Tailoring functional beverages from selected agri-food by-products and lactic acid bacteria

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Abstract: The agroforestry and agri-food industry produce a flow of waste and by-products that have to be dealt with due to their high pollution potential. Value-added wood biomass by-products find applications as a fuel, insulation material, waste-based biosorbents for the removal of a wide range of water pollutants, filler in biogenic plastics and building block. Medicinal mushrooms by-products are commonly employed as animal feed, fertilizer, bio-based materials for bioremediation and energy production. Moreover, residues and the sawdust from medicinal mushroom and wood cask are recognized for their high phenolic content. Consequently, they hold promise as functional components, which supports their integration into various products. Dried, ground, and sieved post-extraction residues from the medicinal mushrooms *Phellinus linteus* and *Phellinus igniarius* (4%), as well as wood sawdust from myrobalan plum (*Prunus cerasifera*), black locust (*Robinia pseudoacacia*), cherry (*Prunus avium*), and mulberry (*Morus alba*) obtained from a Balkan cooperage (2%), were added at the beginning of yogurts fermentation. Fermentation kinetics, syneresis rates and the viability of lactic acid bacteria (LAB) were evaluated. Results obtained indicate that selected agri-food by-products (AFP) do not disturb fermentation dynamics, nor modify syneresis rates. High LAB viability was maintained (up to 9.02±0.48 log CFU/mL). In conclusion, these results provide a good starting point for the development of yogurt formulated with selected agri-food by-products.

Keywords: agri-food by-product, wood fragments, medicinal mushrooms residues, *Phellinus*, yogurt

1. Introduction

From food industries and agroforestry 5 billion tons of biomass residues are estimated worldwide and represent an emission for 3.3 billion tonnes of carbon dioxide each year. Sawdust, barks, leaves, cones, branches, seeds, chips and resins represent wood biomass by-products (Ferreira-Santos *et al.,* 2020). These by-products are used as a fuel, insulation material, waste-based biosorbents for the removal of a wide range of water pollutants, filler in biogenic plastics and a building block for further processing. Next, by-products generated during mushroom production and processing have been utilized as animal feed, fertilizer, cosmeceuticals, bio-based materials for bioremediation and energy production (Antunes *et al.,* 2020). These approaches address the circular economy aspect to use residual material as a novel product. Even so, when observing the composition of wood biomass by-products and the residues of economically important medicinal mushrooms (MMR), it can be seen that selected by-products have a higher content of extractives, which makes this material suitable for the extraction of bioactive compounds (Häsler Gunnarsdottir *et al.,* 2023). Moreover, agri-food by-products (AFP) have the potential to be transformed into edible ingredients that supply dietary fiber and phytochemicals. In previous publications, it has been shown that wood fragments used in Balkan cooperage (WBC) (Veljović *et al.*, 2017; Smailagić *et al.*, 2019) and MMR (Veljović *et al.*, 2023) could be recognized as unusual and overlooked valuable sources of dietary phenolic compounds. Myrobalan plum (*Prunus cerasifera*),

Table 1. Ingredients based on bark, wood fragments and mushrooms used for yogurt supplementation.

Experimental functional ingredient	Form	LAB strains used for fermentation	Research country	Reference*
The cinnamon bark and twig	Freeze-dried extracts	*Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus acidophilus*, *Lactiplantibacillus plantarum*, *Lacticaseibacillus casei*	China, Malaysia	Tang *et al.* (2020)
Birch and spruce sawdust from various threes, including pine (Pinus sylvestris), spruce (Picea abies), and birch (Betula sp.)	Extracts	Low-lactose strawberry yogurt (2.0% milk fat, Valio Ltd)	Finland	Kirjoranta *et al.* (2020)
The bark and wood of black poplar (Populus nigra × P. maximowiczii Henry cv. Max–5) and basket willow (Salix viminalis L.)	Extracts	*S. thermophilus*, *L. delbrueckii* subsp. *bulgaricus*, *Lacticaseibacillus rhamnosus*	Poland	Walter et al. (2021)
Eight ingredients: L-Glutamine, quercetin, slippery elm bark, marshmallow root, N-acetyl-d-glucosamine (NAG), licorice root, maitake mushrooms (Grifola frondosa), and zinc orotate	Powder	*S. thermophilus*, *L. delbrueckii* subsp. *bulgaricus*	Louisiana, U.S.	Aleman *et al.* (2023)
Ganoderma lucidum residues	Powder	*L. delbrueckii* subsp. *bulgaricus*, *S. thermophilus*, *Limosilactobacillus reuteri* DSM 17938	Serbia	Jovanović *et al.* (2022)
Phellinus torulosus and Phellinus igniarius freshly collected fungal fruiting body	Extracts	Probiotic-rich yogurt (Balans, Bimilk, Bitola); *L. acidophilus* LA-5; *Bifidobacterium lactis* BB-12, *S. thermophilus*	Macedonia	Dimitrova-Shumkovska *et al.* (2022)
Pleurotus ostreatus fungal fruiting body	Extracted Crude Polysaccharides	*L. delbrueckii* subsp. *bulgaricus*, *S. thermophilus*	Poland	Radzki et al. (2023)
Tremella fuciformis	Extracted water-soluble polysaccharides	*S. thermophilus*, *L. bulgaricus*, *L acidophilus*, *B. lactis*	China	Wang *et al.* (2023)
Lentinula edodes	Powder	*S. thermophilus* and *L. bulgaricus*	China	Zhu *et al.* (2023)
Lactarius hatsudake	Powder	*L. delbrueckii* subsp. *bulgaricus*, *S. thermophilus*	China	Zhu *et al.* (2025)
Trametes versicolor	Suspension	*S. thermophilus*, *B. animalis* subsp. *lactis*, *L. plantarum*	China	Liu *et al.* (2025)
Morchella esculenta	Mycelia	*L. delbrueckii* subsp. *bulgaricus*, *Streptococcus thermophilus*, together with 10 probiotic varieties Angel Yeast Co., Ltd. (Hubei, China)	China	Tong *et al.* (2022)

* The selected studies are original scientific articles published in the last five years (between 2020 and 2025)

black locust (*Robinia pseudoacacia*), cherry (*Prunus avium*) and mulberry (*Morus alba*) WBC contain protocatechuic acid, *p*-hydroxybenzoic acid, apigenin, and pinocembrin. Additionally, ellagic acid was predominant in myrobalan plum and black locust, while taxifolin was predominant in cherry and mulberry (Veljović *et al.*, 2017). Medicinal mushrooms also represent a valuable source of pharmaceutically important substances with a diverse chemical structure and pronounced bioactivity. Non-edible mushrooms characterized by a coarse texture and therapeutic properties are classified as medicinal mushrooms. *Phellinus linteus,* commonly known as black hoof mushroom, is a wood decay fungus that can grow on *Morus alba*. Numerous studies confirm that *Phellinus linteus* and *Phellinus igniarius* (willow bracket mushroom) contain a wide range of bioactive secondary metabolites, with polysaccharides and phenolic compounds being dominant and showing an absence of toxic compounds (Yang *et al.*, 2016; Thanh *et al.*, 2018; Dimitrova-Shumkovska *et al.*, 2022). Caffeic acid, ellagic acid, *p*-coumaric acid, *trans*-cinnamic acid, hispidin and fumaric acid (Chen *et al.*, 2019; Çayan *et al.*, 2021; Abdelshafy *et al.*, 2022) have been identified in *Phellinus* species including *P. linteus* and *P. igniarius*. Besides providing valuable compounds, AFP are recognized for their ability to improve water-holding capacity, gel-forming properties and syneresis rates. The application of processed AFP along with probiotics is an appropriate approach to confer functional attributes to food products. Yogurt enriched with AFP can serve as a probiotic carrier, and represents an affordable food, available in many varieties and flavors tailored to individual preferences (Borys *et al.*, 2021; Jovanović *et al.*, 2023). Moreover, phenolic compounds may be considered novel second-generation prebiotics (Rodríguez-Daza *et al.*, 2021). As a beverage ingredient, *Phellinus* species are used in the preparation of coffee, milk-based beverages, alcoholic drinks, and yogurt (Dimitrova-Shumkovska *et al.,* 2022; Yang *et al.*, 2025). Additionally, bark bands are used to wrap cheeses during ripening, bark powder is added to cheeses to enhance sensory qualities, and wood casks are valued for improving the flavor of spirits (Aparnna *et al.*, 2024). Only a limited number of studies have focused on the incorporation of wood fragments and medicinal mushrooms into yogurt (Table 1). Research on yogurts enriched with their by-products, WBC and MMR, is even more scarce. Moreover, most available data is based on laboratory-scale experiments and developed products have yet to reach the European market.

It is noteworthy that, recent research has focused on the sustainable utilization of AFP, including their bioactivity, safety for consumption and sensory acceptance, reflecting increasing public awareness of nutrition and environmental sustainability. The aim of this study was to evaluate the functionality of freshly prepared yogurts supplemented with selected AFP, MMR and WBC. Fermentation dynamics, syneresis rate and influence on viability of lactic acid bacteria were analyzed.

2. Materials and Methods

2.1. Materials

Wood samples from Balkan cooperage (WBC) were obtained from the cooperage industry VBX-SRL.D.O.O. from Kraljevo, Central Serbia. Post-extraction solid residues, from medicinal mushrooms (MMR), were obtained from Jingsu Alphay Bio-Tech, Co. Ltd. (PR China).

Pasteurized cow milk, 2.8% milk fat, were obtained from the local market. YC-X11 lyophilized starter culture containing *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* was purchased from Chr. Hansen (Hørsholm, Denmark). *Limosilactobacillus reuteri* DSM 17938 strain was obtained from the Culture Collection of the Department of Microbiology, University of Belgrade, Faculty of Biology, Belgrade, Serbia. De Man-Rogosa-Sharpe broth (MRS broth) and MRS agar were obtained from Torlak (Belgrade, Serbia). Phosphate buffered saline (PBS) was purchased from Sigma-Aldrich (Steinheim, Germany).

2.2. Sample preparation

Processing agricultural residues, WBC and MMR were conducted as reported earlier (Smailagić *et al.*, 2019; Veljović *et al.*, 2023). Regarding WBC, the staves were ground in a wood mill, and the granulation of the obtained sawdust ranged from 0.5–1.5 mm. The sawdust was sterilized by dry heat treatment in a thermal oven (70° C, 30 minutes).

Neat starter cultures and WBC (2%), and MMR (4%) were used to prepare control and supplement yogurt, respectively. WBC and MMR concentration were selected based on a literature search and prior research experience with similar by-products. Cow´**s** milk (2.8% milk fat) was heat-treated at 85 °C for 10 min, cooled to 43°C, and poured into 100 mL glass containers. Subsequently, neat starter culture (0.02 g, initial counts of log 10.7 CFU/g) or WBC (2% w/w) and MMR (4% w/w) were added. After thorough mixing, the samples were subjected to fermentation at 43 °C until a pH < 4.6 was reached. The obtained yogurts were stirred and stabilized by cooling (4 °C for 24 h).

2.3. Viability of Probiotic Bacteria

Yogurt samples with and without AFP were analyzed as described earlier (Jovanović *et al.,* 2022). Viability of probiotic bacteria was estimated using the pour plate technique and serial dilutions in phosphate buffer saline (1% PBS). LAB were enumerated using MRS agar after incubation at 37°C until colony development. Two independent experiments were performed in triplicate. The results were expressed as the logarithm of the mean number of the colony-forming units (log CFU/mL).

2.4. Dynamics of fermentation and syneresis rates

Fermentation kinetics were evaluated by measuring the pH changes at 1h intervals during yogurt fermentation until a pH of 4.6 was reached and after stabilization (cold storage at 4° C, 24 h). The pH value was determined at approximately 10° C using a pH meter with a gel-filled electrode (WTW™ SenTix™ 41 pH, Massachusetts, MA, USA). The susceptibility to syneresis of yogurts samples was estimated as previously described (Jovanović *et al.*, 2021). The samples (2 × 25 g) from each batch of yogurt were weighed in centrifuge tubes, centrifuged at 3000× *g* for 10 min, and the whey was separated. Syneresis (%) was calculated as a weight of generated supernatant per weight of yogurt multiplied by 100. Experiments were performed in duplicate and repeated three times.

2.5. Statistical Analysis

Statistical analysis was performed using the software GraphPad Prism 6.0 (GraphPad Software Inc., San Diego, CA, USA). One-way analysis of variance (ANOVA) was used to determine the significant differences ($p<0.05$) among treatments. Differences between the mean values were analyzed by Tukey's multiple comparison test. The results were expressed as mean value ± standard deviation (SD).

3. Results and discussion

Considering the prevailing trend towards natural compounds that fulfill multiple requirements, as well as health-promoting and functional characteristics of WBC and MMR, it was necessary to select appropriate WBC and MMR materials suitable for yogurt production. Accordingly, the fermentation kinetics, syneresis rates and the viability of lactic acid bacteria were evaluated.

3.1. Fermentation dynamics and syneresis rates

Acidity is an important indicator of yogurt quality. Acidity, more precisely the characteristic acidic taste, results from the presence of lactic acid, diacetyl, and acetaldehyde produced during the fermentation. Supplementation with WBC and MMR does not significantly affect the fermentation time required for yogurt preparation. Time required to reach pH 4.6 ranged between 2 h 55 and 3 h 14 for MMR supplemented probiotic yogurt prepared with YC-X11 starter cultures and *L. reuteri*, and from 3h 54 and 4 h for WBC supplemented yogurt fermented with YC-X11 starter cultures (Figure 1). The addition of MMR from *P. igniarius* and *P. linteus* led to an initial decrease in pH, which is consistent with the results reported by Dimitrova-Shumkovska *et al.* (2022). In contrast, supplementation with polysaccharides extracted from *P. ostreatus* has been reported to increase the total acidity of fermented milk. However, Walter *et al.* (2021) demonstrated that the addition of extracts from the bark and wood basket willow (*Salix viminalis* L.) together with probiotic bacteria resulted in reduction of the milk acidification rate.

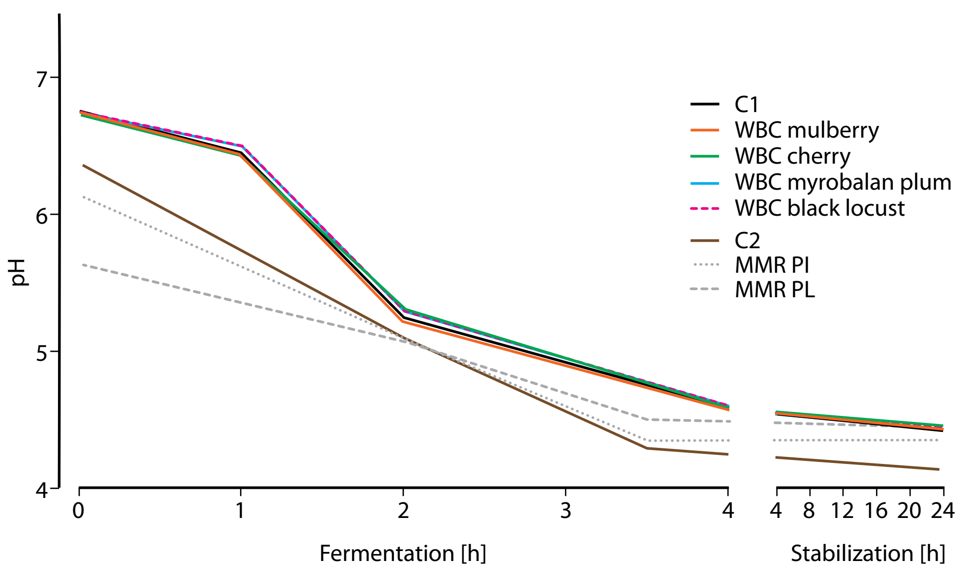

Figure 1. Fermentation kinetics. *C1-control yogurt fermented with *S. thermophilus* and *L. bulgaricus.* C2- control yogurt fermented with *S. thermophilus*, *L. bulgaricus* and *L. reuteri.*

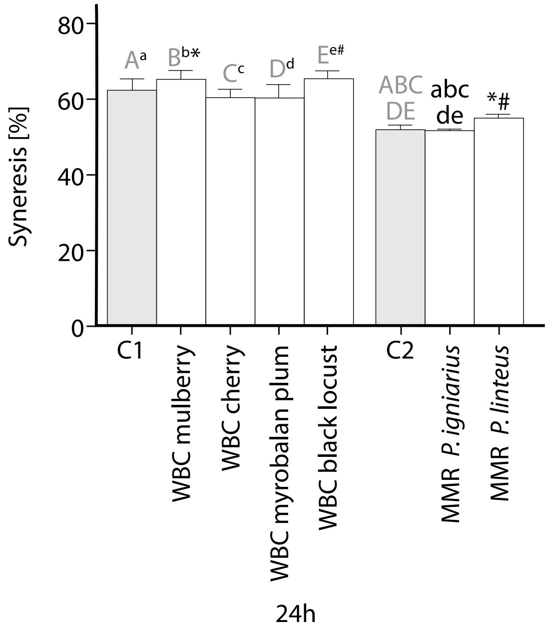

Figure 2. Syneresis rates. [ABCDEabcde*#] significant difference in means between all samples and controls, according to the Tukey's multiple comparison test. Bars with the same mark are significantly different in comparison to each other ($p<0.05$). Exact p value: A p=0.0062;**a** p=0.0099;B p=0.001;**b** p=0.0017; * p=0.01;C p=0.0198; **c** p=0.0294; D p=0.0241; **d** p=0.0353;E p=0.001; **e** p=0.0017; # p=0.01.

Syneresis is also an important characteristic in determining yogurt quality. It is observed as the separation of the surface whey during storage, resulting from the contraction of casein gel. This phenomenon contributes to both chemosensory and psychological aspects of taste perception. Given that syneresis negatively affects consumer perception, it is desirable that functional ingredients added during the yogurt preparation do not increase the syneresis rate. Higher syneresis rates indicate inferior yogurt quality and are attributed to milk preparation, the coagulation process, and the ingredients used (Wang *et al.*, 2020). Selected AFP did not significantly increase syneresis compared to the control (Figure 2). In line with our findings, Dimitrova-Shumkovska *et al.* (2022) showed that yogurt supplementation with the *Phellinus torulosus* and *Phellinus igniarius* water extract (up to 10%) does not increase syneresis. Radzki *et al.* (2023) reported that enriching yogurt with crude polysaccharides extracted from *Pleurotus ostreatus* led to increased syneresis rates, regardless of the starter culture used. For further comparison, fruit and vegetable pomace, such as apple pomace and beetroot pomace, can reduce syneresis owing to their hygroscopic nature. Accordingly, the high water-holding capacity of apple and beetroot pomace may explain the observed reduction in syneresis rates (Wang *et al.*, 2020; Jovanović *et al.*, 2021).

3.2. LAB viability

With the popularization of the Élie Metchnikoff idea that LAB can prevent diseases and delay aging, yogurt has become widely accepted as a regular component of the daily diet (Chandan *et al.* 2017). Numerous epidemiological studies have reported the beneficial effects of yogurt consumption, including a reduction of gastrointestinal discomfort, the elimination of lactose intolerance symptoms, improvement of immune health, reduction in the severity of various infectious diseases, increased metabolic rates, and alterations in mood (Chandan *et al.* 2017; Pannerchelvan *et al.* 2024). These health-promoting effects are strain-specific and rely on LAB amount. According to FAO/WHO, the total viable number of LAB in fermented beverages should exceed 7 log CFU/g (Zhu *et al.*, 2023).

In this study, high LAB viability was observed (Table 2). Consistent with our findings, high LAB viability

Table 2. Probiotic strains viability

Strains	Control yogurt without AFP (log CFU/mL)	Yogurt with selected AFP (log CFU/mL)	Type of AFP
Lactobacillus delbrueckii subsp. *bulgaricus*, *Streptococcus thermophilus*	9.55±0.06	8.78±0.38	WBC Mulberry
		8.58±0.27	WBC Cherry
		9.02±0.48	WBC Myrobalan plum
		8.90±0.32	WBC Black locust
L. delbrueckii subsp. *bulgaricus, S. thermophilus, Limosilactobacillus reuteri* DSM 17938	8.89±0.05	8.54±0.04	MMR *Phellinus igniarius*
		8.81±0.02	MMR *Phellinus linteus*

AFP-Agri-food by-products; WBC-wood fragments used in Balkan cooperage; MMR- medicinal mushroom residues; Amount of selected by-products powder for all WBC samples is 2% while for *P. igniarius* and *P. linteus* residues is (4%).

was detected in yogurt supplemented with *Ganoderma lucidum* residues (up to 9.76±0.11 log CFU/mL) (Jovanović *et al.*, 2022). Moreover, Aleman *et al.* (2023) reported increased growth of *S. thermophilus* in yogurts supplemented with *Grifola frondosa* powder. Yogurt supplementation with *M. esculenta* also promoted LAB viability (Tong *et al.*, 2022).

4. Conclusion

The present circular economy dynamics, sustainability initiatives, and increased awareness on optimal resource use for enhanced sustainable production practices have driven changes and innovations in the by-products management and utilization and support the need to explore more added-value uses for polyphenol-rich agri-food by-products. This study demonstrated that the number and type of innovative ingredients used for yogurt production could be significantly expanded by considering unusual and neglected polyphenol-rich agri-food by-products. Here, selected by-products, residues from the medicinal mushrooms *Phellinus linteus* and *Phellinus igniarius* (4%), as well as wood sawdust from myrobalan plum, black locust, cherry, and mulberry (2%) was added prior to yogurt's fermentation. Such a practice does not disturb fermentation dynamics nor affect syneresis rates in comparison to the control, while a number of the LAB strains remained within the recommended range for LAB cultures.

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Conflicts of Interest: The authors declare no conflicts of interest

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