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APPLICATION OF SPONTANEOUS AND CULTURED FERMENTATION FOR THE SUSTAINABLE PROCESSING OF RASPBERRY POMACE INTO BIOPRODUCTS

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Abstract: Raspberry pomace is a by-product of the berry processing industry, which has limited application due to the high water content and risk of microbiological spoilage. Raspberry pomace is rich in phytochemicals that exhibit antimicrobial and antioxidant properties. Phytochemicals found in raspberry pomace can help to reduce the growth of technologically and nutritionally harmful microorganisms during the fermentation process and increase food functionality. For the production of fermented beverages, a symbiotic culture of microorganisms *Medusomyces gisevii* was utilized, composed of bacteria and yeasts. To ensure microbiological stability and safety of final product, lactic acid bacteria cultures (*Lactobacillus reuteri* and *Lactiplantibacillus plantarum*) were used in the secondary fermentation process. Results indicate that raspberry pomace improved the microbiological stability and functionality of fermented beverages.

Key words: plant by-products, fermentation, symbiotic culture of microorganisms, lactic acid bacteria, ultrasound pretreatment

Introduction

The generation of plant-based waste is a relevant and widespread problem in the biotechnology and food industries. Globally, the largest amount of organic waste is generated in the food industry, especially in the processing of vegetables, fruits and berries (Sarker et al., 2024). As a result, the rational use of plant raw materials, especially their secondary products, has gained considerable attention.

The consumption habits of society are changing, an increasing number of diets aim to balance the ratio of plant and animal food, giving priority to plant-based food (Aschemann-Witzel et al., 2020). Therefore, it is relevant to develop technologies for the processing of plant raw materials and especially their secondary products.

Industrial processing of fruits and berries generates significant amounts of waste. Berry processing yields about 70–80 % of the target product and 20–30 % pomace, which consists of peels, seeds, and pulp (Struck et al., 2016). Raspberries are one of the most widely processed berries, thus large amounts of raspberry pomace is produced. Raspberry pomace has not yet been widely utilized due to the lack of technological solutions and the risk of microbiological contamination (Szymanowska et al., 2021). Pomace, as a by-product of berry processing, is often discarded, resulting in the loss of valuable components such as fibres, sugars, proteins, unsaturated fatty acids, anthocyanins, polyphenols and other secondary metabolites (Sommer et al., 2023). Raspberry pomace is rich in phytochemicals – 100 g of fresh raspberry pomace contains 637,8 mg of polyphenols, 591,7 mg of flavonoids and 65,2 mg of anthocyanins (Vulić et al., 2011). In addition to antimicrobial effect, these phytochemicals have health benefits, such as anti-inflammatory and antioxidant activity in human body (Szymanowska et al., 2021).

Phytochemicals found in raspberry pomace can be used in fermentation processes as antimicrobial agents, thus replacing chemical preservatives or pasteurization (Brodowska et al., 2017). Phenolic compounds found in raspberry pomace exhibit inhibitory effects against *Clostridium*, *Enterococcus*, *Escherichia*, *Mycobacterium*, *Salmonella* and *Staphylococcus* bacteria species (Brodowska et al., 2017).

Symbiotic culture of microorganisms *Medusomyces gisevii* has gained a growing interest in the production of fermented plant-based beverages. *Medusomyces gisevii* is a complex community of microorganisms, composed of bacteria and yeasts (Villarreal-Soto et al., 2018). The primary microorganisms found in *Medusomyces gisevii* include the bacteria *Acetobacter*, *Clostridium*, *Lactobacillus*, *Lactococcus*, and *Gluconacetobacter*, as well as yeasts like *Bretanomyces*, *Candida*, *Saccharomyces*, *Schizosaccharomyces*, *Torulopsis*, *Zygosaccharomyces*, and others (Flyurik et al., 2023). This symbiotic culture of bacteria and yeasts facilitates three types of fermentation: lactic acid, alcoholic, and acetic acid fermentation. Traditionally, *Medusomyces gisevii* is used to ferment sweetened black or green tea. The fermentation process takes place in two stages: during the primary fermentation, the sweetened tea is fermented, and in the secondary fermentation, fruits, berries, or other plant-based ingredients are added. Research has also demonstrated the successful use of alternative substrates instead of sweetened tea in the fermentation process. Several researchers have conducted studies with substrates such as coconut water (Watawana et al., 2015), grape juice (Ayed et al., 2016), and medicinal herbs (Velićanski et al., 2013). These studies suggest that *Medusomyces gisevii* can be successfully adapted for the fermentation of various plant-based raw materials.

Lactic acid bacteria (LAB) can be used in the production of fermented beverages, especially to extend their shelf life and safety. The literature indicates that certain LAB cultures exhibit antimicrobial activity against foodborne pathogenic microorganisms, including bacteria, yeasts and filamentous fungi. In addition, LAB can neutralize mycotoxins released by filamentous fungi (Agriopoulou et al., 2020). LAB are generally considered safe for humans and widely used in the food industry, for example *Lactobacillus reuteri* and *Lactiplantibacillus plantarum*. Also, LAB are naturally found in human gut microbiota (Zapansnik et al., 2022).

The antimicrobial effect of LAB is based on their ability to produce lactic acid and other organic acids, as well as hydroperoxide and bacteriocins, which inhibit the growth of pathogenic microorganisms (Abedi et al., 2020). These properties of LAB are very important and could be used to replace chemical and physical preservation methods (Yadav et al., 2021).

Combination of microorganisms with phytochemicals extracted from plant raw materials can help to ensure the microbiological stability of the product and avoid the use of chemical preservatives. Additionally, the use of biologically active compounds and probiotic bacteria can increase the functionality of the final product.

Material and methods

Microorganisms

Cultures of *Medusomyces gisevii*, *L. plantarum* and *L. reuteri* were taken from the collection of the microbiology science laboratory of Food Institute, Kaunas University of Technology.

Ultrasound pretreatment of raspberry pomace

Ultrasound pretreatment of raspberry pomace was carried out in *Ulsonix Proclean 3.0DSP* 37 kHz (70 W) ultrasonic bath. Fresh raspberry pomace obtained after juice extraction was used for the research. Raspberry pomace was poured into plastic polyethylene bags (8 × 14 cm) with fasteners, 25 grams each. Ultrasound pretreatment was carried out at 35 °C, with ultrasound intensity of 70 % and 45 min pretreatment time.

Primary and secondary fermentation

Primary fermentation. A fermentable solution was prepared under aseptic conditions in the microbiology laboratory. Green tea (3 g/ 1 L) was added into a cylindrical glass container with a capacity of 2.5 L and poured with boiling water. After cooling to 25–27 °C, the tea was strained and unrefined cane sugar (70 g / 1 L) was added. The resulting mixture was homogenized and cooled to a temperature of 20 ± 1 °C. The biological structure of *Medusomyces gisevii* was placed in a 2.5 L container with a cooled mixture of green tea and sugar and fermented for 4 days at a temperature of 20 ± 1 °C.

Secondary fermentation. The fermented liquid obtained after primary fermentation was poured into sterile 200 ml bottles. Raspberry pomace (fresh and ultrasonically treated) and LAB suspensions (*L.plantarum* and *L.reuteri*) were added to the bottles containing fermented liquid. Secondary fermentation was carried out for 3 days at a temperature of 20 °C ± 1 °C. After secondary fermentation, fermented beverages were filtered and stored at 3 °C ± 1 °C.

Microbiological analysis

Microbiological parameters were determined following the LST ISO 21527-1:2008, LST ISO 21527-1:2008, LST ISO 4832:2006, LST ISO 16649-2:2009, LST EN ISO 4833-1:2013 standards.

Fermented beverage samples composition analysis

The total concentration of phenolic compounds was determined by the Folin–Ciocalteu method (Makkar et al., 2007) and the values were expressed as mg of tannic acid equivalent (TAE)/L of fermented beverage.

The total concentration of monomeric anthocyanins was determined by the pH differential method (Lee et al., 2005) and the values were expressed as mg cyanidin-3-glucoside equivalent (CGE)/L of fermented beverage.

Antioxidant activity was determined by the DPPH method (Brand-Williams et al., 1995) and the values were expressed as mg trolox equivalent (TE)/L of fermented beverage.

Evaluation of microbial stability over 20-day storage period

Microbiological stability analysis was performed to evaluate the microbiological stability of fermented beverage samples over a 20-day period. Samples were analyzed at four different time points: day 0, day 6, day 13 and day 20. At each time point, the number of yeasts, moulds, mesophilic lactic acid bacteria, anaerobic microorganisms, coliform bacteria and *E. coli* bacteria were determined.

Results and discussion

Fermented beverage samples analysis

The results of the study show that ultrasound pretreatment of raspberry pomace resulted in significantly higher concentrations of total phenolic compounds, monomeric anthocyanins and antioxidant activity in the fermented beverages.

It was determined that the use of ultrasound pretreated pomace in the production of fermented beverages (samples US, US-R, US-P) resulted in an average of 32,34 % higher concentrations of phenolic compounds and 17,96 % higher concentrations of monomeric anthocyanins compared to the samples prepared with untreated raspberry pomace (RP, RP-R, RP-P). The results are provided in Figure 1.

Anthocyanins were present in the fermented beverage samples due to the inclusion of raspberry pomace. As a result, the control samples (C, CR, CP) did not contain anthocyanins. Additionally, control samples exhibited lowest concentrations of phenolic compounds.

The research revealed that ultrasound pretreatment of raspberry pomace led to an average increase of 4,02 % in antioxidant activity in fermented beverage samples compared to untreated raspberry pomace samples (RP, RP-R, RP-P). The results are presented in Table 1.

Microbiological stability of fermented beverage samples

After the preparation of fermented beverage samples (secondary fermentation stage) microbiological stability studies were performed. To assess the impact of raspberry pomace (both fresh and ultrasound-pretreated) and LAB on the microbiological stability of fermented beverages over 20 days, samples were analyzed at four intervals: day 0, day 6, day 13, and day 20. The number of yeasts, mesophilic LAB, aerobic microorganisms and pH values of samples over a period of 20 days is presented in Figure 2.

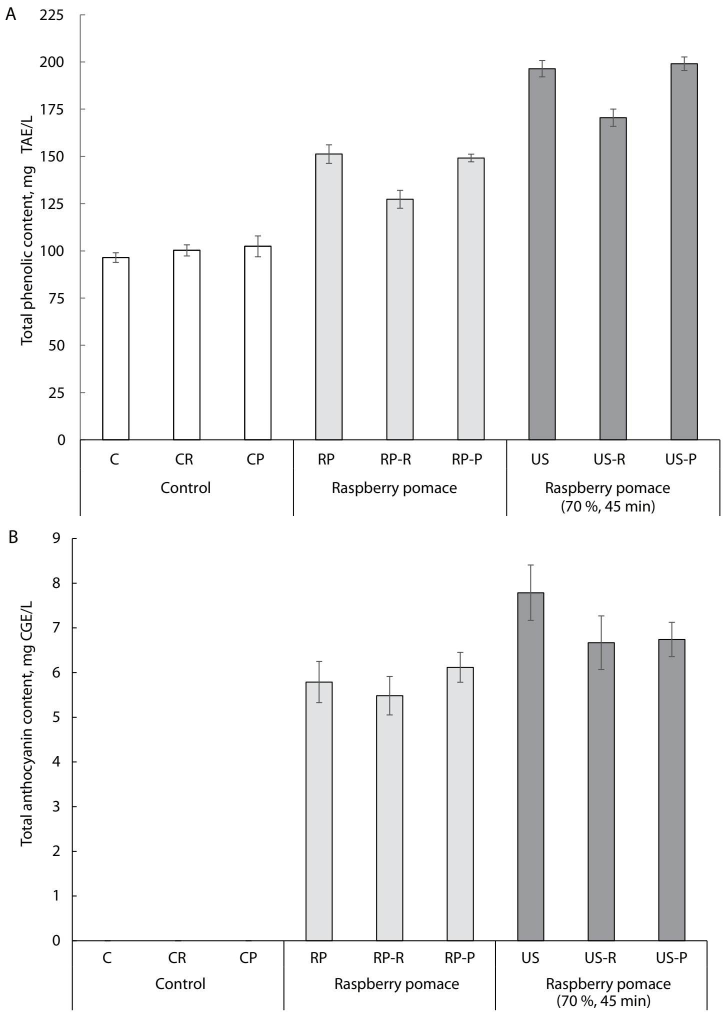

Figure 1. The total concentration of phenolic compounds (A) and monomeric anthocyanins (B) in fermented beverages. Control samples (C, CR, CP) – fermented beverages without raspberry pomace, (RP, RP-R, RP-P) – fermented beverages with raspberry pomace, (US, US-R, US-P) – fermented beverages with ultrasound pretreated raspberry pomace. Samples CR, RP-R and US-R were prepared with addition of *L. reuteri*; samples CP, RP-P, US-P were prepared with addition of *L. plantarum*.

Table 1. Antioxidant activity of fermented beverages expressed as mmol Trolox equivalent (TE/L)

Sample		Antioxidant activity, mmol TE/L
C	Control samples (without raspberry pomace)	0.87 ± 0.01
CR		0.87 ± 0.00
CP		0.88 ± 0.00
RP	Samples with raspberry pomace	0.89 ± 0.00
RP-R		0.89 ± 0.00
RP-P		0.88 ± 0.00
US	Samples with ultrasound pretreated raspberry pomace	0.91 ± 0.01
US-R		0.93 ± 0.00
US-P		0.94 ± 0.00

After 6 days of storage, it was found that samples prepared with raspberry pomace inhibited yeast development (Figure 2, B) and reduced the number of aerobic microorganisms (Figure 2, C), compared to control samples C, CR and CP, which were prepared without raspberry pomace.

However, by the 13th day, there was a decline in LAB concentrations in all fermented beverage samples (Figure 2, A) and an increase of aerobic microorganisms in the samples containing raspberry pomace.

By the 20th day, samples with raspberry pomace had stable levels of aerobic microorganisms, yeast and LAB, similar to the concentrations observed on the 13th day. Results indicate that beverages containing raspberry pomace (both fresh and ultrasound pretreated) were more microbiologically stable, compared to control samples (C, CR, CP).

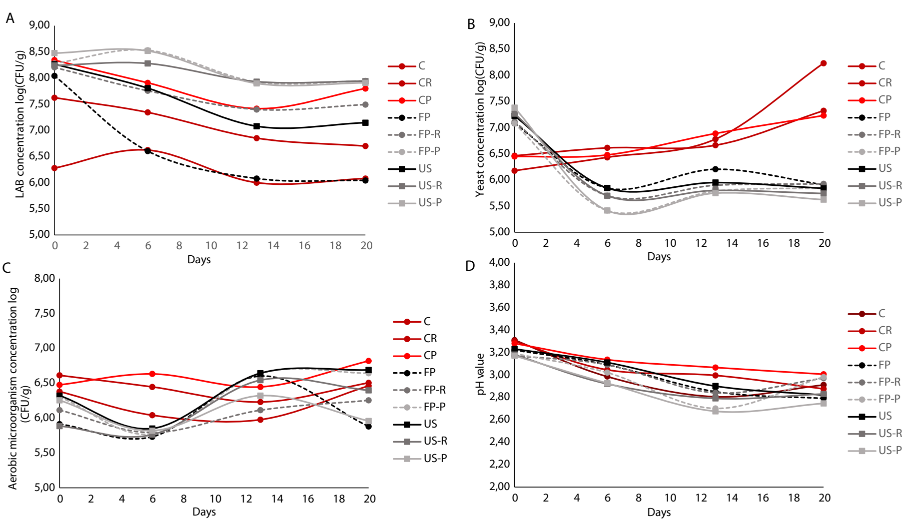

Figure 2. The concentration of LAB (A), yeast (B), aerobic microorganisms (C) and pH values (D) of fermented beverage samples. Control samples (C, CR, CP) – fermented beverages without raspbery pomace, (RP, RP-R, RP-P) – fermented beverages with raspberry pomace, (US, US-R, US-P) – fermented beverages with ultrasound pretreated raspbery pomace. Samples CR, RP-R and US-R were prepared with addition of *L. reuteri*; samples CP, RP-P, US-P were prepared with addition of *L. plantarum*.

Concentrations of coliform bacteria, *Escherichia coli* bacteria and mould remained insignificant (< 1 CFU/g) in all fermented beverage samples throughout the 20-day period.

Conclusions

Research shows that ultrasound pretreated raspberry pomace resulted in higher concentrations of phenolic compounds and anthocyanins in samples of fermented beverages and increased their antioxidant activity. Addition of raspberry pomace resulted in more microbiologically stable fermented beverage samples, compared to control. The results of the study show that the by-product of the berry processing industry can be effectively used for the production of probiotic fermented beverages.

References

Sarker, A., Ahmmed, R., Ahsan, S. M., Rana, J., Ghosh, M. K., Nandi, R. 2024. A comprehensive review of food waste valorization for the sustainable management of global food waste. *Sustainable Food Technology.* 2(1): 48–69.

Aschemann-Witzel, J., Gantriis, R. F., Fraga, P., Perez-Cueto, F. J. A. 2020. Plant-based food and protein trend from a business perspective: markets, consumers, and the challenges and opportunities in the future. *Critical Reviews in Food Science and Nutrition.* 61(18): 3119–3128.

Struck, S., Plaza, M., Turner, C., Rohm, H. 2016. Berry pomace – a review of processing and chemical analysis of its polyphenols. *International Journal of Food Science & Technology. 51.*

Szymanowska, U., Karaś, M., Bochnak-Niedźwiecka, J. 2021. Antioxidant and Anti-Inflammatory Potential and Consumer Acceptance of Wafers Enriched with Freeze-Dried Raspberry Pomace. *Applied Sciences*. 11(15).

Sommer, S., Hoffmann, J. L., Fraatz, M. A., Zorn, H. 2023. Upcycling of black currant pomace for the production of a fermented beverage with Wolfiporia cocos. *Journal of Food Science and Technology*. 60(4): 1313–1322.

Brodowska, A. J. 2017. Raspberry pomace – composition, properties and application. *European Journal of Biological Research.* 7(2): 86–96.

Vulić, J., Tumbas Šaponjac, V., Savatović, S. M., Dilas, S. M., Ćetković, G. S., Ĉanadanović-Brunet, J. M. Polyphenolic content and antioxidant activity of the four berry fruits pomace extracts. *Acta Periodica Technologica*. 2011. 42: 271–279.

Villarreal-Soto, S. A., Beaufort, S., Bouajila, J., Souchard, J. P., Taillandier, P. 2018. Understanding Kombucha Tea Fermentation: A Review. *Journal of Food Science*. 83(3): 580–588.

Flyurik, E. A., Ermakova, O. S. 2023. *Medusomyces gisevii*: cultivation, composition, and application. *Foods and Raw Materials*. 11(1): 152–161.

Watawana, M. I., Jayawardena, N., Gunawardhana, C. B., Waisundara, V. Y. Enhancement of the antioxidant and starch hydrolase inhibitory activities of king coconut water (*Cocos nucifera var. aurantiaca*) by fermentation with kombucha 'tea fungus'. 2015. *International Journal of Food Science & Technology*. 51(2): 490–498.

Ayed, L., Ben Abid, S., Hamdi, M. Development of a beverage from red grape juice fermented with the Kombucha consortium. 2016. *Annals of Microbiology*. 67: 111–121.

Velićanski, A., Cvetković, D., Markov, S. Characteristics of Kombucha fermentation on medicinal herbs from Lamiaceae family. 2013. *Romanian Biotechnological Letters*. 18(1): 8034–8042.

Agriopoulou, S., Stamatelopoulou, E., Sachadyn-Król, M., Varzakas, T. 2020. Lactic Acid Bacteria as Antibacterial Agents to Extend the Shelf Life of Fresh and Minimally Processed Fruits and Vegetables: Quality and Safety Aspects. *Microorganisms*. 8(6): 952.

Zapaśnik, A., Sokołowska, B., Bryła, M. 2022. Role of Lactic Acid Bacteria in Food Preservation and Safety. *Foods*. 11(9): 1283.

Abedi, E., Hashemi, S. M. B. 2020. Lactic acid production – producing microorganisms and substrates sources-state of art. *Heliyon*. 6(10): e04974.

Yadav, R., Gupta, R. 2021. Impact of Chemical Food Preservatives Through Local Product on Human Health – A Review. *High Technology Letters*. 27: 767–773.

Makkar, H. P. S., Siddhuraju, P., Becker, K. 2007. Plant secondary metabolites. Methods in molecular biology (Clifton, N. J.). 393: 1–122.

Lee, J., Durst, R. W., Wrolstad, R. E, Collaborators. 2005. Determination of Total Monomeric Anthocyanin Pigment Content of Fruit Juices, Beverages, Natural Colorants, and Wines by the pH Differential Method: Collaborative Study. *Journal of AOAC INTERNATIONAL*. 88 (5): 1269–1278.

Brand-Williams, W., Cuvelier, M. E., Berset, C. 1995. Use of a free radical method to evaluate antioxidant activity. *LWT – Food Science and Technology*. 28(1): 25–30.

Morales, D., Gutiérrez-Pensado, R., Bravo, F. I., Muguerza, B. 2023. Novel kombucha beverages with antioxidant activity based on fruits as alternative substrates. *LWT*. 189: 115482.