштетів вивчали за допомогою реометра Kinexus Pro+.

Результати і обговорення. Фізико-хімічні показники якості активованої води за всіма характеристиками, окрім рН, відповідають вимогам Директиви Європейського парламенту та Ради щодо якості води, призначеної для споживання людиною. Водопоглинальна здатність крохмалю при приготуванні його суспензій на катоді (отримують при пропусканні постійного електричного струму через воду в катодній камері електролізера) знижується на 26%, а при приготуванні на аноді (отримують у процесі реакцій окислення води на аноді) підвищується на 18%. Значення вологоутримувальної здатності гідратованого крохмалю при застосуванні катодіту знижується на 10%, а при використанні анодіту підвищується на 36%. Показано, що електрохімічно активована вода має значний вплив на реологічні показники крохмальних суспензій: зі збільшенням відсотка комплексної деформації зсуву напруга зсуву пропорційно зростає для зразків суспензій, виготовлених на активованій воді. В'язко-еластичні властивості крохмальних суспензій, виготовлених із застосуванням електрохімічно активованої води як при 25°C, так і при 68°C, тяжіють до ідеально еластичного гелю, тобто мають більш еластичну структуру, ніж контрольні зразки. Під дією деформації зсуву еластичні властивості втрачаються, при цьому суспензії набувають в'язкості (значення фазового кута збільшуються). Максимальне значення вологозв'язувальної здатності паштетів спостерігалось при застосуванні крохмальної суспензії, приготовленої на анодіті. Кращі значення реологічних показників паштетів отримано при застосуванні крохмальної суспензії з 2% крохмалю на анодіті.

Висновки. Електрохімічно активована вода має значний вплив на фізико-хімічні і реологічні характеристики напівфабрикатів, зокрема суспензій кукурудзяного крохмалю, та паштетів з їх використанням, і сприяє кращому структуроутворенню досліджуваних харчових систем.

Ключові слова: крохмаль, паштет, м'ясо, реологія, в'язкість, активована вода, електрохімічне оброблення.

Біоактивний профіль ріжкового дерева (*Ceratonia siliqua* L.), культивованого в агропродовольчому секторі Європи та Північної Африки

Татяна Капканара, Євгенія Ковальова, Ауріка Чірсанова,
Віоліна Попович, Оксана Раду, Родіца Сімініук

Технічний університет Молдови, Кишинів, Республіка Молдова

Вступ. Проведено дослідження фізико-хімічного складу бобів і м'якоті стручків ріжкового дерева, що походять з чотирьох країн.

Матеріали і методи. Охарактеризовано фізико-хімічні властивості ріжкового дерева (*Ceratonia siliqua* L.), культивованого в різних країнах – Молдові, Алжирі, Італії та Іспанії, за вмістом мінералів (Ca, Mg, Fe), каротиноїдів (β -каротин, лікопен, зеаксантин) і вмістом хлорофілу (а-хлорофіл і б-хлорофіл). Антиоксидантну активність біологічно активних сполук визначали моделюванням шлунково-кишкового травлення.

Результати і обговорення. Порівняно з вирощеними в Алжирі, Іспанії та Італії, зразки плодів молдавського ріжкового дерева містять більшу кількість біологічно активних сполук, у деяких позиціях значно перевищуючи показники ріжкового дерева із зазначених регіонів. Так, вміст мінеральних речовин у перерахунку на Ca, Mg і Fe у молдавських зразках ріжкового дерева був вищим у 1,1–1,7 рази. Такі ж тенденції зафіксовані щодо вмісту каротиноїдів у бобах ріжкового дерева Молдови: β -каротин 13,610 мг/100 г сухої речовини (СВ); лікопену 19,882 мг/100 г сухої речовини та зеаксантину 20,709 мг/100 г сухої речовини, які були значно вищими порівняно зі зразками з Алжиру, Іспанії та Італії. Відмінності за вмістом біологічно активних речовин між плодами ріжкового дерева з Молдови та інших регіонів були суттєвими. Найбільшим вмістом хлорофілу відзначалися зразки з Італії – до 1,1 мг/100 г сухої речовини. Еволюція антиоксидантної активності біологічно активних сполук, яка відбувалася через шлунково-кишкове травлення, підтвердила функціональний профіль стручків ріжкового дерева та бобів. Так, антиоксидантна активність DPPH (2,2-дифеніл-1-пікрилгідразил) біоактивних сполук у плодах з різних регіонів світу під час моделювання шлункового травлення зросла з 38–48% до 60–74%.

Висновки. Чотири досліджувані зразки бобів і стручків ріжкового дерева, що походять з різних регіонів світу, були схожими за своїм біоактивним потенціалом. Ріжкове дерево з Молдови є найкращим за вмістом мінеральних речовин, β -каротину, лікопену, зеаксантину та антиоксидантної активності.

Ключові слова: *ріжкове дерево, стручок ріжкового дерева, мінеральна сполука, каротиноїд, хлорофіл, антиоксидантна активність.*

Характеристика протеолітичної активності карпатського традиційного рідкого молокозгортального препарату

Володимир Юкало, Ольга Крупа, Катерина Дацишин, Людмила Сторож
Тернопільський національний технічний університет імені Івана Пулюя

Вступ. В Українських Карпатах для виробництва традиційних м'яких сирів використовують спеціальний рідкий молокозгортальний препарат (КРМП). Вважають, що КРМП забезпечує характерні органолептичні властивості традиційних карпатських сирів, таких як бринзи і будзу. Оскільки КРМП раніше не вивчався, метою досліджень є характеристика його молокозгортальних і протеолітичних властивостей.

Матеріали і методи. Препарати КРМП різного терміну зберігання були отримані з фермерських господарств Ворохти. Молокозгортальну активність визначали за методом Сокслета. Вміст білків у зразках КРМП досліджували за К'ельдалем. Гомогенні α_{S1} -, β - і κ -казеїнові субстрати виділяли за допомогою препаративного електрофорезу. Концентрацію продуктів протеолізу казеїнових субстратів визначали спектрофотометрично. Специфічність протеолізу казеїнових фракцій встановлювали аналітичним електрофорезом.

Результати і обговорення. Для КРМП характерна висока молокозгортальна активність, яка дещо нижча, ніж у стандартного сичужного ферменту, і близька до твердого молокозгортального препарату «Глек». Встановлено, що молокозгортальна активність КРМП зростає більш ніж у 3 рази при його зберіганні, що обумовлено процесами екстракції протеолітичних ензимів з клітин шлунків тварин. Тривалість коагуляції свіжого КРМП становить 159 хв, а після 18 місяців зберігання скорочується до 49 хвилин. Молокозгортальна активність для свіжого препарату становить 2515 SU, тоді як після 18 місяців зберігання зростає до 8100 SU.

При виробництві карпатської бринзи і будзу доцільно використовувати таку кількість КРМП, яка забезпечує початок згортання молока при 33–35 °С через 20–25 хвилин. Аналіз продуктів протеолізу казеїнових субстратів електрофоретичним і спектрофотометричним методами свідчить про високу специфічність КРМП до κ -казеїну. Така специфічність характерна для очищеного природного молокозгортального ферменту – хімосину. Водночас КРМП, на відміну від пепсину і меншою мірою – від стандартного сичужного ферменту, практично не розщеплює α_{S1} - і β -казеїнові субстрати.

Висновки. Виявлено що молокозгортальна активність КРМП у процесі його зберігання до 18 місяців зростає втричі. Зберігання понад 18 місяців не підвищує активність ензимів КРМП, оскільки зупиняється їхня екстракція із секреторних клітин шлунків, проходять лише процеси денатурації. За специфічністю протеолізу α_{S1} -, β - і κ -казеїнових субстратів КРМП близький до хімосину і є високоякісним молокозгортальним препаратом.

Ключові слова: *молоко, коагулянт, протеоліз, казеїнові субстрати, електрофорез.*

Порівняльне дослідження ліпазних препаратів для ензимного дегумінгу соняшникової олії

Тамара Носенко, Діана Жупанова

Національний університет харчових технологій, Київ, Україна

Вступ. Вилучення фосфоліпідів з рослинних олій є важливою стадією виробництва рафінованих олій. Ензимний дегумінг рослинних олій був запропонований для підвищення ефективності вилучення фосфоліпідів. Метою дослідження є визначення впливу нових ліпазних препаратів на ефективність дегумінгу соняшникової олії.

Матеріали і методи. Ферментні препарати Lecitase® Ultra, Quara® Boost та QuaraLowP, одержані від компанії Novozyme (Данія). Вміст фосфору в золі олій визначали фотометричним методом. Кислотне, пероксидне та число омилення визначали стандартними методами. Антиоксидантні властивості олій визначали за реакцією гасіння радикалів 2,2-дифеніл-1-пікрілгідразилу (DPPH).

Результати і обговорення. Ензимний дегумінг соняшникової олії приводив до збільшення виходу олії порівняно з водним дегумуванням. Вихід гідратованої олії становив 98% при використанні ензимного препарату Quara®Boost, що на 1,5% вище порівняно з водним дегумінгом. Використання Lecitase® Ultra та QuaraLowP збільшувало вихід олії на 1 і 0,5% відповідно порівняно із водною гідратацією. Вміст фосфоліпідів зменшувався з 0,4% у сирій олії до 0,2% після водного дегумінгу. Додавання ензимних препаратів суттєво зменшувало вміст фосфоліпідів, ензимний дегумінг з Lecitase® Ultra і QuaraLowP зменшував вміст фосфоліпідів до 0,08 та 0,06% відповідно. Гідратація фосфоліпідів із фосфоліпазою С (Quara®Boost) мала найвищу ефективність, залишковий вміст фосфоліпідів становив 0,04% у перерахунку на стеароолеолецитин, що відповідає 16 мг/кг вмісту фосфору. Зменшене значення числа омилення (191,5 мг КОН/г) зразків соняшникової олії, гідратованих за допомогою фосфоліпази С, підтвердило утворення діацилгліцеролів. Найвищий вміст вільних жирних кислот у соняшниковій олії був після дегумінгу з препаратом Lecitase® Ultra, кислотне число збільшувалось від 0,86 в сирій олії до 2,7 мг КОН/г. Препарат QuaraLowP, який також має активність фосфоліпази А1, несуттєво збільшував кислотне число олії порівняно із сирою олією. Дегумінг із препаратом Quara®Boost не впливав на вміст вільних жирних кислот, кислотне число було навіть нижче, ніж у зразку олії після водного дегумінгу. Пероксидне число олії внаслідок ензимного дегумінгу було нижчим за значення 1 мМоль 1/2О/кг, водночас після водного дегумінгу це значення становило 2,6 мМоль 1/2 О/кг. Всі зразки соняшникової олії мали подібну антиоксидантну здатність – 30–36% погашених вільних радикалів DPPH протягом 30 хвилин.

Висновки. Отже, найвища ефективність дегумінгу соняшникової олії була з препаратом Quara®Boost, який має активність фосфоліпази С. Дегумінг за допомогою Quara®Boost супроводжувався найвищим виходом олії, найменшим вмістом фосфору та вільних жирних кислот, низьким значенням пероксидного числа та високою антиоксидантною здатністю одержаної олії.

Ключові слова: *ензими, дегумінг, фосфоліпаза, соняшникова олія.*

Аналіз молочної сировини, що використовується для виробництва швейцарського сиру «Дорна» в різні сезони

Некула Дору^{1,2}, Тамас-Крумпе Октавія¹, Фенешан Дарія¹,
Мадаліна Унгуряну-Юга^{2,3}, Огнян Лауренціу¹

1 – Університет аграрних наук і ветеринарної медицини Клуж-Напока, Румунія

2 – Національний інститут економічних досліджень, Ватра Дорней, Румунія

3 – Університет «Штефана чел Маре», Сучава, Румунія

Вступ. Метою досліджень є аналіз хімічних і гігієнічно-санітарних властивостей молока, що використовується у виробництві швейцарського сиру «Дорна» за два сезони, а також документування особливостей технології.

Матеріали і методи. Зразки молока, що використовуються у виробництві швейцарського сиру, досліджували два сезони (влітку та взимку). Визначали хімічний склад, рН та гігієнічно-санітарні показники.

Результати і обговорення. Основною особливістю оброблення швейцарського сиру «Дорна» є коагуляція в мідних резервуарах суміші непастеризованого молока (70%) з повільно пастеризованим молоком за температури 65 °С (30%). Хімічний склад і гігієнічно-санітарні показники молочної сировини мають вирішальне значення для отримання високоякісного швейцарського сирного продукту. Еволюція основних хімічних властивостей молочної сировини виявила значні варіації в літній і зимовий сезони щодо вмісту жиру, загального білка і казеїну. Значення загального соматичного числа було вищим у літній сезон, якщо порівняти із зимовими показниками ($201,75 \times 10^3$ проти $78,45 \times 10^3$ клітин/мл). Загальна кількість бактерій також демонструвала низькі значення із дуже щільними коливаннями в літній і зимовий сезони ($81,70 \times 10^3$ проти $87,65 \times 10^3$ КУО/мл), які були нижчими порівняно з показниками, представленими в європейських стандартах. Вміст жиру влітку коливався від 3,32 до 4,67%, а взимку – від 3,86 до 3,25%. Відмінності ($p < 0,05$) щодо вмісту білка і казеїну спостерігалися між сезонами (3,15% і 2,55% відповідно влітку проти 3,32% і 2,68% взимку). Вміст лактози був вищим у літній період (4,62%), у зимовий –4,25%. Вміст загальної сухої речовини і знежиреної сухої речовини молока влітку були нижчими порівняно із зимовими показниками (11,98% та 8,54% відповідно проти 12,86% та 8,95% відповідно). Зразки молока показали нижчі значення рН влітку (6,37) порівняно з зимовими (6,60), різниця була достовірною при $p < 0,05$. Отримано достовірні кореляційні зв'язки ($p < 0,05$) між хімічними характеристиками молока і сезоном. Загальний аналіз основних хімічних складів і санітарно-гігієнічних показників молочної сировини виявив невеликі індивідуальні коливання, що виключає будь-який ризик впливу на виробника, переробника або споживача.

Висновок. Динаміка гігієнічно-санітарних показників молока показала, що вони відповідають загальноприйнятим вимогам. Особливості виробництва швейцарського сиру «Дорна» полягають у поєднанні якості молочної сировини та використання мідних резервуарів, а також повільної часткової пастеризації, що сприяє унікальним характеристикам кінцевого продукту.

Ключові слова: сир «Ементаль», швейцарський сир, сир «Дорна», молоко.

Харчова хімія

Визначення форми харчової добавки цинк-кобальт(II) фосфату, стійкої до дії високих температур

Надія Антрапцева¹, Олена Подобій², Галина Біла²

1 – Національний університет біоресурсів і природокористування України, Київ, Україна

2 – Національний університет харчових технологій, Київ, Україна

Вступ. Досліджено процес зневоднення цинку-кобальту(II) фосфатів під час теплової обробки.

Матеріали і методи. Як основні об'єкти дослідження використовували цинку-кобальту(II) фосфати тетрагідрати. Вміст інгредієнтів у складі фосфатів такий, % мас.: Zn – 41,8–23,5; Co – 2,3–12,8; P – 13,6–13,8; H₂O – 16,1–16,3. Дослідження процесу і продуктів дегідратації виконували спектральними методами. ІЧ спектри поглинання записували при 20 °C і –190 °C, а також у процесі нагрівання фосфатів. Виконували рентгенівський фазовий аналіз.

Результати і обговорення. В структурі цинку-кобальту(II) фосфатів існують два види кристалографічно неіdentичних молекул води, ОН-групи яких утворюють жорстку систему водневих зв'язків, різних за міцністю і спрямованістю (від 29,35–30,48 кДж/моль у групі ОН...ОРО₃ до 12,48–13,31 кДж/моль у групі ОН...H₂O). Енергія Н-зв'язків, зв'язків катіон-вода та асиметрія молекул води посилюються зі збільшенням вмісту кобальту від 2,3 до 12,8 % мас., що пов'язано з більш сильною поляризаційною дією кобальту на молекули води, які складають найближче координаційне оточення катіона.

Під час теплової обробки цинк-кобальт (II) фосфати термічно стійкі до 85–90 °C. При подальшому нагріванні відбувається їх зневоднення з попарним видаленням чотирьох молекул води й утворенням як проміжного продукту однієї гідратної форми – цинку-кобальту фосфату дигідрату. Температурні режими процесу зневоднення фосфатів різного катіонного складу корелюють з енергетичним станом молекул води і максимальні у фосфату, що містить 12,8 % мас. кобальту. Він стійкий під час термообробки до 90 °C. В інтервалі 90–125°C відбувається видалення двох молекул води з утворенням у продуктах термообробки цинку-кобальту(II) фосфату дигідрату. Останній стійкий у температурному інтервалі 125–250 °C. Подальше підвищення температури супроводжується виділенням останніх двох моль води й утворенням повністю зневодненого цинку-кобальту(II) фосфату. Температурні інтервали термічної стабільності цинку-кобальту(II) фосфатів і продуктів їх часткового і повного зневоднення на 15–20 °C вищі у фосфатів з більшим вмістом кобальту.

Висновки. Цинк-кобальт(II) фосфат, що містить максимальну кількість кобальту, є найбільш термостабільною формою. Внаслідок видалення двох молекул води при 90–125 °C утворюється лише один стійкий продукт – цинк-кобальт(II) фосфат дигідрат.

Ключові слова: фосфат, цинк, кобальт, тепла обробка, дегідратація.

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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.
2. **Назва статті.**
3. Автори статті (ім'я та прізвище повністю, приклад: Денис Озеряно).
4. *Установа, в якій виконана робота.*
5. Анотація. **Обов'язкова** структура анотації:
 - Вступ (2–3 рядки).
 - Матеріали та методи (до 5 рядків)
 - Результати та обговорення (пів сторінки).
 - Висновки (2–3 рядки).
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:
 - Вступ
 - Матеріали та методи
 - Результати та обговорення
 - Висновки
 - Література.

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).
9. Контактні дані автора, до якого за необхідності буде звертатись редакція журналу.

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.

Оформлення цитат у тексті статті:

Кількість авторів статті	Приклад цитування у тексті
1 автор	(Arych, 2019)
2 автора	(Kuievda and Bront, 2020)
3 і більше авторів	(Bazopol et al., 2022)

Приклад тексту із цитуванням: It is known (Arych, 2019; Bazopol et al., 2022), the product yield depends on temperature, but, there are some exceptions (Kuievda and Bront, 2020).

У цитуваннях необхідно вказувати одне джерело, звідки взято інформацію.

Список літератури сортується за алфавітом, літературні джерела не нумеруються.

Правила оформлення списку літератури

В Ukrainian Food Journal взято 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, DOI.

Ініціали пишуться після прізвища.

Всі елементи посилання розділяються комами.

Приклад:

Popovici C., Gitin L., Alexe P. (2013), Characterization of walnut (*Juglans regia* L.) green husk extract obtained by supercritical carbon dioxide fluid extraction, *Journal of Food and Packaging Science, Technique and Technologies*, 2(2), pp. 104–108, <https://doi.org/5533.935-3>.

2. Посилання на книгу:

Автори (рік), Назва книги (курсивом), Видавництво, Місто.

Ініціали пишуться після прізвища.

Всі елементи посилання розділяються комами.

Приклад:

Deegan C. (2000), *Financial Accounting Theory*, McGraw-Hill Book Company, Sydney.

3. Посилання на розділ у редакovanій книзі:

Автори (рік), Назва глави, In: Редактори, Назва книги (курсивом), Видавництво, Місто, сторінки.

Приклад:

Fordyce F.M. (2013), Selenium deficiency and toxicity in the environment. In: O. Selinus (Ed.), *Essentials of Medical Geology*, Springer, pp. 375–416, https://doi.org/10.14453/10.1007/978-94-007-4375-5_16

4. Тези доповідей конференції:

Arych M. (2018), Insurance's impact on food safety and food security, *Resource and Energy Saving Technologies of Production and Packing of Food Products as the Main Fundamentals of Their Competitiveness: Proceedings of the 7th International Specialized Scientific and Practical Conference, September 13, 2018*, NUFT, Kyiv, pp. 52–57, <https://doi.org/5533.935-3>.

5. Посилання на електронний ресурс:

Виконується аналогічно посиланню на книгу або статтю. Після оформлення даних про публікацію пишуться слова **Available at:** та вказується електронна адреса.

Приклад:

Cheung T. (2011), *World's 50 most delicious drinks*, 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.

Зручний сайт для транслітерації з української мови: <http://translit.kh.ua/#lat/passport>

Стаття надсилається за електронною адресою:

ufj_nuft@meta.ua

Ukrainian Food Journal публікує оригінальні наукові статті, короткі повідомлення, оглядові статті, новини та огляди літератури.

Тематика публікацій в **Ukrainian Food Journal**:

Харчова інженерія	Процеси та обладнання
Харчова хімія	Нанотехнології
Мікробіологія	Економіка та управління
Фізичні властивості харчових продуктів	Автоматизація процесів
Якість та безпека харчових продуктів	Упаковка для харчових продуктів

Періодичність виходу журналу 4 номери на рік.

Результати досліджень, представлені в журналі, повинні бути новими, мати чіткий зв'язок з харчовою наукою і представляти інтерес для міжнародного наукового співтовариства.

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 Open Access scholarly Resources (ROAD) (2014)
- 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)
- Web of Science (Emerging Sources Citation Index) (2018)
- Scopus (2022)

Рецензія рукопису статті. Матеріали, представлені для публікування в «Ukrainian Food Journal», проходять «Подвійне сліпе рецензування» двома вченими, призначеними редакційною колегією: один є членом редколегії і один незалежний учений.

Авторське право. Автори статей гарантують, що робота не є порушенням будь-яких авторських прав, та відшкодовують видавцю порушення даної гарантії. Опубліковані матеріали є правовою власністю видавця «Ukrainian Food Journal», якщо не узгоджено інше.

Детальна інформація про Журнал, інструкції авторам, приклади оформлення статті та анотацій розміщені на сайті:

<http://ufj.nuft.edu.ua>

Редакційна колегія

Головний редактор:

Олена Стабнікова, д-р., *Національний університет харчових технологій, Україна*

Члени міжнародної редакційної колегії:

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Члени редакційної колегії:

Агота Гедре Райшене, д-р., *Литовський інститут аграрної економіки, Литва*
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Йорданка Стефанова, д-р, *Пловдивський університет "Паїсій Хілендарскі", Болгарія*
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Крістіна Попович, д-р., доцент, *Технічний університет Молдови*
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Нур Зафіра Нур Хаснан, доктор філософії, *Університет Путра Малайзії, Селангор, Малайзія*
Октавіо Паредес Лопес, д-р., проф, *Центр перспективних досліджень Національного політехнічного інституту, Мексика.*
Олександр Шевченко, д.т.н., проф., *Національний університет харчових технологій, Україна*
Ольга Рибак, канд. техн. наук, доц., *Тернопільський національний технічний університет імені Івана Пулюя, Україна*
Рана Мустафа, д-р., *Глобальний інститут продовольчої безпеки, Університет Саскачевана, Канада*
Семіх Отлес, д-р., проф, *Університет Еге, Туреччина*

Соня Амарей, д-р., проф., *Університет «Штефан чел Маре», Сучава, Румунія*
Станка Дам'янова, д.т.н., проф., *Русенський університет «Ангел Канчев», філія Разград, Болгарія*
Стефан Стефанов, д.т.н., проф., *Університет харчових технологій, Болгарія*
Тетяна Пирог, д.т.н., проф., *Національний університет харчових технологій, Україна*
Тетяна Пирог, д-р. біол. наук, проф., *Національний університет харчових технологій, Україна*
Умезуруйке Лінус Опара, професор, *Стелленбошський університет, Кейптаун, Південна Африка*
Шейла Кілонзі, *Університет Каратіна, Кенія*
Юлія Дзязько, д-р. хім. наук, с.н.с., *Інститут загальної та неорганічної хімії імені В. І. Вернадського НАН України*
Юн-Хва Пеггі Хсі, д-р, професор, *Університет Флориди, США*
Ясмiна Лукiнак, д-р, проф., *Осієкський університет, Хорватія.*

Олексій Губеня (відповідальний секретар), канд. техн. наук, доц., *Національний університет харчових технологій, Україна.*

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