THE PROSPECTS OF USING LACTOBACILLUS DELBRUECKII FOR APPLE JUICE FERMENTATION

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Abstract. *Functional foods are a crucial component for maintaining individuals' well-being and nutrition in individuals with specific constraints, for example, gastrointestinal issues, intestinal disorders, lactose and gluten intolerance. The ability to modify the composition of food products by lactic acid bacteria (LAB) fermentation processes makes it possible to obtain functional products that could be the basis of healthy or wellness nutrition. The study examined the potential use of Lactobacillus delbrueckii for the fermentation of apple juice and investigated the organoleptic properties, as well as the concentration of macro- and microelements, total acidity, and vitamin C content. Organoleptic indicators are better in fermented juices than in non-fermented ones because they have a richer taste and aroma. The apple aroma is less strong in the sample without glucose added at the beginning of fermentation than in the sample with glucose added at the beginning of fermentation. The work showed that fermented L. delbrueckii apple juice with pre-added glucose at a final concentration of 2% before the start of fermentation, has more potassium and vitamin C content than unfermented juice. There was no significant influence on the content of magnesium and glucose in fermented apple juice. During fermentation, the total acidity of fermented apple juice with pre-introduced glucose decreased by 12%. The obtained results indicate that adding glucose at the beginning of fermentation allows for better modification of the final properties of the juice. It has been demonstrated that this method could be used to develop a functional product based on apple juice.*

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Introduction. Functional foods are not only an essential component of a healthy diet, but also have potential for use in the treatment of a variety of chronic diseases. Furthermore, functional products help maintain a healthy balance of vitamins, minerals, antioxidants, fiber, phenolic compounds and omega-3 fatty acids in the diet of people with restrictions, such as lactose intolerance and celiac disease (Arshad et al, 2021).

One example of functional products can be apple juice fermented by lactic acid bacteria. Due to fermentation processes, apple sugar is transformed into lactic acid, which leads to a sharp sour taste. Furthermore, the fermentation of LAB juice may confer enhanced cellular antioxidant activity and radical scavenging activity (Zhang et al, 2021). The fermentation of apple juice with lactic acid bacteria such as *Lactobacillus plantarum* ATCC14917 increases the content of 5-O-caffeoylquinic acid, quercetin, and phloretin, which are compounds with strong antioxidant properties. Despite a reduction in the total phenolic and flavonoid content, fermentation by lactic acid bacteria can increase the bioavailability of polyphenols in apples, making them more available for consumption as functional food (Li et al, 2018). The range of LAB organisms suitable for juice fermentation includes *Lactobacillus collinoides*, *Oenococcus oeni*, *Pediococcus parvulus*, *Lactobacillus casei*, *Lactobacillus plantarum*, *Lacticaseibacillus paracasei*, or *P. ethanolidurans* (Liang et al, 2022;

Guiné et al, 2021; Cousin et al, 2017; Dimitrovski et al, 2015). The research team of Chen S. and co-authors examined the effects of four LAB strains on the distinctive taste profile of fermented apple juice in a study. The diverse assortment of strains included *Lactobacillus acidophilus*, *L. rhamnosus*, *L. casei*, and *L. plantarum*. In apple juice, all four strains showed good growth ability, but *L. casei* and *L. rhamnosus* showed higher microbial viability and produced higher total acid concentration than the other strains. Lactic acid formation from malic acid was a common feature of the fermented samples. This trend was most obvious in those samples with *L. acidophilus* (Chen et al, 2019).

The fermentation process by lactic acid organisms has a significant impact on the quantity of essential nutrients present in apple juice. During fermentation, changes in organic acids such as succinic acid, tartaric acid, malic acid, and pyruvic acid affect the taste and aroma of apple juice. Also, fermented apple juice has a higher level of polyphenols, such as gallic acid and protocatechuic acid, which makes it more antioxidant. The fermentation process also influences the distinct phenolic compounds present in apple juice and its sugar content (Yang et al, 2022).

Lactobacillus delbrueckii is a probiotic strain of lactic acid bacteria that has been used in yogurt production for hundreds of years. *L. delbrueckii* has an immunomodulatory effect, has beneficial properties for innate and acquired immune responses of the elderly (Moro-García et al, 2013), and has inhibitory properties against other bacteria such as E. coli and Salmonella spp. (Nicdao et al, 2022; Abedi et al, 2013), has the potential to reduce lead toxicity and improve immune function *in vivo* (Evivie et al, 2020). In addition, yogurt prepared using *L. delbrueckii* ssp. Bulgaricus is more effective at improving lactose digestion because it has a higher level of β-galactosidase activity. Several studies have shown that this probiotic strain can improve lactose digestion in people who have lactose malabsorption (Oyeniran et al, 2020).

The importance of developing and implementing functional products for human health and the proven benefits of fermented *L. delbrueckii* dairy products prompted the creation of this study, which sought to create fermented *L. delbrueckii* apple juice and analyze its properties.

Materials and Methods. The main fermentation was conducted in an environment based on fruit juice, which consisted of freshly squeezed apple juice of the Golden variety. The juice was obtained using a home juicer (TEFAL, China). The juice was pasteurized before starting fermentation according to the scheme of double heating to 90°C and sharp cooling to 20°C. The fermentation of a 250-ml volume of juice was carried out in flasks, and glucose was added to one of the samples at a final concentration of 2%. The fermentation was carried out without the use of forced aeration in closed containers without air access. The volume of the container was 80%. The choice of this concentration was based on the goal not to increase the glycemic index of the juice in order to keep it positioned as a dietary product.

In the study, we used a strain of *Lactobacillus delbrueckii* ssp. Bulgaricus isolated from Vivo's commercial yogurt starter, which contained only two strains of *Streptococcus thermophilus* and *L. delbrueckii* ssp. Bulgaricus. *L. delbrueckii* was isolated using repeated streaks by the depletion streak method on MRS (Condalab, Spain) medium at 37°C culture conditions. The purity of the culture was checked by microscopy and Gram staining of cells. Before fermentation experiment the culture of *L. delbrueckii* was previously grown in static anaerobic conditions on liquid MRS (Condalab, Spain) medium for 48 h at 37°. When the culture reached 2 OD (measured by spectrophotometry, UOSLab, Ukraine), it was sterilely added to apple juice in a ratio of 1:50 and fermented for 48 hours at a temperature of 37°C.

The following groups were formed in the studies: group 1 - juice before fermentation, group 2 - juice after fermentation.

Using a spectrophotometer and titrimetric methods, magnesium, glucose, vitamin C, potassium, and total acidity were measured before and after fermentation in two experiments: without pre-added and with pre-added glucose.

The concentration of magnesium was determined by spectrophotometric analysis using xylidyl blue (Chromý et al, 1973), glucose by the glucose oxidase method (Kubihal et al, 2021), and potassium by the turbidimetric method (Tubino et al, 1992).

Iodometric titration was used to quantify vitamin C (Abe-Matsumoto et al, 2020), and acidity was measured using the potentiometric method (MIT, 2019).

The organoleptic properties of apple juice were evaluated using the descriptor-profile method with extended taste indicators according to taste sensations (Lilishentseva and Smoliar, 2019).

All data are presented as medians with interquartile range. For statistical evaluation, Wilcoxson signed rank test has been used in comparison of the control and experimental values of the same group's variables. Statically significant level was set at 0,05.

Results and Discussion. To compare the properties of apple juice before and after two days of *L. delbrueckii* fermentation with and without the addition of glucose, organoleptic indicators, the amount of magnesium, potassium, glucose, vitamin C, and total acidity were compared.

The organoleptic indicators are a key component of comparing apple juice before and after fermentation, with and without the addition of glucose. The results of these indicators are illustrated graphically in Figures 1 and 2.

In the study of organoleptic indicators, it was found that the juice without pre-added glucose after *L. delbrueckii* fermentation loses the apple smell by 45.0% ($p < 0.05$). However, total saturation and salinity increased by 25.0% and 100.0%, respectively ($p < 0.05$). No significant changes in the sensation of sweetness, tartness, and acidity were observed (see Fig. 1).

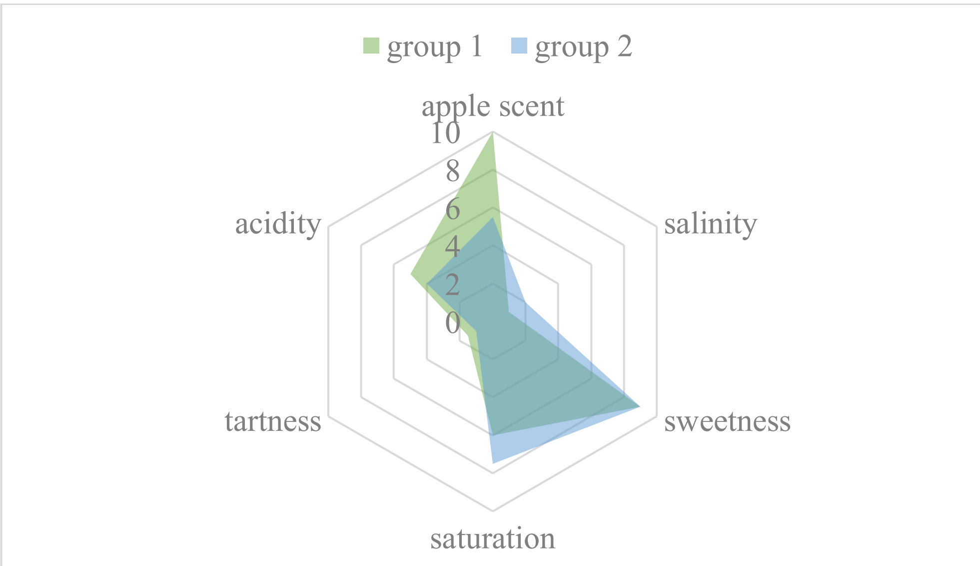


Fig. 1. Organoleptic indicators of apple juice without the addition of glucose before (group 1) and after fermentation (group 2)

When studying the organoleptic properties of fermented *L. delbrueckii* apple juice with the addition of glucose at the beginning of fermentation, it was established that the sensation of saltiness and sweetness decreased significantly – by 100.0% and 12.5%, respectively ($p < 0.05$) (Fig. 2).

Moreover, a significant increase in the apple aroma and overall saturation was observed – by 33.3% and 57.9% ($p < 0.05$) respectively, compared to the original juice. There were no significant changes in the sensation of tartness and acidity (see Fig. 2).

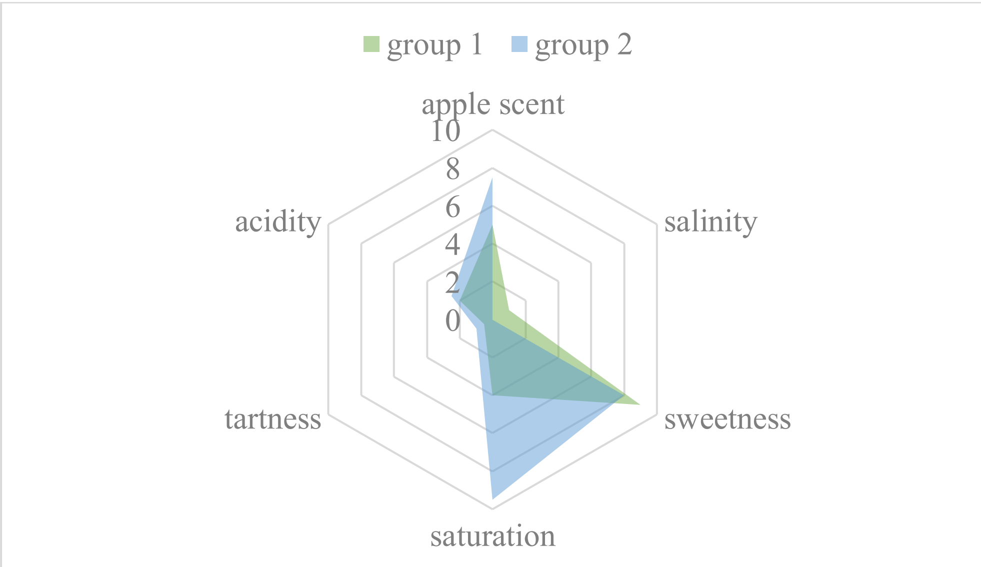


Fig. 2. Organoleptic parameters of apple juice with added glucose before (group 1) and after fermentation (group 2)

The results of the experiment suggest that the addition of glucose to the beginning of the fermentation of apple juice enhances the apple scent and saturation of the juice, thereby improving consumer perception. Without adding sugar at the beginning of the fermentation, the apple scent is lost, but the overall saturation increases. In both versions of the research, acidity indicators did not change. The flavor of fermented apple juice can be stronger than that of regular apple juice. During fermentation, organic acids, like citric acid, can be formed, which can make the product taste sour. Furthermore, fermentation may contribute to the degradation of sugars, which may have impact on the flavor of the product (Swain et al, 2014). Our research suggests that during fermentation, the acidity index does not change reliably, which may be a feature of the *L. delbrueckii* strain. It is possible for lactic acid bacteria to produce specific aromatic compounds, such as ethyl esters, aldehydes, and ketones, which give the product a special smell (Shokri et al, 2020). The rise in the overall concentration of the apple's distinctive scent and flavor after fermentation is a result of the possible accumulation of aromatic compounds, which could explain the observed intensification of the apple's distinctive fragrance.

The amount of glucose in fruit juices is an important indicator. The concentration of glucose in the samples with pre-added and non-added glucose was 5,9 mmol/L and 5.7 mmol/L respectively (see Fig. 3).

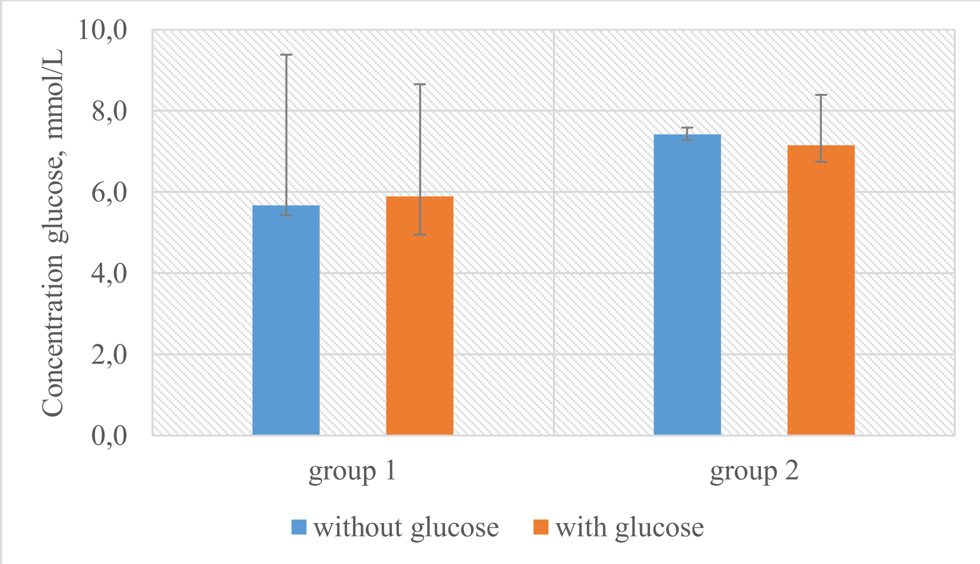
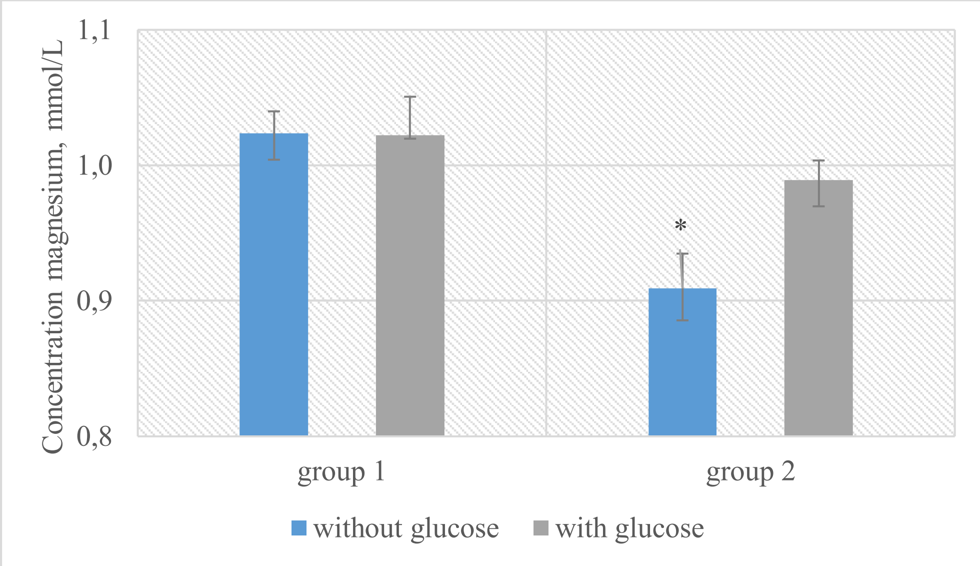


Fig. 3. Glucose concentration in the studied samples of apple juice before (group 1) and after fermentation (group 2)

After fermentation, there was a slight increase in its concentration in the samples with and without glucose before fermentation – 7.2 mmol/L and 7.4 mmol/L respectiveky. This difference in indicators, however, is not statistically reliable. The use of *Lactobacillus delbrueckii* to ferment apple juice could potentially change the sugar content of the drink. Research has shown that this bacterium is capable of quickly consuming the sugar in tomato juice (Kaprasob ey al, 2017), and other fruits, including oranges and apples (Pakbin et al, 2014). A significant decrease in the amount of sugar in the juice occurs only after four days of fermentation, according to another study. *L. delbrueckii* ATCC 9646 was used for apple juice fermentation at 37 °C and pH 6.5. The glucose content significantly decreased after four days of fermentation. Conversely, the sugar content of apple juice did not show significant changes during the first four days of spontaneous fermentation (Al Daccache ey al, 2020). Our research findings align with those found in the existing academic literature. Furthermore, the daily consumption of glucose ranges from 25 to 37.5 grams (138.8 to 205.4 mmol) (Johnson et al., 2009). Based on the obtained results, we can conclude that fermented *L. delbrueckii* apple juice can be a component of a healthy diet.

In two experiments, the magnesium index was analyzed before and after fermentation, without the addition of glucose and with the addition of glucose (see Fig. 4). The magnesium concentration was found to be significantly decreased by 12.6% when using juice without pre-added glucose ($p < 0.05$). But in the juice with the previous addition of glucose, there is no significant change in the magnesium index. The level of magnesium in the juice before fermentation with and without pre-added glucose is at the same level – 1.0 mmol/L.

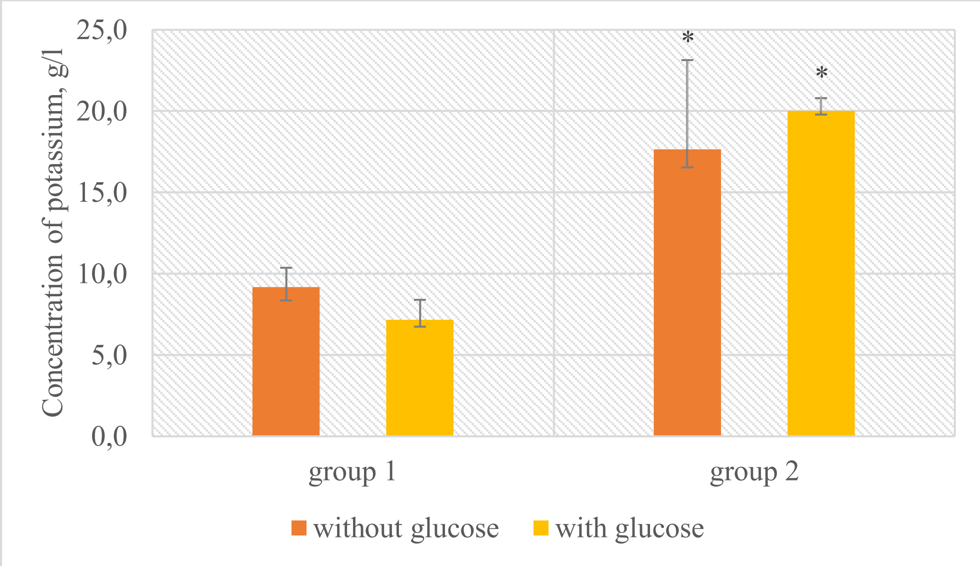


p < 0.05

Fig. 4. Concentration of magnesium in the investigated samples of apple juice before (group 1) and after fermentation (group 2)

Magnesium is an important mineral for human health. The presence of a sufficient level of magnesium in the body is associated with a reduced risk of developing cardiovascular diseases, such as hypertension, coronary heart disease, and stroke (Del Gobbo et al, 2013). Furthermore, magnesium has a significant impact on the regulation of glucose and insulin in the body. There is an increased risk of type 2 diabetes and insulin resistance associated with low magnesium levels Magnesium is an essential mineral for maintaining healthy bones and can be beneficial in the prevention of osteoporosis, especially when combined with vitamin D and calcium (Rude et al, 2009). It also plays an important role in the functioning of the nervous system, reducing stress and improving mood and sleep. Magnesium can also have a positive effect on the prevention of depression and anxiety disorders (Serefko et al, 2016). Magnesium affects many other body functions, including energy metabolism, protein synthesis, muscle function, and the immune system (Volpe, 2013). In a study with fermented *L. plantarum* NRRL-D-14768 fruit juices, the magnesium content increases in all the studied juices: in orange, pineapple, pear and tomato juices, except for watermelon juice - the magnesium content decreases from 37.1 mmol/L to 35.4 mmol/L (Zeng ey al, 2021). The results of this study demonstrate the possible consequences of diminishing the concentration of magnesium in the juice, which could be influenced by the composition of the juice. On the other hand, the daily magnesium requirement for an adult is 12,3-14,4 mmol (Fiorentini et al, 2021). In our study, we obtained quite low levels of magnesium. Just like in other studies, we found a pattern of decline in this indicator. It is important to investigate this indicator more deeply and analyze possible ways to preserve it.

The concentration of potassium in fermented juice without and with pre-added glucose was increased by 48.0% and 64.2%, respectively ($p < 0.05$) (Fig. 5).



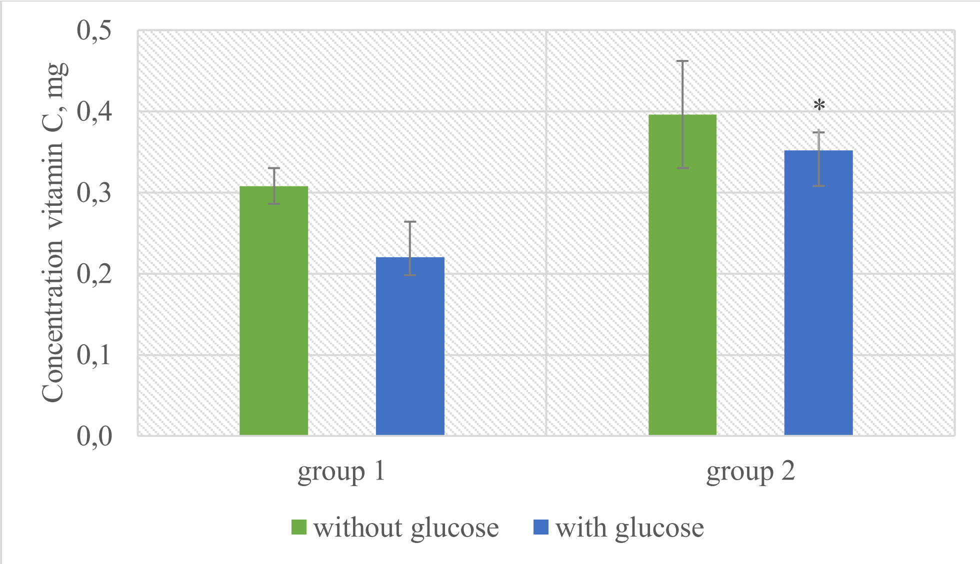
p < 0.05

Fig. 5. The concentration of potassium in the apple juice samples that were studied before (group 1) and after fermentation (group 2)

The addition of glucose to the juice resulted in a slight decrease in the potassium concentration, from 9.2 g/L to 7.2 g/L. However, the increase in potassium concentration after fermentation was greater in the juice with the previous addition of glucose – 20.0 g/L compared to 17.7 g/L in the juice without the previous addition of glucose.

A reduced risk of developing high blood pressure (hypertension) is associated with potassium consumption, which may have a positive effect on heart and blood vessel health (Aburto et al, 2013). Potassium affects the work of muscles, including the heart muscle. It helps ensure normal muscle coordination and contraction (McCartney et al, 2007). A high level of potassium in the diet can help reduce the risk of kidney stones and improve kidney function in people with chronic kidney disease (Siener et al, 2006). Potassium exerts a beneficial effect on the regulation of body fluid levels, thereby promoting a healthy balance of electrolytes. This is particularly important for maintaining normal blood pressure and kidney function (Geleijnse et al, 2003). Potassium can help reduce body fat and increase muscle mass (Kim et al, 2016). The high content of potassium in products has a positive effect on human health. An adult requires 90 mmol of potassium every day (Turck et al, 2016). The resultant fermented juice has more potassium than unfermented juice, even if the juice has additional glucose in it. This presents considerable prospects for the applying of fermented apple juice as an effective therapeutic agent.

An additional indicator determined in the work was the concentration of vitamin C. After adding sugar to the juice, we observed a decrease in the vitamin C content. According to the literature, the effect of glucose on vitamin C is poorly described. There is information about the effect of ascorbic acid on glucose oxidizing enzymes (Lielpetere et al., 2023), as well as the effect of sugars (sucrose, fructose and glucose) in the presence of ascorbic acid on anthocyanins (Gérard et al., 2019). However, we cannot confirm our hypothesis about the possible destruction of vitamin C by glucose when exposed to temperature. This issue requires additional research. The concentration decreased by 37.5% ($p < 0.05$). The concentration of vitamin C was not significantly changed in the sample without the previous introduction of glucose (Fig. 6)



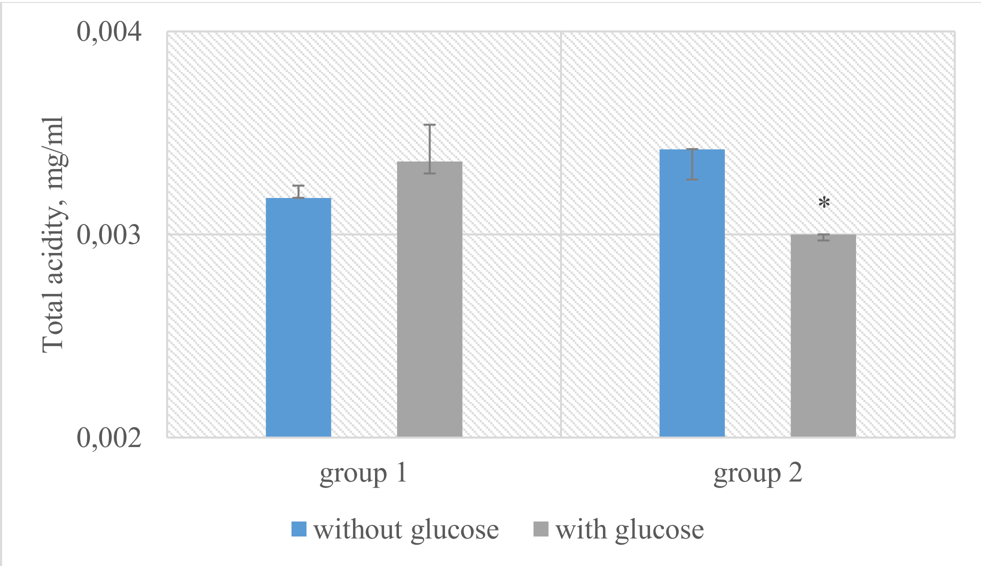
$p < 0.05$

Fig. 6. The concentration of vitamin C in the studied samples of apple juice before (group 1) and after fermentation (group 2)

Vitamin C plays a crucial role in supporting the immune system. It helps to fight against infectious agents by improving the barrier function of the skin and promoting the formation and functioning of immune cells (Carr et al, 2017). Vitamin C is a powerful antioxidant that protects cells from free radical damage and promotes vascular health. It contributes to maintaining a normal tone of blood vessels, reducing the risk of developing cardiovascular diseases, and improving the function of the endothelium (Ashor et al, 2015). Vitamin C plays an important role in the production of collagen, which promotes skin health, delays the aging process, and improves wound healing. Furthermore, it may help to mitigate the harmful effects of ultraviolet radiation and maintain a healthy complexion (Pullar et al, 2017). Vitamin C plays a role in the synthesis of collagen, which is the main component of the bone matrix. It also contributes to the maintenance of bone mineral density and may have a protective effect against the development of osteoporosis (Mosdøl et al, 2010). Vitamin C is involved in the protection of the nervous system, participating in the fight against oxidative stress and protecting neurons from damage. The findings of some studies indicate that vitamin C may improve mood, reduce the risk of depression, and enhance cognitive function (Padayatty et al, 2003).

A decrease in vitamin C content is caused by the fermentation of apple juice by *L. delbrueckii* (Pereira et al, 2011). A temperature of 30-34 °C also negatively affects the process of vitamin C accumulation (Nguyen et al, 2013). In addition to apple juice (Yim et al, 2016), it was also shown that the reduction of vitamin C after fermentation by lactic acid bacteria was also seen in kiwi juice (Kaprasob et al, 2017). The increased content of glucose at the beginning of fermentation may encourage the production of a larger amount of vitamin C. This may be due to the peculiarities of the biosynthesis of vitamin C in *L. delbrueckii*. According to literature, *L. delbrueckii* has been shown to accumulate ascorbic acid during fermentation of sheep milk (Bonczar et al, 2004). It is worth noting that the average daily recommended amount of vitamin C is 90 mg (NIH, 2020). The obtained fermented juice, of course, is not capable of providing such a quantity, since the maximum concentration of glucose in a sample with pre-introduced glucose is only 0.4 mg. The ability of *L. delbrueckii* to accumulate ascorbic acid in the juice with the previous addition of glucose can be used to further develop functional products.

The last indicator that was found for juice samples is the amount of total acidity. During fermentation, it was established that the total acidity in the sample to which glucose was added at the beginning of fermentation was significantly reduced. The decrease is by 12.0% ($p < 0.05$). No significant change in total acidity was observed in the sample without the previous addition of glucose (Fig. 7).



p < 0.05

Fig. 7. The total acidity of the studied samples of apple juice before (group 1) and after fermentation (group 2)

According to scientific studies, the fermentation of apple juice with *Lactobacillus delbrueckii* can result in a decrease in pH, which indicates an increase in the acidity of the juice. Lactic acid bacteria, particularly *L. delbrueckii*, are capable of converting sugars such as glucose and fructose into lactic acid through fermentation. This process contributes to the acidic taste of the juise by lowering the pH (Sossa et al, 2017). One 2017 study looked at the effects of *L. delbrueckii* fermentation on apple juice production. As a result of fermentation, a decrease in pH was observed from 3.8 to approximately 3.4, which indicates an increase in the acidity of the juice. The study also indicated that fermented apple juice had a sour taste because it had lactic acid (Chaves-López et al, 2018). However, in our experimentation, we found the opposite outcome. Total acidity decreased during fermentation, which was not reflected in the organoleptic indicators. The obtained data requires further analysis and research. It may be necessary to supplement the glucose-rich environment with lactose and other sugars to produce acids.

Conclusions. Due to the fermentation process of *L. delbrueckii* apple juice, it is possible to obtain a product with positive organoleptic properties, namely a rich aroma and taste. Adding more glucose at the beginning of fermentation makes the apple taste salty, while adding more glucose at the beginning of fermentation improves the apple scent. In juice with glucose introduced at the beginning of fermentation, there is no decrease in magnesium concentration, but a significant increase in potassium concentration. The concentration of glucose in both types of fermented juices is below the recommended limit. It is observed that juice with pre-added glucose has a greater accumulation of vitamin C. Furthermore, this sample shows a significant decrease in total acidity. Furthermore, the obtained results demonstrate that it is possible to use the bacterium *L. delbrueckii* for the fermentation of apple juice, as it provides more accumulation of potassium and vitamin C. This is also positive for the development of functional products.

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