



Article

Innovative Functional Lactic Acid Bacteria Fermented Oat Beverages with the Addition of Fruit Extracts and Lyophilisates

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Featured Application: In response to the growing consumer demand for dairy-free and vegan products, the presented study demonstrates the potential for producing innovative fermented plant-based beverages with the addition of fruit extracts and lyophilisates. The application of highly biologically active plant compounds as well as the fermentation of oat-based beverages indicate a new direction in the functional food design process. This approach influences the antioxidant activity, sensory, and health-promoting value of the final product.

Abstract: Nowadays, plant-based fermented products are attracting a lot of consumer interest due to their probiotic and health-promoting properties. The aim of this study was to evaluate the microbiological quality and antioxidant activity of innovative fermented oat beverages with the addition of extracts and freeze-dried local fruit. In the first step, chokeberry and hawthorn were selected based on their antioxidant and antimicrobial properties. The final study material consisted of oat beverages fermented with the use of the *Lactiplantibacillus plantarum* DKK 003 strain for 20 h with the addition of 1 and 5% of extracts and freeze-dried fruits. It was found that freeze-dried chokeberry and chokeberry extracts showed a higher content of polyphenolic compounds than freeze-dried hawthorn and hawthorn extracts. After the fermentation process of the innovative beverages, the content of polyphenolic compounds remained the same or there was a slight decrease depending on the additive type. Antioxidant activity significantly decreased after 20 h of fermentation in all enriched oat beverages with no significant differences observed compared to control samples. The obtained fermented beverages were characterised by a high lactic acid bacteria count (above 8 log CFU/mL), a low pH (approximately 4.15), and no microbiological contamination. Oat fermented beverages with fruit additives can be good dietary enrichment products.

Keywords: antimicrobial properties; antioxidants; fermented beverages; functional food; plant-based food; product innovation; sustainable food



Citation: Marchwińska, K.; Gwiazdowska, D.; Juś, K.; Gluzińska, P.; Gwiazdowska, J.; Pawlak-Lemańska, K. Innovative Functional Lactic Acid Bacteria Fermented Oat Beverages with the Addition of Fruit Extracts and Lyophilisates. *Appl. Sci.* **2023**, *13*, 12707. <https://doi.org/10.3390/app132312707>

Academic Editor: Anna Lante

Received: 1 October 2023

Revised: 19 November 2023

Accepted: 21 November 2023

Published: 27 November 2023



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1. Introduction

Nowadays, the prevalence of cow's milk allergies, lactose intolerance, and hypercholesterolemia directs the food industry and the global market to design, supply, and produce novel milk alternatives. This is also supported by emerging consumer trends in the field of plant-based diets. Such substitutes are obtained from plant-based derivatives including cereals, nuts, legumes, and fruits. In particular, the most popular commercial plant-based beverages are obtained with soy, oat, rice, coconut, almond, hazelnut, quinoa,

sesame, and hemp. Currently, worldwide, non-dairy plant-based milk alternatives are a fastgrowing segment in the newer food product development category of specialty beverages. Furthermore, Transparency Market Research (TMR) reported that the plant-based beverages market was forecasted to have approximately 8% of its annual growth during the period from 2019 to 2029 [1]. Designing these innovative food products is in line with current trends aiming to reduce animal production and increase plant production, as a solution for cutting carbon emissions and, therefore, caring for the climate. It is worth underlining that the agri-food industry and global economy place a growing emphasis on the idea of sustainable development. International efforts are made to improve the efficiency of the food industry while reducing its harmful impact on the environment [2]. Literature data indicate that future perspectives on plant-based beverages should focus on their fermentation, as these processes benefit the final product parameters. Popova et al., based on compiled data including minerals, vitamins, and phytochemicals, stated that the enrichment of plant-based beverages should also take place for the design of novel products [3].

New products among the plant-based milk alternatives are fermented beverages. The fermentation process enhances the final product's taste, structure, and health-promoting properties. To add further value to the product, the non-dairy fermented beverages are produced using starter cultures with specific probiotic properties. Fermentation with selected lactic acid bacteria (LAB) may improve the nutritional profile of the product mainly due to the production of amino acids and various bioactive compounds [4,5]. What is important is that due to consumer awareness, fermented plant-based products are gaining popularity as the process of fermentation is one of the oldest, easiest, and most affordable ways to produce and preserve food and beverages. Moreover, this process also enhances the nutritional, sensory, and shelf-life qualities of such products [6]. The consumer's need to live a healthy lifestyle has increased interest in superfoods; therefore, plant-based beverages are being enriched with, among others, nutrients such as proteins and vitamins, as well as plant extracts. A popular group of fermented beverages are products made from cereals such as barley, maize, oats, rye, or wheat [7] due to their properties suitable for fermentation. Non-alcoholic malt beverages, especially from barley, are gaining increasing interest due to their lack of alcohol and their nutritional properties. It is worth underlining that cereals may also constitute the basis for preparing functional probiotic drinks as they are rich in nutrients appropriate for the growth of probiotic strains [8,9].

Fermented OBBs despite the usage of LAB strains are also considered to be functional foods due to the content of prebiotic fibre β -glucan from the cereal. The advantage concerning using oat matrix for fermented beverages lies within their composition as these cereals are rich in not only fibre but also proteins, dietary lipids, as well as micronutrients such as calcium, zinc, and iron, and essential amino acids [6,7,10]. Oat-based fermented beverages appeared first on the Swedish market in 1994, based on the oatmeal gruel fermented with the *Lactobacillus plantarum* 299 v/L and the addition of malted barley as well as different fruit drinks in an amount of 5%. Among the additives responsible for the taste of the beverage were strawberries, blueberries, black currant, rose-hip, and other tropical fruits [6].

Moreover, plants are a source of varied potent bioactive compounds that exhibit high health-promoting and antioxidant potential [11]. Of particular interest in this group are fruits, which, when added in different forms to various products, increase their nutritional and functional value due to the high content of bioactive compounds. The most frequently used are, among others, blueberry, apple, pomegranate, strawberries, mango, peach, plums, grapes, cherries, acai, acerola, guarana, and blackcurrant [12]. Due to their bioactive properties, they are desirable additions to the production of nutraceuticals and functional foods, defined as 'industrially processed or natural foods that when regularly consumed within a diverse diet at efficacious levels have potentially positive effects on health beyond basic nutrition'. It could be achieved by the incorporation of some health-promoting ingredients such as fruit extracts [13,14].

In the presented work, local fruits such as black chokeberry, red currant, blueberry, hawthorn, quince, and sea buckthorn, which are considered to be valuable sources of highly bioactive polyphenols, were selected as enrichments to the designed oat-based beverages (OBBs). Oat is popular worldwide and consumed as a part of the diet. It is worth emphasising that due to the content of soluble fibre, essential amino acids, vitamins, minerals, and unsaturated fatty acids, it demonstrates its health-promoting and nutritional value [10,15]. What is important is that oat may be consumed by people suffering from celiac disease because it does not contain gluten [16,17]. One of the most attractive forms of the functional food category are beverages, and this form is proposed in the presented study. It is worth underlining that functional beverages are becoming a more and more popular group of beverages, next to fruit beverages, such as juices, squashes, or nectars, and stimulated beverages like tea, coffee, or sports beverages [18].

In response to the above considerations, the purpose of this study was to evaluate the potential functional properties of fruits such as chokeberry, red currant, hawthorn, quince, sea buckthorn, and blueberry as additives for designing innovative oat-based fermented beverages. Finally, an approach was proposed to improve plant-based milk alternatives through fermentation of oat beverages with the addition of selected fruit extracts and freeze-dried fruits with the intention of increasing functional properties.

2. Materials and Methods

2.1. Materials, Microorganisms, and Chemicals

2.1.1. Chemicals

Microbial media were purchased from BIOMAXIMA (Lublin, Poland): Endo Agar, TBX, TSA, TSB, BHI Agar, BHI Broth, NA, NB, Sabouraud with Chloramphenicol, Mueller–Hinton (MH) broth; and Sigma-Aldrich (Steinheim, Germany): MRS broth, MRS agar. Folin–Ciocalteu reagent (Sigma-Aldrich, Steinheim, Germany) and sodium carbonate (POCH, Gliwice, Poland) were used to determine total phenolic content, with gallic acid (Sigma-Aldrich, Steinheim, Germany) as a reference. For the Trolox equivalent antioxidant capacity (TEAC) method, the ABTS* + radical cation was prepared using 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS)) diammonium salt from Roche (Mannheim, Germany) and potassium persulfate from Fluka (Buchs, Switzerland). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was purchased from Sigma-Aldrich and used as a standard. Methanol (98%) and ethanol (98%) (POCH, Gliwice, Poland) were used as solvents in antioxidant assays and in the extraction process. For acidification of the ethanol during extraction, trifluoroacetic acid (Merck, Darmstadt, Germany) was used. All chemicals used for the studies were of analytical grade.

2.1.2. Extracts and Lyophilisates

The first step of the experiment was preparing and examining the six local fruits as beverage additions, with a potentially high content of polyphenols. Fresh fruits of black chokeberry (*Aronia melanocarpa*), red currant (*Ribes spicatum* Robson), blueberry (*Vaccinium myrtillus* L.), and quince (*Cydonia oblonga*) were cultivated in home conditions. The plant material was collected in the one growing season (2019) and frozen at $-22\text{ }^{\circ}\text{C}$ for further investigation. Hawthorn fruit (*Crataegus* L.) and whole sea buckthorn berries (*Hippophaës rhamnoides*) were purchased. After crushing, samples of fruits were frozen and then subjected to a freeze-drying process in a CHRIST ALPHA 1–2 LO freeze-drier at $-70\text{ }^{\circ}\text{C}$ under reduced pressure. Lyophilised samples were stored at $-22\text{ }^{\circ}\text{C}$.

For the preparation of extracts, 100 g of shredded samples were placed in a flat-bottom flask with 150 mL of acidified ethanol (80%) and subjected to ultrasound-assisted extraction for 20 min in Bandelin Sonorex Rk 100 H (Sigma-Aldrich, Steinheim, Germany) at room temperature ($22.0 \pm 2.0\text{ }^{\circ}\text{C}$). The samples were filtered and 100 mL of acidified ethanol (80%) was added. The ultrasound-assisted extraction was carried out again for 20 min. After filtration, both filtrates were combined and made up to 300 mL in volume. The prepared extracts were filtered and distilled to reduce the content of ethanol in the samples

in a BÜCHI Heating Bath B-490 (BÜCHI Labortechnik AG, Flawil, Switzerland) at 45 °C. After condensing ethanol, the extracts were frozen and lyophilised in a CHRIST ALPHA 1–2 LO freeze-dryer (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) at –70 °C under reduced pressure. Freeze-dried extracts were stored at –22 °C until use. Twelve different samples including six extracts and six freeze-dried samples were examined in this study.

2.1.3. Microorganisms

The effect of fruit extracts and lyophilisates on the growth of microorganisms was examined using strains obtained from the American Type Culture Collection (ATCC), Polish Collection of Microorganisms (PCM), and its own collection (Department of Natural Science and Quality Assurance Collection—DKK). Gram-positive bacteria included *Bacillus subtilis* ATCC 11774, *Enterococcus faecalis* ATCC 19433, *Micrococcus luteus* ATCC 4698, *Staphylococcus aureus* ATCC 33862, and *Staphylococcus epidermidis* ATCC 12228 as well as *Lactiplantibacillus plantarum* DKK 003, *Lactobacillus rhamnosus* DKK 004, *Lacticaseibacillus paracasei* DKK 002, *Pediococcus pentosaceus* DKK 001, and *Lactobacillus reuteri* DKK 008. Gram-negative bacteria were represented by *Escherichia coli* ATCC 25922, *Salmonella enterica* ser. Enteritidis ATCC 13076, *Serratia marcescens* ATCC 8100, *Moraxella catharalis* ATCC 25238, *Proteus vulgaris* PCM 542, and *Pseudomonas aeruginosa* ATCC 9027. *L. plantarum* DKK 003 was selected as a starter culture for the fermentation of oat beverages. All strains used in the studies were stored at –22 °C using Microbank® cryogenic beads (BIOMAXIMA, Lublin, Poland). Before each experiment, beads were subcultured onto the appropriate broth medium and re-subcultured onto agar medium. The incubation conditions and time depended on the bacterial strain. Microbiological media were used as follows: for *S. marcescens*, *M. luteus*, *M. catharalis*—Trypticasein Soy Broth (TSB) and Agar (TSA) were used; for *S. enterica* ser Enteritidis, *E. faecalis*, *P. vulgaris*—Brain Heart Infusion (BHI) broth and agar were used; for *E. coli*, *P. aeruginosa*, *S. aureus*, *B. subtilis*—Nutrient Broth (NB) and Nutrient Agar (NA) were used; and lastly, for LAB—DeMan, Rogosa and Sharpe (MRS) broth and agar were used.

2.1.4. Fermented Oat-Based Beverages

Commercial oat (*Avena sativa*) flakes were used for the fermented OBBs. The nutritional value of oat flakes, according to the manufacturer, per 100 g was as follows: total fat 6.7 g; total carbohydrate 61 g; dietary fibre 9 g; protein 13 g, and salt < 0.01 g. The mineral content per 100 g, according to the manufacturer, was as follows: calcium 68 mg; iron 4 mg, and potassium 460 mg. Initially, matrix selection was based on the non-published, pilot studies including different approaches while preparing six different samples, with a fixed 10% oat value. Finally, the chosen oat beverage matrix was prepared with 10 g of milled oat flakes and 90 mL of warm water. The flakes were milled using a basic analytical mill (IKA® A11, Sigma-Aldrich, Steinheim, Germany) to produce bran flour with particle size < 1 mm determined through a sieving test. Samples were inoculated with 24 h *L. plantarum* culture on MRS broth in the amount of 10%. Enrichment of fermented OBBs was conducted within the black chokeberry and hawthorn fruit extracts and lyophilisates. The set of samples was prepared for each additive (extract and lyophilisate) in concentrations as follows: 0% (without addition—control sample), 1%, and 5% *v/w*. The process was carried out using sterile Simax glass bottles. Beverages were fermented for 20 h, and the samples were aseptically collected at the beginning and at the end of the process. The microbial quality of all six samples was determined, including the number of LAB, yeast and moulds, Enterobacteriaceae, and the presence of *E. coli*.

2.2. Methods

2.2.1. Antibacterial Activity Assay

The effect of freeze-dried fruits and ethanolic extracts of fruits on the growth of microorganisms was determined using the broth microdilution method according to Gwiazdowska et al. [19] towards 11 pathogenic or spoilage bacteria and 5 LAB. Samples

were dissolved in water at a concentration of 10 mg/mL, centrifuged in sterile Eppendorf centrifuge tubes (10,000 rpm/min, 10 min) in a Centrifuge 5804R, Eppendorf AG, and filtered through the sterile syringe-controlled filter module (MILLEX GP, 33 mm, 0.22 µm, Merck Millipore, Darmstadt, Germany). Twofold dilutions of the extracts were prepared in 96-well microtiter plates in broth media appropriate for each microorganism. Microbiological broth media were used as follows: for *S. marcescens*, *M. luteus*, *M. catharalis*—Mueller–Hinton broth (MH) with a 10% addition of TSB was used; for *S. enterica* ser Enteritidis, *E. faecalis*, *P. vulgaris*—MH with a 10% addition of BHI broth was used; for *E. coli*, *P. aeruginosa*, *S. aureus*, *B. subtilis*—MH was used; and for LAB—MH with a 10% addition of MRS broth was used. The final concentration of tested lyophilisates and extracts was established in the 0.04–5 mg/mL range. Next, microbial suspensions in a medium with an optical density of 0.5 based on the McFarland turbidity standard were prepared from 24 h cultures and 100 µL of the microorganism solutions was added to each well. The final culture density in the well was at the level of 5×10^5 CFU/mL. The negative control was the culture media containing lyophilisates or extracts without microbial inoculum and the positive control was a bacterial culture without any inhibitory substance. The microplates were incubated for 24 h at 30 °C or 37 °C. The optical density of the cultured bacteria was measured at 600 nm wavelength using a BioTek Epoch 2 (BioTek Instruments Limited, Winooski, VT, USA) microplate spectrophotometer. The Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) values were defined as the lowest concentration of extract that exhibited at least 90% and 100% growth inhibition, respectively. All tests were triplicate and performed in parallel. The results were calculated according to the following equation:

$$A_{\%} = \left(1 - \left(\frac{\bar{X}_{OD_s} - \bar{X}_{OD_{c2}}}{\bar{X}_{OD_B} - \bar{X}_{OD_{c1}}} \right) \right) \times 100\% \quad (1)$$

where $A_{\%}$ is the antibacterial activity of the tested substance (extract or lyophilisate), \bar{X}_{OD_s} is the mean optical density of the bacterial inoculate with the tested substance (extract or lyophilisate), $\bar{X}_{OD_{c2}}$ is the mean optical density of the medium with the tested substance (extract or lyophilisate), \bar{X}_{OD_B} is the mean optical density of the bacterial inoculate (without tested substance), and $\bar{X}_{OD_{c1}}$ is the mean optical density of the medium.

2.2.2. Total Phenolic Content Determination

The TPC of extracts, lyophilisates, and OBB samples was spectrophotometrically determined according to Singleton and Rossi [20] with modifications including 48-well microplates as Włodarska et al. [21] previously reported. Tested extracts and lyophilisates were prepared at a concentration of 10 mg/mL in water, while the oat-based fermented beverages fortified by extract and lyophilisates were measured without dilution. The fermented OBB control samples were prepared without the addition of fruit lyophilisates or extracts. All samples were centrifuged before the analysis (5 min, 14,000 rpm/min). The absorbance measurements at a wavelength of 765 nm were conducted using a BioTek Epoch 2 microplate spectrophotometer. Obtained results are expressed as mg gallic acid equivalents (GAE) per g of the extracts or lyophilised fruits, and mg GAE per litre of the OBB. The calculation of the results was made using the following formula:

$$TPC = \frac{(c \times v)}{m} \quad (2)$$

where c is the concentration of gallic acid established from the calibration curve (mg/mL), v is the volume of extract or lyophilised fruits solution (mL), and m is the weight of the sample (g) or volume of the sample for OBB (L) [22].

2.2.3. Antioxidant Activity Assay

The antioxidant activity of extracts and lyophilisate samples as well as fermented OBB was determined through the TEAC assay according to Re et al. [23], based on the ABTS + absorption decrease in the reaction, with previously centrifuged samples ($14,000\times g$, 5 min; MiniSpin plus centrifuge, Eppendorf, Hamburg, Germany). The measurements were carried out using a Milton Roy Spectronic Genesys 2 (Houston, TX, USA) spectrophotometer as described previously [24]. Samples of extracts and lyophilisates were prepared at a concentration of 10 mg/mL in water, whereas the oat-based fermented beverages fortified by extracts and fruit lyophilisates were tested without dilution. The fermented OBB control samples were prepared without the addition of fruit lyophilisates or extracts. All of the examined samples were subjected to studies after centrifugation (5 min, $14,000\times g$ rpm/min). The TEAC values are expressed in mmol Trolox equivalent in g of extracts and lyophilisates; however, for the fermented OBB, the TEAC values are expressed in mM Trolox equivalent per litre of the OBB. The TEAC values were calculated as an equation of the coefficient of sample dilution ratio (extract or OBB) and the corresponding Trolox standard curve.

2.2.4. Microbial Quality Determination

The microbial quality of the oat matrix and fermented OBB was tested using the standard plate technique. The 10 g of the tested sample was placed in sterile blender bags (BagFilter S) with 90 mL of sterile saline solution and homogenised in the stomacher (BagMixer 400 W, Interscience, Saint Nom, France) for 5 min. The microbial quality was determined, including the number of LAB on MRS Agar (37 °C for 48 h), yeast and moulds on Sabouraud Agar with Chloramphenicol (25 °C for 5 days), Enterobacteriaceae on Endo Agar (37 °C for 24 h), and the presence of *E. coli* on TBX Agar (37 °C for 24 h). The results are presented as an average from three parallel repetitions and expressed as the microbial number logarithm.

2.2.5. pH Measurements of Oat-Based Fermented Beverages

The pH was measured using a Mettler-Toledo pH meter after the preparation and fermentation processes of the beverages. pH is the actual acidity, which is defined as the negative logarithm of the hydronium ion concentration (H_3O^+) (EN 1132:1999 [25]). The average laboratory sample of the beverage was thoroughly mixed and placed in a measuring vessel. The measurement was made by immersing the combination glass electrode in the test sample and the result was read after the readings stabilised. The measurement was carried out at room temperature (22.0 ± 2.0 °C).

2.2.6. Statistical Analysis

The results are presented as the mean (\pm standard deviation) of three (for microbiological analysis) or five (for TPC and TEAC analysis) parallel replicates. The obtained results were estimated through one-way analysis of variance (ANOVA). The homogeneity of variance was tested using Levene's test and, based on the results for homogeneous samples and nonhomogeneous samples, Tukey's test and the Games–Howell test with a p -value < 0.05 were applied, respectively. Analyses were conducted using the IBM SPSS Statistics 28 software (PS IMAGO PRO 8.0).

3. Results and Discussion

3.1. Biological Activity of Fruit Extracts and Lyophilisates

In the first part of our investigation, the antioxidant and antibacterial activity of fruit extracts obtained through extraction with acidified ethanol (80%) assisted by ultrasound from frozen and dried fruits (described in Section 2.1.2), and freeze-dried fruits obtained through freeze-drying of non-extracted fruit material were determined. The TPC and antioxidant activity of extracts and lyophilisates are presented in Table 1. The results showed that the polyphenol content, as well as the antioxidant activity, were higher in extracts

compared to freeze-dried fruits. Significant differences in these parameters between individual fruits were also observed. Considering the TPC value and antioxidant activity, the obtained results for the tested fruit may be ranked in decreasing order as follows: black chokeberry > blueberry > hawthorn > red currant > sea buckthorn > quince. It is worth underlining that the antioxidant capacity of black chokeberry extracts and lyophilisates is significantly higher than those of the other fruits, while differences between other fruits are not always so clear. Extracts and freeze-dried blueberry and hawthorn demonstrated a similar antioxidant activity, although they significantly differed in the polyphenol content. Similarly, extracts from quince and sea buckthorn exhibited comparable activities while the polyphenol content was different, whereas the lyophilisates obtained from these fruits did not differ in terms of phenolic compound amount and antioxidant activity. The obtained results are in line with the literature data. The berries such as black chokeberry, blueberry, and red currant are some of the richest sources of polyphenol compounds [26–31]. Chokeberry is an abundant source of phenolic compounds, including procyanidins, anthocyanins, phenolic acids, and their analogues with a relatively high content of the major bioactive components of Aronia berries, from 10 mg to 5500 mg per 100 g of the dried fruits. Polyphenol compounds determine not only the biological activity of chokeberry but also influence the interest in this fruit as a therapeutic agent [32–34]. A high amount of anthocyanins is also found in other dark berries such as blueberry along with other flavonoids including procyanidins, catechin, and epicatechin, as well flavonols (quercetin, kaempferol, myricetin) and nonflavonoid compounds such as phenolic acids or stilbenes [35–37]. Similarly, hawthorn is known for its antioxidant properties due to the presence of polyphenols such as epicatechin, chlorogenic acid, and hyperoside [38]. Also, quince is a good source of antioxidants including flavonoids, phenolic acids, quercetin, rutin, or kaempferol, which makes it useful in various applications including functional food product design [39–42]. The sea buckthorn used in the presented study is an interesting plant, contains a high level of carotenoids, acts mainly as an antioxidant, but also influences its potential to reduce the risk of some diseases such as cancers and eye diseases [43]. Moreover, it contains different flavonoids and polyphenolic acids [44]. It is worth underlining that the comparison of different fruits in terms of their composition and biological activity can significantly vary depending on geographical factors, climate conditions, as well as variety [45].

Table 1. Antioxidant activity and total content of polyphenolic compounds in freeze-dried fruits and fruit extracts.

Raw Material	Fruit Lyophilisates		Fruit Extracts	
	TPC (mg GAE/g of Lyophilisate)	Antioxidant Activity (mM Trolox/g of Lyophilisate)	TPC (mg GAE/g of Extract)	Antioxidant Activity (mM Trolox/g of Extract)
black chokeberry	26.1 ± 0.2 ^a	20.31 ± 1.3 ^a	42.6 ± 0.3 ^a	42.23 ± 0.9 ^a
red currant	10.1 ± 0.1 ^c	10.49 ± 1.0 ^c	11.7 ± 0.3 ^e	17.34 ± 0.4 ^c
Blueberry	13.8 ± 0.0 ^b	14.82 ± 1.0 ^b	26.1 ± 0.2 ^c	35.98 ± 2.9 ^b
Hawthorn	9.1 ± 0.1 ^c	15.05 ± 2.8 ^b	32.2 ± 0.1 ^b	33.26 ± 0.9 ^b
Quince	4.4 ± 0.2 ^d	2.76 ± 0.8 ^d	8.7 ± 0.1 ^e	4.29 ± 0.4 ^d
sea buckthorn	4.2 ± 0.2 ^d	3.82 ± 1.5 ^d	16.9 ± 0.2 ^d	7.31 ± 0.4 ^d

Results are reported as mean ± standard deviation; ^{a–e}—mean values with different letters were significantly different ($p < 0.05$).

Therefore, the content of TPC as well as the antioxidant properties of extracts described in the literature vary due to different methods of their preparation and even part of the plant used for extraction. Vázquez-Espinosa [46], by working on the optimal extraction conditions, obtained a total average TPC concentration in aronia extract at a level of 38.057 mg/g, quite similar to that of the presented work. Bamba et al. [47] used the ultrasound-assisted extraction of phenolic compounds from blueberry pomace, as in the

presented study, and stated that the results depended on the process conditions. The extraction in 50% ethanol/water was the most efficient, with the TPC at a level of 22.33 mg/g dry matter. Ngoc et al. [48] used different extraction methods including ultrasound-assisted extraction and obtained a TPC that varied between 12.6 and 34.7 mg eq. GA/g dry plant. The highest value obtained was comparable to that observed in the presented study. In turn, Sonmez and Sahin [49] demonstrated different TPC values in quince extracts depending on the part of the plant used for extraction, from 9.80 ± 1.41 mg GAE/g extract (from seeds) to 113.47 ± 9.35 mg GAE/g extract (from leaves).

Some reports indicate that processing treatments may influence the quantity of phenolic compounds such as anthocyanins; however, freeze-drying used in the presented study is reported as the best method to protect them. This method is useful to preserve colour and flavour and shows no negative impact on the antioxidant and nutritional properties of fruits [50,51]. The studies of different authors indicated a great variation in results; therefore, it could be quite difficult to compare the obtained results. Du et al. [52] showed that the TPC value differed depending on the species of *C. japonica*, from 19.35 to 46.92 GAE mg/g FW. In freeze-dried quince, Turkiewicz et al. [53] observed 57 g/kg DW, and Antoniewska et al. [54] observed 4165 ± 24 mg GAE/100 mg DM. Téllez-Pérez et al. [55] reported 51.32 mg GA eq./100 g dry matter in freeze-dried chokeberry, while in the research of Thi and Hwang [56], the total phenolic contents reached 919.7 ± 16.9 mg GA eq/g dry matter. In Horszward et al. [57], the content of total polyphenols in freeze-dried aronia powder was 27.63 mg GAE/100 mg DM. The authors also underlined the high antioxidant activity of freeze-dried fruits. For example, Antoniewska et al. [54] and Turkiewicz et al. [55] confirmed the high antioxidant activity of freeze-dried quince. Antioxidant activity has also been preserved in the samples studied in the presented study; however, the extracts, due to their concentrated form, demonstrated a higher TPC value and antioxidant activity. Difficulties in comparing the level of polyphenol content and biological activity between different authors may result from differences in the material as well as process or extraction conditions.

The antibacterial activity of the prepared extracts and lyophilisates was assessed towards pathogenic Gram-positive and Gram-negative bacteria (Table 2, Figures 1 and 2), as well as towards some strains of LAB (Table 3, Figures 3 and 4), commonly used in the fermented products. Comparing the effect of extracts and lyophilisates against pathogenic bacteria, it can be stated that the highest activity was shown by black chokeberry extracts; however, red currant and sea buckthorn extracts also demonstrated a high activity towards the tested bacteria. The lyophilisates obtained from all fruits generally showed a lower activity than the extracts and the determination of the MIC value was impossible. The tested microorganisms differed in their sensitivity to the extracts and lyophilisates used. The most susceptible bacteria were *S. epidermidis* and *M. catarrhalis*, for which the MIC and MBC values for chokeberry extract were set at 0.625 and 2.5 mg/mL, respectively. These microorganisms also demonstrated a high susceptibility to red currant, blueberry, hawthorn, and sea buckthorn extracts (MIC values ranged from 1.25 to 2.5 mg/mL). A high sensitivity to extracts and lyophilisates was also observed in the case of *M. luteus* with an MIC value in the range from 2.5 to 5 mg/mL except for quince, which showed no inhibitory effect at the tested concentrations.

Literature data indicate quite similar values of MIC/MBC of fruit extracts as presented in Table 2; however, it should be noted that it strongly depends on the type of fruit, type of processing, and extraction method. In the presented work, MIC and MBC for most microorganisms were above 5 mg/mL, both in the case of lyophilisates and extracts. Only in the case of a few bacteria, MIC and MBC were established at levels from 0.625 to 5 mg/mL. In the research of Zhang et al. [58], MIC and MBC values for extracts of hawthorn obtained with different solvents (methanol, ethanol, acetone) ranged from 1.25 to 40 mg/mL and from 1.25 to 80 mg/mL, respectively. Similarly, Salmanian et al. [59] reported MIC and MBC values ranging from 2.5 to 40 mg/mL depending on the indicator microorganism and the kind of extract (from pulp or seed). Jeong et al. [60] obtained methanol extracts from

Sea buckthorn and noticed MIC values at levels ranging from 125 to 1000 µg/mL towards Gram-positive and Gram-negative bacteria. In the research of Xiaoyong and Luming [61], blueberry leaf extracts demonstrated antimicrobial activity with MIC values ranging from 12.5 to 50 mg/mL. It is worth underlining that the authors usually observed a stronger inhibition of Gram-positive than Gram-negative bacteria, which is usually explained by a difference in the structure of the cell wall.

Table 2. Antibacterial activity of fruit extracts and lyophilisates towards pathogenic bacteria (mg/mL).

Microorganism	MIC/MBC	Black Chokeberry		Red Currant		Blueberry		Hawthorn		Quince		Sea Buckthorn	
		E	L	E	L	E	L	E	L	E	L	E	L
<i>E. faecalis</i>	MIC	2.5	5	>5	>5	>5	>5	>5	>5	>5	>5	5	>5
	MBC	5	>5	>5	>5	>5	>5	>5	>5	>5	>5	5	>5
<i>B. subtilis</i>	MIC	5	>5	>5	>5	>5	>5	>5	>5	>5	>5	>5	>5
	MBC	5	>5	>5	>5	>5	>5	>5	>5	>5	>5	>5	>5
<i>S. epidermidis</i>	MIC	0.625	5	2.5	5	2.5	>5	1.25	>5	>5	>5	2.5	5
	MBC	2.5	>5	5	>5	5	>5	>5	>5	>5	>5	2.5	5
<i>S. aureus</i>	MIC	2.5	>5	5	>5	>5	>5	>5	>5	>5	>5	5	>5
	MBC	5	>5	>5	>5	>5	>5	>5	>5	>5	>5	5	>5
<i>M. luteus</i>	MIC	2.5	5	2.5	5	5	5	>5	5	>5	>5	2.5	5
	MBC	5	>5	5	>5	>5	>5	>5	>5	>5	>5	5	5
<i>P. aeruginosa</i>	MIC	>5	>5	>5	>5	>5	>5	>5	>5	>5	>5	5	>5
	MBC	>5	>5	>5	>5	>5	>5	>5	>5	>5	>5	5	>5
<i>P. vulgaris</i>	MIC	5	>5	5	>5	>5	>5	>5	>5	>5	>5	2.5	5
	MBC	5	>5	5	>5	>5	>5	>5	>5	>5	>5	5	>5
<i>S. marcescens</i>	MIC	>5	>5	>5	>5	5	>5	>5	>5	>5	>5	>5	>5
	MBC	>5	>5	>5	>5	>5	>5	>5	>5	>5	>5	>5	>5
<i>S. enteritidis</i>	MIC	1.25	5	5	>5	5	>5	>5	>5	>5	>5	5	>5
	MBC	2.5	>5	5	>5	>5	>5	>5	>5	>5	>5	5	>5
<i>M. catarrhalis</i>	MIC	0.625	2.5	2.5	1.25	>5	>5	2.5	5	5	>5	2.5	>5
	MBC	2.5	5	5	2.5	>5	>5	5	5	>5	>5	5	>5
<i>E. coli</i>	MIC	>5	>5	>5	>5	>5	>5	>5	>5	>5	>5	>5	>5
	MBC	>5	>5	>5	>5	>5	>5	>5	>5	>5	>5	>5	>5

E—fruit, extracts; L—fruit, lyophilisates.

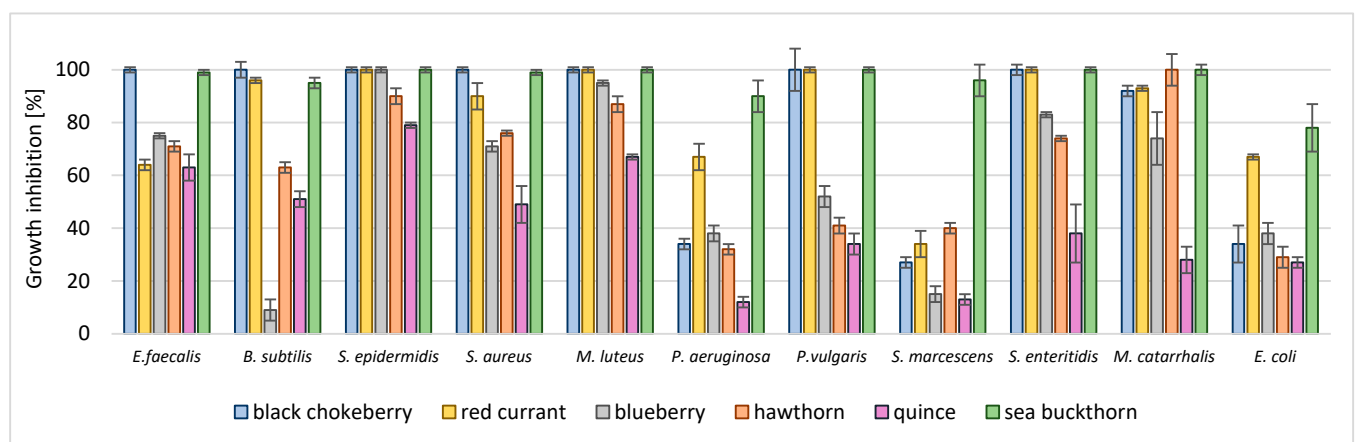


Figure 1. The percentage of growth inhibition of indicator microorganisms by fruit extracts at a concentration of 5 mg/mL.

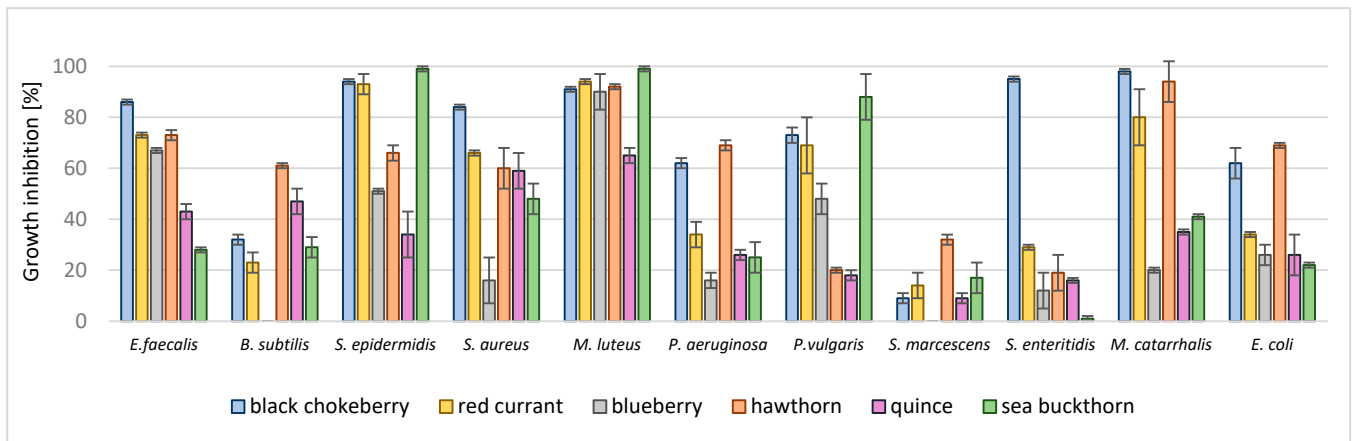


Figure 2. The percent of growth inhibition of indicator microorganisms by fruit lyophilisates at a concentration of 5 mg/mL.

Table 3. Antibacterial activity of fruit extracts and lyophilisates towards LAB (mg/mL).

Microorganism	MIC/MBC	Black Chokeberry		Red Currant		Blueberry		Hawthorn		Quince		Sea Buckthorn	
		E	L	E	L	E	L	E	L	E	L	E	L
<i>Lactiplantibacillus plantarum</i>	MIC	>5	>5	>5	>5	>5	>5	>5	>5	>5	>5	>5	>5
	MBC	>5	>5	>5	>5	>5	>5	>5	>5	>5	>5	>5	>5
<i>Lactobacillus rhamnosus</i>	MIC	>5	>5	>5	>5	>5	>5	>5	>5	>5	>5	>5	>5
	MBC	>5	>5	>5	>5	>5	>5	>5	>5	>5	>5	>5	>5
<i>Lacticaseibacillus paracasei</i>	MIC	>5	>5	>5	>5	>5	>5	>5	>5	>5	>5	>5	>5
	MBC	>5	>5	>5	>5	>5	>5	>5	>5	>5	>5	>5	>5
<i>Pediococcus pentosaceus</i>	MIC	2.5	>5	5	>5	>5	>5	>5	>5	>5	>5	5	>5
	MBC	5	>5	>5	>5	>5	>5	>5	>5	>5	>5	5	>5
<i>Lactobacillus reuteri</i>	MIC	2.5	5	2.5	5	5	5	>5	5	>5	>5	2.5	5
	MBC	5	>5	5	>5	>5	>5	>5	>5	>5	>5	5	5

E—fruit, extracts; L—fruit, lyophilisates.

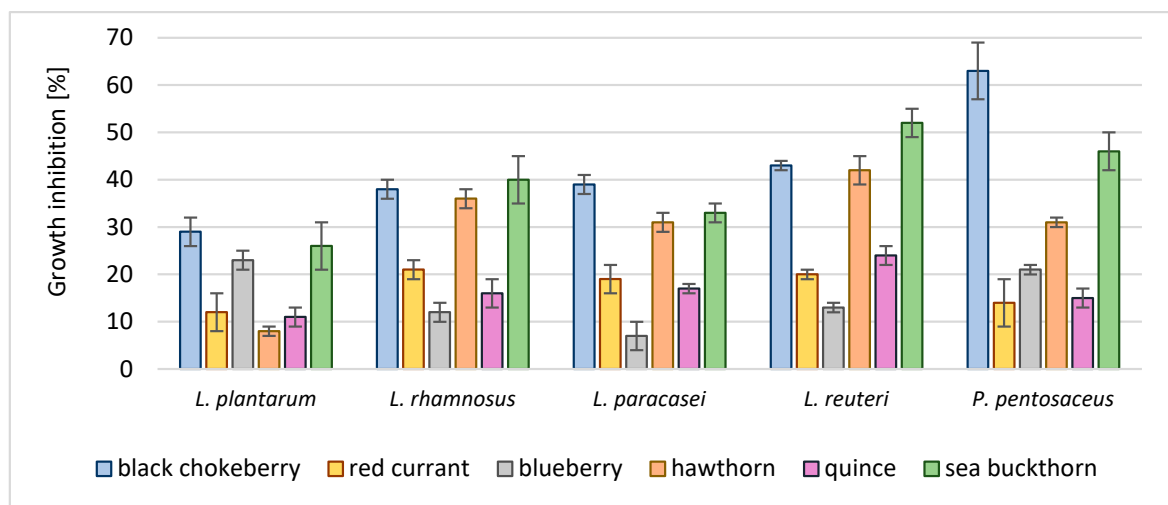


Figure 3. The percentage of growth inhibition of LAB by fruit extracts at a concentration of 5 mg/mL.

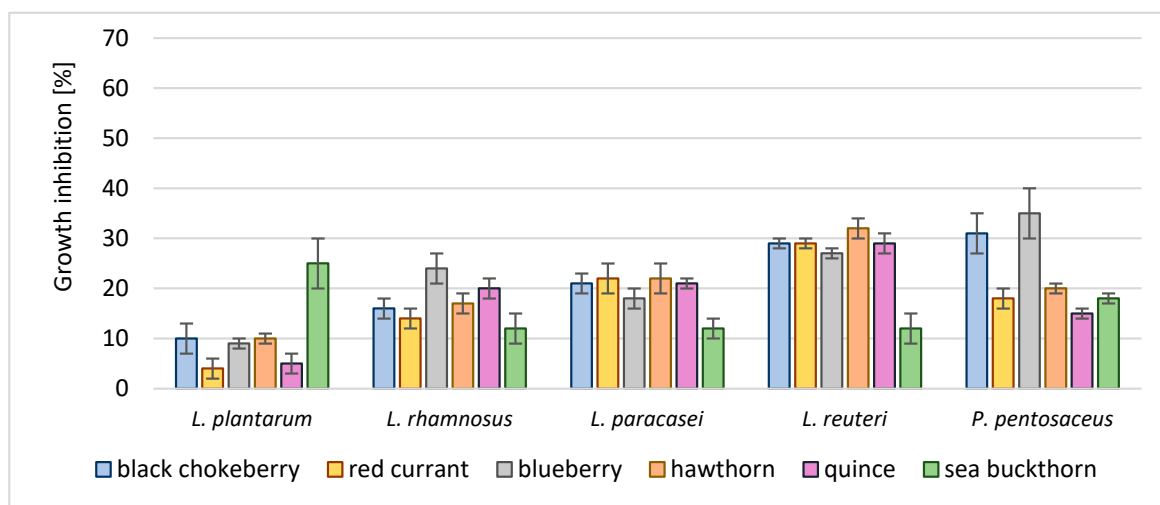


Figure 4. The percentage of growth inhibition of LAB by fruit lyophilisates at a concentration of 5 mg/mL.

Taking into account that MIC and MBC values were not measurable for most microorganisms, as it is shown in Table 2, but the inhibition of the growth of microorganisms was observed, the inhibition degree by the highest tested concentration is presented in Figures 1 and 2. The highest activity, as shown above, demonstrated that the extracts obtained from chokeberry, sea buckthorn, and red currant had an inhibition degree reaching 100% against a majority of indicator bacteria excluding *P. aeruginosa*, *E. coli*, *M. catarrhalis*, and *S. marcescens*. However, it is worth underlining that blueberry and hawthorn extracts exhibited a high activity towards *E. faecalis*, *S. epidermidis*, *S. aureus*, *M. luteus*, *S. Enteritidis*, and *M. catarrhalis*, causing more than 50% of inhibition of growth. Quince extract showed an activity higher than 50% of growth inhibition only towards *E. faecalis*, *S. epidermidis*, and *M. luteus*. Comparing the sensitivity of microorganisms, it could be stated that Gram-positive bacteria were more susceptible to fruit extracts. Among the Gram-negative bacteria, *P. aeruginosa*, *S. marcescens*, and *E. coli* were least sensitive.

Lyophilisates demonstrated a lower activity towards tested indicator bacteria (Figure 2) with the best results obtained for chokeberry lyophilisate, exceeding 80% of growth inhibition in the case of *E. faecalis*, *S. epidermidis*, *S. aureus*, *M. luteus*, *S. Enteritidis*, and *M. catarrhalis*. Also, sea buckthorn lyophilisates showed a high activity towards *S. epidermidis*, *M. luteus* (up to 99%), and *P. vulgaris* (above 80%). Hawthorn lyophilisates inhibited the growth of *M. luteus* and *M. catarrhalis* by at least 80%, while the inhibition degree of *E. faecalis*, *S. epidermidis*, *P. aeruginosa*, and *E. coli* exceeded 60%. Similar to extracts, Gram-negative bacteria, especially *P. aeruginosa*, *S. marcescens*, *S. Enteritidis*, and *E. coli*, were least sensitive. Among Gram-positive bacteria, *B. subtilis* was the least susceptible.

The antibacterial activity of fruit extracts and lyophilisates was also examined towards lactic acid used usually as a starter culture in fermented food (Table 3 and Figures 3 and 4). According to the data in Table 3, fruit extracts and lyophilisates did not show a high activity against the tested LAB strains. The MIC and MBC values were determined only in the case of the effect of black chokeberry, red currant, and sea buckthorn on *P. pentosaceus* and *L. reuteri*. The highest activity was demonstrated in extracts from all fruits mentioned above with an MIC value of 2.5 mg/mL towards *L. reuteri* as well as chokeberry extracts against *P. pentosaceus*. In relation to other tested LAB strains (*L. plantarum*, *L. rhamnosus*, and *L. paracasei*), MIC and MBC values were not measurable.

Similarly to the results concerning antibacterial activity towards pathogenic strains, MIC and MBC values in the case of LAB inhibition were not measurable for most microorganisms. Therefore, the inhibition degree of LAB strains by the highest tested concentration of extracts and lyophilisates is presented in Figures 3 and 4, respectively. The highest activ-

ity was demonstrated in extracts obtained from chokeberry, sea buckthorn, and hawthorn; however, the inhibition degree was usually below 50% excluding the activity of sea buckthorn towards *L. reuteri* and chokeberry extract towards *P. pentosaceus*, inhibited in 52 and 62%, respectively. The inhibition degree of red currant, blueberry, and quince extracts was much lower and did not exceed 30%. It can be noticed that *L. plantarum* showed the least sensitivity to the extracts.

Lyophilisates demonstrated a lower activity towards tested LAB bacteria (Figure 4) with the results not exceeding 40% of growth inhibition. The differences between lyophilisates were not very noticeable; however, differences in the sensitivity of strains were clearly visible. As can be observed, *L. reuteri* showed the greatest sensitivity, while the least susceptibility was demonstrated by *L. plantarum*.

In the presented work, all examined extracts and lyophilisates exhibited antibacterial properties against Gram-positive and Gram-negative bacteria. The inhibition degree strongly depended not only on the fruit type but also the sample preparation as well as the susceptibility of indicator microorganisms. The highest activity towards pathogenic bacteria was demonstrated by chokeberry, sea buckthorn, and red currant extracts; however, hawthorn and blueberry were also active towards the majority of pathogens. In the case of lyophilisates, chokeberry was also the strongest inhibitor; however, blueberry and hawthorn also exhibited a high activity towards pathogenic bacteria. Similar results were reported in the literature. It should be noted that the results described by various authors often indicate very diverse effects due to different methods of sample preparation such as a different extraction method, which usually results in a different composition and concentration of main compounds. Sandulachi et al. [62] examined the antimicrobial effects of powder and hydroalcoholic extracts of berries (rose-hip, aronia, sea buckthorn, and hawthorn) on the growth of antibiotic-resistant *L. monocytogenes* and observed that the highest bacteriostatic and bactericidal effects were demonstrated in sea buckthorn and rosehip. Aronia and hawthorn did not exhibit antimicrobial activity against *L. monocytogenes* in their study. The authors explained this by the cumulative effect of the composition of these berries due to the high content of antioxidant compounds and organic acids. Georgescu et al. [63] conducted research to determine the antioxidant and antimicrobial activities of eight types of berries, including bilberry, black currant, red currant, raspberry, gooseberry, sea buckthorn, sour cherry, and strawberry, harvested from two different geographical regions. The results showed that bilberries and raspberries had the highest antioxidant activity. The extracts from the tested berries demonstrated antibacterial activity against pathogenic bacteria, such as *E. coli*, *B. subtilis*, and *S. aureus*, while the inhibitory effect on *Salmonella* Typhi and fungi including *Candida albicans* and *Aspergillus niger* was absent or very low. The authors underlined that depending on the place of origin, different phytochemical compositions could be observed, which can influence the biological activity. Moreover, the environmental and cultivation conditions in which the berries grow may affect variations in their chemical composition. In contrast to the presented study, the authors observed that the most susceptible indicator microorganism was *E. coli*. Literature data indicate that tannins from berries, especially the pro-anthocyanidins, may be responsible for the strong inhibition of this bacteria [64]. Some authors suggest that antimicrobial activity mainly relates to the presence of hydroxyl groups with polyphenolic compounds and the interaction with the cell envelope, although the mode of action still remains unclear. Studies indicate that phenolic extracts may cause outer membrane disintegration, LPS release, and an increase in membrane permeability. Moreover, a disturbance in the ion exchange through the cell envelope inhibiting the metabolism of the bacterial cell is also described [65,66].

3.2. Microbiological Quality and Biological Activity of Fermented OBB

In the second part of the presented work, oat fermented beverages were produced with the addition of selected fruit extracts and lyophilisates and chosen LAB strains. Oat was chosen as a fermentation matrix due to its good nutritional properties including,

among others, soluble fibre, essential amino acids, vitamins, minerals, and unsaturated fatty acids. Due to its pro-health and nutritional value [10,15], it could gain acceptance by consumers. The addition of fruit extracts of lyophilisates was proposed to increase the polyphenol content and antioxidant activity, enhancing health-promoting properties. It is worth underlining that the availability of similar products on the market, which could be substitutes for fermented milk products, is still limited, although the group of consumers interested in such a solution is growing. Based on the results from the first part of this study, chokeberry as well as hawthorn extracts and freeze-dried fruits were chosen due to their high antioxidant properties and antibacterial activity towards pathogenic bacteria as the additives to the prepared oat fermented beverage. The choice of chokeberry preparations was due to its high content of polyphenolic compounds and antioxidant potential, also confirmed in the literature as a material with extremely high biological potential [27]. Although blueberry extracts and lyophilisates demonstrated a higher antioxidant activity than hawthorn, their antibacterial properties were similar to hawthorn in the case of extracts and weaker than hawthorn in the case of lyophilisates. Moreover, hawthorn is used much less frequently than blueberry; therefore, it is more interesting to use it as an additive to design oat fermented beverages. As hawthorn is one of the oldest known medicinal plants in Europe and presents different pro-health properties such as neuroprotective, hepatoprotective, or cardioprotective effects, an increase in interest in using this plant in food applications including yogurts and tonic wines is observed [67–69]. In turn, *L. plantarum* was chosen as a starter culture due to its low sensitivity to the extracts and lyophilisate activity, which suggests that its growth would not be inhibited in the prepared oat beverages with fruit additives.

The addition of chokeberry extract and lyophilisate (in amounts of 1% and 5%) significantly increased the TPC in oat beverages both immediately after application and after 20 h of fermentation compared to control samples (without any addition). The results are presented in Table 4. The increase in TPC at 0 h of fermentation was 132.04 and 120.83 mg GAE/L in samples with the 1% addition in comparison with the control, while in samples with the 5% addition, it was 130.76 and 158.01 mg GAE/L for the chokeberry extract and lyophilisate, respectively. After 20 h of fermentation, it was noticed that the TPC decreased both in control samples and in oat beverages with the addition of chokeberry extract and lyophilisate. However, a significant decrease in TPC was observed only in control samples (decrease from 84.93 to 64.92 mg GAE/L) and samples with the 5% addition of chokeberry lyophilisate (decrease from 242.94 to 213.71 mg GAE/L). Comparing the TPC values in oat drinks depending on the amount of additive (1% and 5%) as well as on the type of additive (extract/lyophilisate) and fermentation time (0 h and 20 h), no significant differences were noticed, except for the 5% addition of chokeberry lyophilisate in 0 h of fermentation where the TPC content was significantly higher than those of the other variants. The antioxidant activity of the prepared oat drinks was significantly higher compared to control samples only in the case of the 5% addition of chokeberry extract (0.792 mM) as well as 1 and 5% additions of chokeberry lyophilisate (0.750 and 0.847 mM, respectively) immediately after application (at 0 h of fermentation). In all examined variants (except for control samples), a significant decrease in antioxidant activity was concluded after 20 h of fermentation.

Table 4. The effect of chokeberry extracts and lyophilisate addition on total polyphenol content and antioxidant activity of fermented oat beverages.

Oat Beverages Samples	Chokeberry Extract		Chokeberry Lyophilisate	
	Fermentation Time			
	0 h	20 h	0 h	20 h
TPC (mg GAE/L)				
Control	84.93 ^{bB} ± 1.89	64.92 ^{aA} ± 3.50	84.93 ^{bB} ± 1.89	64.92 ^{aA} ± 3.50
1% addition	216.97 ^{cC} ± 11.85	208.53 ^{cC} ± 5.58	205.76 ^{cC} ± 2.51	201.29 ^{cC} ± 5.83
5% addition	215.69 ^{cC} ± 9.99	201.86 ^{cC} ± 9.05	242.94 ^{dD} ± 9.18	213.71 ^{cC} ± 8.31
Antioxidant activity (mM Trolox/L)				
Control	0.657 ^{bB} ± 0.034	0.603 ^{bB} ± 0.033	0.657 ^{aB} ± 0.034	0.603 ^{aB} ± 0.033
1% addition	0.648 ^{bB} ± 0.005	0.517 ^{aA} ± 0.017	0.750 ^{bC} ± 0.021	0.627 ^{aB} ± 0.021
5% addition	0.792 ^{cCD} ± 0.007	0.489 ^{aA} ± 0.011	0.847 ^{cD} ± 0.022	0.638 ^{aB} ± 0.056

Averages with different small letters (^{a-c}) are significantly different at $p < 0.05$ (comparison within a group of samples with the addition of extract and lyophilisate separately). Averages with different capital letters (^{A-D}) are significantly different at $p < 0.05$ (comparison between groups of samples with the addition of extract and lyophilisate).

In the oat beverage samples with the addition of hawthorn extract and lyophilisate (in the amounts of 1% and 5%), the TPC was significantly higher compared to control samples. The results are presented in Table 5. A higher TPC was observed immediately after application and remained at a high level after 20 h of fermentation. The increase in TPC at 0 h of fermentation was 154.46 and 134.67 mg GAE/L in samples with the 1% addition in comparison with the control, while in samples with the 5% addition, it was 158.72 and 129.2 mg GAE/L for the hawthorn extract and lyophilisate, respectively. After 20 h of the fermentation, a significant decrease in TPC was observed in control samples as well as in samples with the 5% extract and 1% lyophilisate. Comparing the TPC values in oat drinks depending on the amount of additive as well as on the type of additive (extract/lyophilisate) and fermentation time (0 h and 20 h), significant differences were noticed between the samples with 1 and 5% of hawthorn extract and lyophilisate after fermentation. The antioxidant activity of the prepared oat drinks was significantly higher compared to control samples only in the case of the 5% addition of chokeberry extract (0.743 mM) as well as 1 and 5% additions of chokeberry lyophilisate (0.808 and 0.732 mM, respectively) immediately after application (at 0 h of fermentation). In all examined variants (except for control samples), a significant decrease in antioxidant activity was concluded after 20 h of fermentation. The fortification of dairy or non-dairy beverages with plant material was reported in the literature. Some experiments were conducted with yogurts fortified with hawthorn extracts, where authors observed a dose-dependent linear effect in the amount TPC and antioxidant activity as in the presented paper. Dabija et al. [69] prepared yogurt with the addition of plant extracts, including hawthorn, and observed a significant increase in TPC, while Herrera et al. [70] prepared yogurts with the addition of hawthorn and strawberry extracts in amounts of 8 and 12 mg/mL, noticing a tendency to increase the TPC value and antioxidant activity; however, the differences were not significant. In the work of Yaneva et al. [71], chokeberry juice was added to oat fermented beverage, where the values for the antioxidant activity increased with the increase in the concentration of chokeberry juice. Lactic acid fermentation can lead to changes in the amount and profile of biologically active compounds such as phenolic acids, sterols, and vitamins, increasing or decreasing their levels in the product [72,73]. Due to the microbial metabolism, polyphenol compounds may be released from conjugated forms and become available, influencing the antioxidant properties. However, it should be underlined that the metabolism of polyphenols by LAB strongly depend on the composition of the raw material used for fermentation as well as the microbial strain responsible for the process [74–76]. Therefore, different variations are described in the literature during the fermentation with plant material or milk with the addition of plant extracts. Similar effects of increasing and

decreasing the total polyphenol content and antioxidant activity were observed during the fermentation of blueberry juices [77] and beetroot and carrot juices with LAB [78] or vegetable and fruit beverages with *L. plantarum* [77]. For example, Szutowska et al. [76] observed a decrease in TPC value after the fermentation of curly kale juice, indicating changes in the amount of some phenolic compounds including sinapic acid, quercetin, or kaempferol. These changes may result from the activity of phenolic compounds acting as reducing agents, singlet oxygen quenchers, or hydrogen donors, causing fluctuations in the TPC and antioxidant capacity [78]. The decrease in phenolic content is most often explained by the bioconversion of phenolic compounds and the rearrangement of their structure due to the acidic environment resulting from the fermentation process. The self-polymerisation or interaction with other compounds such as amino acids is also proposed [79–81].

Table 5. The effect of hawthorn extract and lyophilisate addition on total polyphenol content and antioxidant activity of fermented oat beverages.

Oat Beverages Samples	Hawthorn Extract		Hawthorn Lyophilisate	
	Fermentation Time			
	0 h	20 h	0 h	20 h
TPC (mg GAE/L)				
Control	84.93 ^{bB} ± 1.89	64.92 ^{aA} ± 3.50	84.93 ^{bB} ± 1.89	64.92 ^{aA} ± 3.50
1% addition	239.39 ^{dEF} ± 5.90	251.88 ^{dF} ± 12.29	219.60 ^{dDE} ± 13.91	193.42 ^{cC} ± 5.27
5% addition	243.65 ^{dF} ± 15.50	199.73 ^{cCD} ± 7.50	214.13 ^{dCD} ± 9.39	215.41 ^{dD} ± 2.51
Antioxidant activity (mM Trolox/L)				
Control	0.657 ^{bBC} ± 0.034	0.603 ^{abAB} ± 0.033	0.657 ^{bcBC} ± 0.034	0.603 ^{bAB} ± 0.033
1% addition	0.677 ^{bcCD} ± 0.039	0.557 ^{aA} ± 0.026	0.808 ^{eE} ± 0.013	0.674 ^{cdBCD} ± 0.020
5% addition	0.743 ^{cDE} ± 0.032	0.543 ^{aA} ± 0.018	0.732 ^{dD} ± 0.011	0.532 ^{aA} ± 0.009

Averages with different small letters (^{a–e}) are significantly different at $p < 0.05$ (comparison within a group of samples with the addition of extract and lyophilisate separately). Averages with different capital letters (^{A–F}) are significantly different at $p < 0.05$ (comparison between groups of samples with the addition of extract and lyophilisate).

The prepared oat beverages, both without and with the addition of chokeberry extracts and lyophilisates, were characterised by good microbiological quality. *E. coli*, *Enterobacteriaceae* rods, as well as fungi including yeast and filamentous fungi were not detected in any of the beverages. Therefore, it could be stated that the obtained product does not pose a threat to consumers' health. In turn, the number of LAB was around 10^7 – 10^8 CFU/mL after inoculating the drinks with starter cultures and increased significantly after 20 h of fermentation in all variants (Figure 5). Due to the high number of the selected LAB strains, the obtained product may be considered potentially probiotic. After the fermentation process in oat beverages with the addition of chokeberry extract or lyophilisate, the number of LAB was comparable (in the case of the lyophilisate and a 1% extract addition) or significantly higher (for a 5% extract addition) compared to the control sample. Analysing the effect of the additive type (extract or lyophilisate), it can be noticed that a significantly higher number of LAB was noted in samples with a 5% addition of chokeberry extract.

Similarly to the oat beverages with chokeberry extracts and lyophilisates, the samples with hawthorn demonstrated high microbiological quality since *E. coli*, *Enterobacteriaceae* rods, and fungi were not observed. The number of LAB was initially at a level of 10^7 – 10^8 CFU/mL; however, a significant increase was noted after 20 h of fermentation, which proves that both hawthorn extract and lyophilisate have no inhibitory effect on LAB (Figure 6). After the fermentation process, the number of LAB in all variants of beverages with hawthorn additions was comparable (without statistically significant differences). The results of the microbiological analysis confirmed that the oat fermented beverage with hawthorn does not pose a health hazard to consumers.

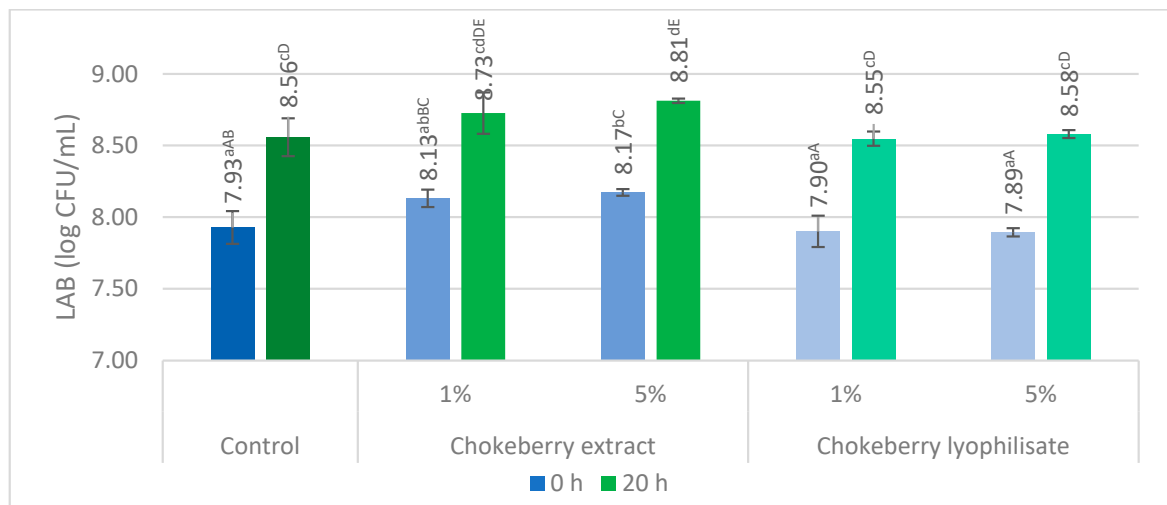


Figure 5. Total number of LAB in oat beverages with the addition of chokeberry extract and lyophilisate. Averages with different small letters (^{a-d}) are significantly different at $p < 0.05$ (comparison within a group of samples with the addition of extract and lyophilisate separately). Averages with different capital letters (^{A-E}) are significantly different at $p < 0.05$ (comparison between groups of samples with the addition of extract and lyophilisate).

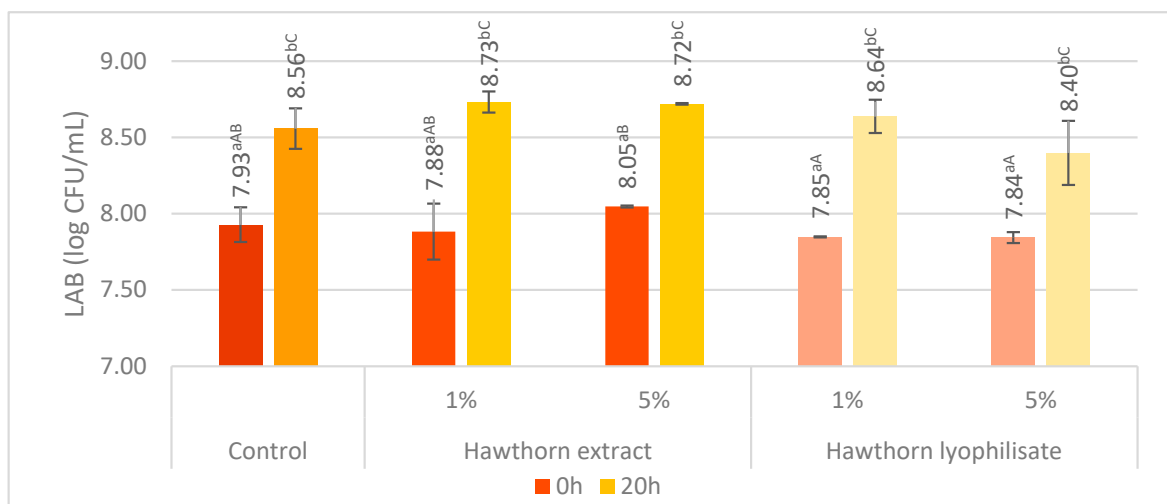


Figure 6. Total number of LAB in oat beverages with the addition of hawthorn extract and lyophilisate. Averages with different small letters (^{a,b}) are significantly different at $p < 0.05$ (comparison within a group of samples with the addition of extract and lyophilisate separately). Averages with different capital letters (^{A-C}) are significantly different at $p < 0.05$ (comparison between groups of samples with the addition of extract and lyophilisate).

During the OBB fermentation by *L. plantarum* DKK 003, a decrease in pH value was observed for all tested samples. The measurements were conducted both at the beginning of the fermentation and after the process. The pH value of the control samples, without fortification, decreased from 5.08 ± 0.05 to 4.15 ± 0.03 after 20 h of fermentation. The same tendency was observed for all of the fortified OBB samples, independently of the used additive and its amount. For OBBs with black chokeberry extracts, the pH value decreased from 5.00 ± 0.03 to 4.13 ± 0.02 and, for the freeze-dried fruits, from 5.03 ± 0.04 to 4.14 ± 0.02 during the fermentation process. As for the hawthorn fortification of OBBs, the pH value of samples with added extract acidified from 5.04 ± 0.04 to 4.14 ± 0.01 , and for the lyophilisates, from 5.03 ± 0.04 to 4.14 ± 0.02 .

Literature data indicate that different LAB species may be successfully used as starter cultures for the preparation of cereal fermented beverages [82]. In the presented study, *L. plantarum* was used due to the dynamics of its growth and reached a high cell number in the matrix created on the basis of oat flakes. Among LAB species, *L. plantarum* is part of the natural microbiota occurring in spontaneously fermented cereals; however, it should be underlined that the technological properties are strongly dependent on the strain [83]. Nionelli et al. [84] prepared a fermented oat beverage based on oat flakes with different strains of *L. plantarum* and obtained a beverage with good nutritional and sensory properties with one of the tested strains, *L. plantarum* LP09. The fermentation process decreased the pH to 4.2, similar to the presented work, and increased antioxidant activity, while in this study, a decrease in antioxidant activity was observed. After the fermentation, the LAB cell number was at a level of 6.5×10^8 CFU/mL, even as the process was shorter than in the presented work, and took 12 h. Similar results concerning LAB cell numbers were obtained in the presented work. However, some authors obtained even higher results. In the work of Angelov et al. [85], a whole-grain oat substrate was fermented with LAB for 8 h to obtain a drink, with the probiotic culture and the oat prebiotic beta-glucan. The LAB cell number was about 7.5×10^{10} CFU/mL. In their study, the addition of some sweeteners did not influence the dynamics of the fermentation process and viability of bacteria. Gupta et al. [86] obtained an oat fermented drink using oat flour and *L. plantarum* as a starter culture, indicating that oat and sugar content had the greatest impact on the growth of bacterial strains. The fermentation was carried out at 37 °C for 8 h, achieving 10.4 log CFU/mL. In turn, Nionelli et al. [84] prepared a beverage from oat flakes milled into flour and conducted fermentation with *L. plantarum*, *L. casei*, and *L. paracasei* strains. Similar results were also presented by Aparicio-García et al. [87], who studied fermented drinks based on oat sprout flour. The authors showed that after 4 h of fermentation with *L. plantarum* WCFS1, the number of LAB in the beverages was approximately 9 log CFU/mL, while the number of fungi, *E. coli*, total coliforms, and *B. cereus* was below 1 log CFU/mL. They also did not note the presence of pathogens such as *L. monocytogenes* or *Salmonella* spp. in the prepared beverages. It is worth underlining that a high number of LAB, observed also in this study, guarantees the appropriate properties of the beverages because, during their development, bacteria lower the pH and produce metabolites that inhibit the growth of undesirable microorganisms. In the case of the beverages tested in the presented work, an additional factor influencing the microbiological quality was the addition of extracts and lyophilisates, demonstrating antibacterial properties.

4. Conclusions

Using freeze-dried fruits, their extracts, and by-products rich in bioactive compounds to enrich the basic food products is an emerging topic in food science research and widely used in various food products [88]. From a nutritional point of view, this kind of additive provides important benefits: it improves the microbiological stability and antioxidant activity of the final product, but also changes the colour and flavour. Furthermore, the use of bacteria starter cultures for the production of fermented food products increased their probiotic properties, which is very health-promoting and quality-improving.

The current study demonstrated the potential for producing innovative vegan plant-based beverages with the addition of highly biologically active fruit extracts and lyophilisates, such as a chokeberry or hawthorn with LAB starter cultures. This paves the way for the future development of functional plant-based products with enhanced bioactive properties, both through LAB fermentation and the addition of fruit extracts and lyophilisates. The application of fruit extracts and lyophilisates as well as the fermentation of OBBs indicate a new innovative direction in the functional food design process as it influences the antioxidant activity, sensory value, and health-promoting value of the final product.

Author Contributions: Conceptualization, K.M., D.G. and K.P.-L.; methodology, K.M., D.G. and K.P.-L.; validation, K.M., D.G., K.J. and K.P.-L.; formal analysis, K.M., D.G., K.J., P.G., J.G. and K.P.-L.; investigation, K.M., D.G., K.J., P.G., J.G. and K.P.-L.; resources, K.M., D.G. and K.P.-L.; data curation, K.M., D.G., P.G. and K.P.-L.; writing—original draft preparation, K.M., D.G., K.J., P.G., J.G. and K.P.-L.; writing—review and editing, K.M., D.G., K.J. and K.P.-L.; visualization, K.M., D.G. and K.J.; supervision, K.M., D.G. and K.P.-L.; project administration, K.M. and D.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available in the article.

Conflicts of Interest: The authors declare no conflict of interest.

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