Article

Effect of Sea Buckthorn (*Hippophae rhamnoides* L.) Mousse on Properties of Probiotic Yoghurt

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Abstract: The stability of the physico-chemical and sensory characteristics of yoghurts during refrigerated storage is important for industry and the consumer. The aim of the study was to evaluate the nutritional value, microbiological quality, sensory properties, and structure of natural probiotic yoghurts made with the addition of sea buckthorn fruit mousse during refrigerated storage. In this study, we produced natural, probiotic organic yoghurts with the addition of superfoods, i.e., sea buckthorn (*Hippophae rhamnoides* L.) fruit mousse, using ABT-1 probiotic yoghurt starter culture based on *Lactobacillus acidophilus* LA-5, *Bifidobacterium lactis* BB-12, and *Streptococcus thermophilus*. Physico-chemical (acidity, nutritional value, and structure) as well as microbiological and sensory changes occurring during 21-day refrigerated storage were determined. The yoghurts were characterized by high sanitary and hygienic quality during the whole refrigerated storage. Fourier-Transform Infrared (FTIR) spectra were also used in the analyses of the obtained yoghurt samples. The applied yoghurt additive was visible at the spectral level as an increase in the intensity of the characteristic bands for vibrations related to protein, fat, and polysaccharide structures. Sea buckthorn can be used as a modern and unconventional addition to yoghurts with health-promoting properties. Micrographic studies have shown that the addition of sea buckthorn mousse significantly changes the microstructure of the yoghurt. The structure of sea buckthorn to yoghurt seems to be more susceptible to the influence of damaging factors, which is also confirmed by the FTIR test conducted during the storage.

Keywords: yoghurt; storage; nutritional value; sensory properties; microbiological quality; scanning electron microscopy; FTIR spectroscopy

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1. Introduction

Dairy products, and in recent years, yoghurt in particular, are important components of the human diet. For this reason, manufacturers of dairy products are making an effort to meet the needs and expectations of the contemporary consumer by offering a growing assortment of products [1,2]. Consumers increasingly consider the quality of fermented milk products [3–5], and expect a high level of innovation. Chemically, yoghurt is a complex gel system incorporating protein, lipids, and polysaccharide in its structure. According to the definition given by the FAO/WHO, yoghurt is milk that has been fermented and coagulated using *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* bacteria [6]. It

should be mentioned that *Str. thermophilus* bacteria primarily produce lactic acid, while *L. delbrueckii* subsp. *bulgaricus* are responsible for the aroma of yoghurt [7–9]. Lactic acid fermentation is one of the oldest and most widespread methods for preserving milk.

Among the food, yoghurt most frequently contains probiotics [10], which are defined as “live microorganisms, which when administered in adequate amounts confer a health benefit on the host” [11]. Lactic acid bacteria, mainly from the genera *Bifidobacterium* and *Lactobacillus*, are widely recognised as probiotics, as they commonly possess probiotic characteristics. These genera are usually assigned the generally-recognised-as-safe (GRAS) status, which indicates low or no health risk to the host upon consumption, with positive health effects [10,12,13]. Probiotic bacteria that are added to yoghurts colonize the human gut, thereby increasing immunity, reducing the effects of lactose intolerance, and protecting against diarrhoea and constipation. They also facilitate the absorption of minerals (iron and calcium) and vitamins [12,14]. Yoghurt is recommended for children, people with allergies or digestive disorders, the elderly, and patients undergoing antibiotic treatment [12,14–16]. The human body digests yoghurt three times faster than milk [17].

Producers increasingly often meet the expectations of consumers and enrich fermented milk beverages with plant additives. The application of additives primarily affects the sensory features of the final product, i.e., flavour, aroma, colour, and consistency, as well as texture and storage capacity. According to the idea of functional food, a plant additive also increases the content of bioactive compounds. When assessing the perceived quality of fermented dairy products, the most important are consumer quality attributes, i.e., sensory characteristics, mainly flavour, as well as availability, especially packaging and convenience of use, which determine the purchase decision. Healthiness, including product composition, is becoming increasingly important, especially among more informed consumers, taking into account not only the basic nutritional value, but also the content of biologically active ingredients positively influencing the human body. Therefore, fermented milk drinks enriched with plant additives may be more attractive to consumers, also contributing to increasing the market offer [2,4].

In the recent years, it has been reported that fortification of yoghurts using natural resources (fruits, vegetables, cereals, nuts, seeds, oil, botanical or herbal extracts, fibre from fruits or vegetables, fruit processing wastes, and whey protein isolates) is necessary to improve its quality and bioactivity, including nutrient intake with minimal health side effects [2,18–20]. However, the base is natural, plain yoghurt, which should be of good quality.

In our research, we proposed sea buckthorn (*Hippophae rhamnoides* L.) as a new yoghurt additive. Sea buckthorn was known already in ancient Greece, where it was used as a medicine for horses. Its Latin name comes from Greek (hippos-horse, phao-shine). The treatment consisted of giving the animal leaves and young shoots of sea buckthorn, thanks to which the horses quickly gained mass and their fur was shiny [21]. Currently, sea buckthorn grows in Asia and Europe, including the Baltic Sea coast. It is cultivated on an industrial scale in Russia, Germany, China, Estonia, and Finland. In Poland, sea buckthorn can be found growing wild on the coast of the Baltic Sea, and there are few, small plantations of this plant in the rest of the country. Sea buckthorn belongs to the olive family and its fruits are drupes. Sea buckthorn berries are soft, ellipsoid, juicy, fine, and orange in colour, densely covering the shoots with a characteristic citrus aroma. It is a plant of increasing importance because of the content of numerous biologically active components. The extract content in the fruit ranges from 8.67 to 10.17%. It is characterized by a high content of vitamins C, A, B1, B2, K, and PP; a precursor of folic acid; and high antioxidant activity (because of the presence of numerous anthocyanins, flavonoids, and polyphenols). Sea buckthorn berry is one of the few fruits that contains 3.67–6.17% fat. The total fat content depends on the variety and degree of fruit ripeness. The presence of fat means that fruits can be used to obtain oil and fatty acids present in it, the content of which is approximately 77%, of which unsaturated acids constitute from 50% to even 70% of all fatty acids. Among the active substances, one can also distinguish carotenoids (7.94–28.16 mg/100 g), which

give the fruit its characteristic colour. The fruit is also a valuable source of amino acids and minerals. Substances obtained from sea buckthorn berries exhibit antioxidant, antitumor, hepatoprotective, or immunomodulating activity [22–25]. Sea buckthorn preparations are used in the food, cosmetic, and pharmaceutical industries. In Poland, sea buckthorn is a still underrated raw material. The unique chemical composition of sea buckthorn allows it to act as a preservative and additive, as well as an ingredient improving the nutritional quality of the product.

So far, only a few studies have been published proposing the use of sea buckthorn in dairy products, including its fruit in cheese [26] and yoghurt [25,27–29]. These are papers aimed at assessing the acidity, nutritional value, and content of bioactive compounds. In the current study, particular attention was paid not only to the nutritional value, but also to sensory attractiveness, including an extensive picture of the structure. Great attention is paid to these very features when designing new products. Moreover, a new form of filling has been proposed, i.e., fruit mousse. Therefore, the aim of the study was to evaluate the nutritional value, microbiological quality, sensory properties, and structure of natural probiotic yoghurts made with the addition of sea buckthorn fruit mousse during refrigerated storage.

2. Materials and Methods

2.1. Research Material

The organic milk for yoghurt production was obtained from the dairy located in Jasienica Rosielna in southern Poland (mountainous areas). Raw material was delivered mainly from the Simmental breed raised on the certified local farms. Milk was normalized (3.2% fat content) and pasteurized (very high temperature (VHT) pasteurization: 85 °C for 20–25 s). Milk was collected two times in the spring/summer (pasture) season. It was transported to the laboratory under refrigeration and immediately used for analysis and yoghurt production.

2.2. Milk Analysis

Acidity and Basic Composition

In each sample of milk, the following parameters were determined: active acidity (pH value), by pH-meter CP-401 (Elmetron, Poland); potential acidity (in °SH), titration method according to Polish Standard PN-68/A-86122 [30]; contents of fat, total protein, lactose, and dry matter, using the infrared spectroscopy method by infrared milk analyzer (Bentley Instruments, Chaska, MN, USA); somatic cell count (SCC) using the flow cytometry method, Somacount 150 apparatus (Bentley, Chaska, MN, USA); and casein percentage according to the Association of Official Agricultural Chemists (AOAC) [31].

The total bacterial count (TBC) in the raw milk was determined (according to Polish Standard [32]) in order to verify whether it met the requirements for microbiological quality (TBC < 100,000/mL).

2.3. Yoghurt Production

The yoghurts were produced by the water bath method (the thermostatic method). Ten (10) litres of milk was heat-treated to 43 °C and the sea buckthorn mousse was added at a rate of 5%. The mousse is produced at a small processing facility (Sea buckthorn and Friends Producer, Warsaw, Poland) from its own raw material. Whole sea buckthorn fruits are grated together with the seeds. Then, the water is evaporated at low temperature (32 °C) and reduced pressure in order to thicken the product. The blended mix was inoculated with starter strains of yoghurt bacteria from Chr. Hansen (Denmark) in the amount of 0.15 g/L [15]. Thermophilic, probiotic starter yoghurt cultures, i.e., FD-DVS ABT-1, based on *Lactobacillus acidophilus* LA-5, *Bifidobacterium lactis* BB-12, and *Streptococcus thermophilus*, were used to yoghurt production. The blended mix was portioned into 100 mL plastic containers and incubated 6 h until pH = approximately 4.6 was attained. The products

were then cooled to 20 °C to discontinue the incubation. The yoghurts were stored at 4–6 °C until the next day (approximately 14 h) for analysis.

The yoghurts were retested every 7 days, for 21 days (day 0, 7th, 14th, and 21st). The yoghurts were stored in the closed cups under refrigerated conditions (4–6 °C). A new cup was opened at each stage.

The description of yoghurt samples is presented in Table 1.

Table 1. The description of yoghurt samples.

Characteristic	Description
Milk type	Organic
Season of milk production	Spring/summer (pasture) season
Milk thermal treatment	Very high temperature (VHT) pasteurization (85 °C for 20–25 s)
Addition	Sea buckthorn mousse (5%)
Starter strains of yoghurt bacteria	Thermophilic, probiotic starter yoghurt cultures, i.e., ABT-1 (0.15 g/L)
Yoghurt type	Plain, natural yoghurt Yoghurt with sea buckthorn fruit mousse
Day of storage	0 7 14 21

2.4. Yoghurt Analysis

2.4.1. Acidity and Nutritional Value

The potential acidity (°SH) was determined by the titration method [33] and expressed as the content of lactic acid (%). The yoghurts were analysed for content of protein (Kjeldahl method [34]), fat (van Gulik's method), and dry matter (oven-drying at 102 °C) [33]. The measurements were taken in triplicate.

2.4.2. Sensory Evaluation

Sensory evaluation of yoghurts was carried out using the scaling method. A sensory evaluation of the yoghurts was performed by a suitably prepared twenty-person panel. All persons were trained according to recommendations pertaining to the scaling method used. Prior to the evaluation, the samples were coded and left to stand for 1 h at room temperature to reach a suitable temperature for eating, and then they were presented to the testers together with a questionnaire. A five-point scale was used to evaluate the colour, consistency, flavour, aroma, and general acceptance of the products, in which 1 designated disqualifying quality (unsuitable) and 5 indicated very good quality (natural, characteristic for the product) [35]. The studies were performed taking into account the requirements for fermented milk [33].

2.4.3. Microbiological Evaluation

Each sample of yoghurt was homogenized prior to testing; subsequently, 10 g of material was measured and placed in sterile bottles containing 90 mL of Ringer's solution [36,37]. The solution was shaken for 5 min and allowed to sediment for 15 min. Then, a series of ten-fold dilutions of the samples were prepared in Ringer's solution and spread onto previously prepared Petri dishes with an appropriate microbiological medium. The following was determined in each material: total number of mesophilic aerobic bacteria on enriched agar medium for 48 h at 30 °C (BTL Ltd., Warsaw, Poland) according to PN-ISO 7889:2007 [38]; total number of fungi on Sabouraud medium for 5–7 days at 30 °C (BTL Ltd., Warsaw, Poland.) [39]; total number of coliform bacteria on Endo les medium for 24 h at 37 °C (BTL Polska Sp. z o.o.) [38]; total number of fecal *Escherichia coli* on mFC medium for 18–24 h at 44 °C (BTL Ltd., Warsaw, Poland) [40]; total number of *Clostridium perfringens*, sulfate reducing bacteria (IV) growing in anaerobic conditions on the Iron Sulfide Agar

(TSC) for 48 h at 37 °C (Biomerieux Poland Ltd., Warsaw, Poland) [41]; total number of lactic acid bacteria of the genus *Lactobacillus* sp. on the De Man, Rogosa and Sharpe Medium Agar—MRS medium for 3–5 days at 30 °C (BTL Ltd., Warsaw, Poland) [42]; bacilli of the genus *Listeria* sp. on the *Listeria* Selective Medium Agar—LSA medium (Merck, Darmstadt, Germany); total number of bacteria of the genus *Bifidobacterium* sp. on the Bifidus Selective Medium Agar—BSM medium (Sigma-Aldrich, St. Louis, MO, USA) [43]; and presence of *Salmonella* bacilli on the *Salmonella-Shigella* Medium Agar—SS medium and Xylose Lysine Deoxycholate Agar—XLD after prior sample multiplication in buffered peptone water and Rappaport–Vassiliadis medium (BTL Ltd., Warsaw, Poland) for 24 h at 37 °C. Final identification was performed using the available API tests (BioMerieux Poland Ltd., Warsaw, Poland) and polyvalent sera (Biomed S.A.) [44].

Each previously prepared sample was plated in triplicate. After incubation, the colonies were counted using an automatic Scan 300 counter (Innerscience Laboratories, Saint-Nom-la-Bretèche, France) and the number of individual morphological types was counted as the number of colony forming units in 1 g of the yoghurt (cfu/g).

2.4.4. Microstructure Analysis

A yoghurt sample (0.25 g) was mixed with an equal weight of 2.5% aqueous agar. Then, it was rested to solidify by cooling at room temperature. After the formation of a stabile gel, the samples were cut into specimens with $2 \times 2 \times 2$ mm using a surgery lancet and immersed in 4% glutaraldehyde (prepared using a 1.0 M phosphate buffer, 7.0 pH) solution overnight. The fixed specimens were washed twice (5 min each time) using phosphate buffer (pH 7.0) and then dehydrated in a graded ethanol series of 30, 50, 70, 90, 96, and 99.8% for 20 min intervals. The samples were critical-point dried using a critical point dryer (model Emitech K850, Quorum Technologies Ltd., Sussex, UK) and pieces with reduced height were mounted on the aluminum stubs. The mini-cubes were coated with a thin layer of gold in a sputter coater (model Emitech K550X, Quorum Technologies Ltd., Sussex, UK) and transferred into a high-vacuum scanning electron microscope (Tescan VEGA\\LMU, Brno, Czech Republic). Measurements using the scanning electron microscope were carried out at 30 kV and images were recorded at 200, 1000, and 3500 $\times$ magnifications. The evaluation was taken in triplicate.

2.4.5. FTIR Measurements

Measurements of Attenuated Total Reflection FTIR (ATR-FTIR) background-corrected spectra (25 scans for each sample) were carried out with the use of an HATR Ge through a crystal plate (45° cut, yielding 10 internal reflections) at 20 °C, and were recorded with a 670-IR spectrometer (Agilent, Santa Clara, CA, USA). The Ge crystal was cleaned with ultra-pure organic solvents (Sigma-Aldrich, St. Louis, MO, USA). The instrument was continuously purged with argon for 40 min. before and during the measurements. Absorption spectra, at a resolution of one data point per 1 cm⁻¹, were obtained in the region between 4000 and 600 cm⁻¹. Scans were Fourier-transformed and averaged with Grams/AI 8.0 software (Thermo Fisher Scientific, Waltham, MA, USA). The methodology was developed on the basis of Ahmed et al. [45] and Chen et al. [46].

2.5. Statistical Analysis

Statistical analysis of the results was performed using in StatSoft Inc. Statistica ver. 13.1 (Dell, Round Rock, TX, USA), by one- and multi-way analysis of variance (ANOVA). Homogeneity of variance was tested by Levene's test and the Brown–Forsythe test. Significance of differences was determined at a level of p (alpha) = 0.05 and 0.01. The results are presented as mean $\pm$ standard deviation (SD).

3. Results

3.1. Basic Physico-Chemical Parameters in Milk

The evaluation of nutritional value of pasteurized organic milk showed that it contained 12.16% dry matter, including 3.06% crude protein, 3.20% fat, and 4.75% lactose (Table 2). The acidity of milk, expressed as active acidity (pH value) and potential acidity (titrimetric, in °SH), was 6.70 and 6.82 °SH, respectively. The number of somatic cells, proving the cytological quality, amounted to 61 thous./mL of milk. The total bacteria count was not detected (0 cfu/mL).

Table 2. Results of physico-chemical analysis of pasteurized organic milk obtained from the dairy used in the production of yoghurts (mean $\pm$ SD).

Active Acidity (pH)	Potential Acidity (°SH)	Total Protein (%)	Casein (%)	Fat (%)	Lactose (%)	Dry Matter (%)
6.70 $\pm$ 0.05	6.82 $\pm$ 0.08	3.06 $\pm$ 0.10	2.48 $\pm$ 0.08	3.25 $\pm$ 0.08	4.75 $\pm$ 0.06	12.16 $\pm$ 0.15

3.2. Yoghurts

3.2.1. Acidity

The initial active acidity in pure natural yoghurts was 4.64, while it was 4.58 with the addition of sea buckthorn (Figure 1). Thus, the applied plant additive had a significant effect on yoghurt acidity, i.e., it lowered the initial pH value ($p \leq 0.05$). The acidity of both products generally decreased with time (increase in pH on day 7 of storage followed by a gradual decrease). Changes also concerned potential acidity expressed as lactic acid content (Figure 2). Yoghurt without addition contained 0.72% lactic acid, and that with sea buckthorn contained 0.69%, and the difference was not statistically significant.

Figure 1. Changes of pH value in the analysed yoghurts during 21 days of storage in the refrigerated conditions. A, B, C, D—differences between the pH value within a yoghurt type, significant at $p \leq 0.01$. x, y, X, Y—differences between days of storage regardless of yoghurt type; x, y—significant differences at $p \leq 0.05$; X, Y—significant differences at $p \leq 0.01$.

3.2.2. Nutritional Value

The results of the basic nutritional value assessment are presented in Table 3. Products containing mousse additive were characterized by a higher dry matter content, including total protein and fat. However, it should be noted that the differences between yoghurt types were not statistically confirmed. It was demonstrated that, regardless of the presence of the additive (yoghurt type), the content of the mentioned components changed during the refrigerated storage, as it decreased in all cases. These differences were statistically significant within the type of yoghurt. In general, yoghurts with sea buckthorn mousse

addition contained the highest dry matter content, including total protein and fat, on the initial day of storage—day “0”. Pure natural yoghurt was the poorest source of these components on the last (21st) day of storage.

Figure 2. Changes of lactic acid content (%) in the analysed yoghurts during 21 days of storage in the refrigerated conditions. A, B, C, D—differences between the lactic acid content within a yoghurt type, significant at $p \leq 0.01$. X, Y—differences between days of storage regardless of yoghurt type, significant at $p \leq 0.01$.

Table 3. Results of basic chemical composition analysis of yoghurts during 21 days of storage in the refrigerated conditions (mean $\pm$ SD).

Yoghurt Type	Day of Storage	Total Protein (%)	Fat (%)	Dry Matter (%)
Plain, natural yoghurts	0	3.07 ^b $\pm$ 0.07	3.24 $\pm$ 0.08	12.21 ^B $\pm$ 0.15
	7	3.05 ^{ab} $\pm$ 0.10	3.18 $\pm$ 0.10	12.08 ^B $\pm$ 0.20
	14	3.01 ^{ab} $\pm$ 0.14	3.16 $\pm$ 0.09	11.49 ^B $\pm$ 0.18
	21	2.80 ^a $\pm$ 0.15	3.10 $\pm$ 0.12	10.10 ^A $\pm$ 0.23
Yoghurts with sea buckthorn fruit mousse	0	3.11 ^b $\pm$ 0.10	3.33 ^b $\pm$ 0.07	12.38 ^C $\pm$ 0.19
	7	3.09 ^{ab} $\pm$ 0.13	3.30 ^b $\pm$ 0.11	12.20 ^C $\pm$ 0.17
	14	3.04 ^{ab} $\pm$ 0.18	3.23 ^{ab} $\pm$ 0.09	11.64 ^B $\pm$ 0.20
	21	2.82 ^a $\pm$ 0.16	3.15 ^a $\pm$ 0.12	10.29 ^A $\pm$ 0.23

a, b, A, B, C—differences between the basic chemical composition within a yoghurt type; a, b—significant differences at $p \leq 0.05$; A, B—significant differences at $p \leq 0.01$.

3.2.3. Sensory Evaluation

The results of sensory evaluation are shown in Figure 3a,b. Among all analysed variants (type of yoghurt and day of storage), the highest scores were given to yoghurt without sea buckthorn addition on the first day of the research, i.e., 5 points for colour, consistency, flavor, and aroma. On the other hand, the lowest (4.03, 4.25, 4.40, and 4.45 points, respectively) were obtained by yoghurt with the addition of sea buckthorn mousse on the last day, i.e., on the 21st day of storage. In general, yoghurt containing the additive, regardless of the day of storage, received statistically significantly ($p \leq 0.05$) lower scores for all analysed characteristics. These results translated into a general acceptance note, as yoghurts without an additive obtained 4.81 points/5 points max, and those with mousse obtained 4.54 points. The least rated parameter was colour—yoghurts with mousse on day 21 of storage received 4.03 points. However, it should be emphasized that the results obtained for the yoghurts with the addition of sea buckthorn mousse were relatively high—above 4 points/5 points max for each characteristic.

Figure 3. (a) Changes in sensory quality (colour, consistency, flavour, aroma, and general acceptance) of the plain, natural yoghurts during 21 days of storage in the refrigerated conditions. (b) Changes in sensory quality (colour, consistency, flavour, aroma, and general acceptance) of the yoghurts with sea buckthorn fruit mousse during 21 days of storage in the refrigerated conditions.

3.2.4. Microbiological Evaluation

Analysing the count of mesophilic aerobic bacteria, significant statistical differences were found in both the analysed yoghurt types and individual days of sample storage (Table 4). Plain, natural yoghurt showed the lowest abundance of this group of microorganisms on the day of starting the analyses. This value was statistically significantly lower than in the corresponding testing period of yoghurt with sea buckthorn mousse and other values obtained during the storage of material type ($p \leq 0.01$). Statistical analysis of the results obtained from subsequent time points of microbiological experiments (day 7, 14, 21) did not show any significant effect of storage time on the number of mesophilic aerobic bacteria in the plain, natural yoghurt. On the other hand, the addition of sea buckthorn mousse had a statistically significant effect on the decrease in the concentration of the analysed group of microorganisms already on the 7th day of storage. This relationship was preserved and statistically confirmed on both day 14 and 21 of analyses at the level of statistical significance ($p \leq 0.01$), where no increase in this group of microorganisms was observed. Yoghurts with the addition of sea buckthorn mousse were characterized by a statistically significantly higher level of yeast-like fungi development ($p \leq 0.01$). However, no statistically significant differences in the number of these microorganisms were recorded throughout the storage period. No fungal growth was observed in plain, natural yoghurt at any of the analysis time points. The total number of lactic acid bacteria reached a high and similar concentration both in plain, natural yoghurt with sea buckthorn mousse

addition, and the differences did not exceed the statistical significance threshold in any of the analysed materials. When analysing typical yoghurt flora, statistically significant differences were observed on the 7th day of storage between yoghurts in the number of bacteria of the genus *Bifidobacterium* sp. There was a statistically lower number of these microorganisms in the yoghurts enriched with sea buckthorn mousse ($p \leq 0.05$).

3.2.5. Microstructure Analysis

The microstructure of the products was recorded using a scanning electron microscope (SEM) at various magnifications (Figure 4a–d). Yoghurt images showed a completely different microstructure of the yoghurt with sea-buckthorn (Figure 4c,d). The addition of sea-buckthorn to yoghurt contributed to the production of a gel during fermentation, stabilized by a greater number of bonds. On the other hand, yoghurt without the addition of sea buckthorn (Figure 4a,b) had smaller clusters of casein micelles, thick chains, and smaller and less frequent empty spaces. Faster degradation rate of yoghurt with the addition of sea buckthorn compared with yoghurt without the additive could also be observed after three weeks of storage of yoghurts in refrigerated conditions (Figure 4a,b). The yoghurt with the addition of sea buckthorn after 21 days of refrigerated storage lost its initial structure faster compared with the yoghurt on day 0 and yoghurt without the addition of sea buckthorn.

Figure 4. Scanning electron microscopy (SEM) of yoghurts, magnification 3500 $\times$. Microstructure: (a) the plain, natural yoghurt during 0 day of storage in the refrigerated conditions; (b) the plain, natural yoghurt during 21 days of storage in the refrigerated conditions; (c) the yoghurt with sea buckthorn mousse during 0 day of storage in the refrigerated conditions; (d) the yoghurt with sea buckthorn mousse during 21 days of storage in the refrigerated conditions.

Table 4. Microbiological evaluation of the analysed yoghurts during 21 days of storage in the refrigerated conditions.

Yoghurt Type	Day of Storage	Total Bacterial Count	Total Number of Fungi	Total Number of Lactic Acid Bacteria	Total Number of Bacteria of the Genus <i>Clostridium</i> sp.	Total Number of Coli Bacteria (Endo)	Total Number of Faecal Coliforms (mFC)	Total Number of Bacteria of the Genus <i>Bifidobacterium</i> sp.	Presence of Salmonella Bacilli	Presence of Campylobacter Bacilli
Plain, natural yoghurts	0	$<1.5 \times 10^1$ A	0 *	8.2×10^7	0	0	0	7.6×10^6	0	0
	7	8.0×10^5 B **	0 *	5.6×10^7	0	0	0	1.6×10^7 *	0	0
	14	2.2×10^6 B **	0 *	4.7×10^7	0	0	0	2.4×10^6	0	0
	21	3.2×10^5 B **	0 *	6.7×10^7	0	0	0	3.1×10^7	0	0
Yoghurts with sea buckthorn fruit mousse	0	4.5×10^1 B	7.2×10^2 **	8.1×10^7	0	0	0	7.8×10^7	0	0
	7	$<1.5 \times 10^1$ B **	$<1.0 \times 10^2$ **	8.9×10^7	0	0	0	7.2×10^5 *	0	0
	14	0 A **	$<1.0 \times 10^2$ **	8.2×10^7	0	0	0	1.2×10^6	0	0
	21	0 A **	1.0×10^4 ** yeast	5.1×10^7	0	0	0	2.9×10^7	0	0

"0" means "not detected". A, B—differences between day of storage within a yoghurt type, significant at $p \leq 0.01$. *, ** differences between yoghurt type within a day of storage; * significant differences at $p \leq 0.05$; ** significant differences at $p \leq 0.01$.

3.2.6. FTIR Measurements

Figure 5 presents the FTIR absorption spectra measurements for the yoghurt samples selected for testing: plain, natural probiotic (black line in each panel), and probiotic yoghurt with sea buckthorn mousse (red line in each panel). A comparative analysis of the spectra was conducted at every stage of the study to identify spectroscopic differences in the initial phase between the selected samples. Secondly, the spectra were also collected at weekly intervals in order to find spectroscopic markers of aging. The samples were placed on an HATR Ge crystal and tested under an N₂ atmosphere (as explained in the Materials and Methods section). Table 5 presents all the characteristic bands recorded in the selected samples, as well as those of the corresponding functional groups identified based on a detailed review of the available and plentiful literature data. It is worth noting that all FTIR infrared spectra analysed in the study of the yoghurt samples, for both plain probiotic yoghurt and probiotic yoghurt with sea buckthorn mousse, had very intense and distinct bands that could be quite precisely attributed to the specific vibrations of functional groups present in ingredients typical of this type of food product. Additionally, for ease of comparison and interpretation, the spectra were normalized at an approximate wavelength of 3280 cm⁻¹. The spectra of all the tested yoghurts showed very similar absorption bands, consistent with literature reports, which indirectly also proved their good quality. We observed only slight differences in specific spectral regions within individual samples, and the differences progressively decreased over the course of the study—the biggest differences occurred at the very beginning of the experiment. The bands characteristic of the ν(-OH) stretching vibrations, occurring as a very wide band with a maximum at about 3280 cm⁻¹, could be clearly observed [45,47]. They might originate from the small amounts of water present in the tested samples and, above all, from the constituent sugar residues. Another very important area of vibrations, with the maximum at between 3000 and about 2800 cm⁻¹, is often called the fatty acid region for this type of sample. The bands with the maxima at around 2923 and 2853 cm⁻¹ are asymmetric vibrations of the C-H methylene group in the cholesterol structure [45,47–49]. Vibrations of this group cause stretching vibrations of CH₂ and CH₃ groups of fatty acids present in yoghurt samples. In turn, symmetrical vibrations in these groups were reflected in a sharp band with the maximum at 2956 cm⁻¹. All yoghurt samples tested at identical time intervals showed distinct vibrations in this region. The next very sharp and very distinct band with the maximum at approximately 1744 cm⁻¹ was most likely related to stretching vibrations of the C=O carbonyl group, derived from a fragment of the ester group or carboxylic acids [50–52]. For all tested samples, the band was quite important and provided important information, even after normalizing the spectra at the wave number related to the stretching vibrations of the -OH group. The region between 1700 and 1240 cm⁻¹ is very important in the description and interpretation of this type of sample, as it is a heterogeneous area containing vibration bands characteristic mainly of fatty acids, protein molecules, and polysaccharides. The band with the maximum at between 1535 and approximately 1570 cm⁻¹ for protein was derived from the vibration of amide II, while between ~1620 and 1690 cm⁻¹ was from amide I structural vibration [43,48]. Amide I vibrations were represented mainly by stretching vibrations of the amide C=O group coupled with in-plane stretching vibrations of the N-H group and stretching vibrations of the C-N group. Overall, this area did not differ significantly between the two types of yoghurt, and the observed slight differences disappeared very rapidly during the experiment. Clear differences between the samples were observed only in the third week of the experiment. On the other hand, the area ranging from 1150 to 1000 cm⁻¹ was dominated by bands originating from polysaccharide systems [52–55]. This location looked different for the sample of probiotic yoghurt containing sea buckthorn mousse, especially in the initial stage of the experiment.

Figure 5. Panel (A)—28.09 (“0” day of storage), panel (B)—5.10 (“7” day of storage), panel (C)—12.10 (“14” day of storage), and panel (D)—19.10 (“21” day of storage). Black line—plain, natural probiotic yoghurt; red line—probiotic yoghurt with sea buckthorn mousse.

Table 5. The location of the maxima of the Fourier Transform Infrared (FTIR) absorption bands, with assignment of particular vibrations to the respective samples, registered within the spectral range of 700–3700 cm^{-1} [45,47–59].

FTIR	Type and Origin of Vibrations
3280	$\nu(\text{-O-H})$
2956	$\nu(\text{-CH}_2)$ and $\nu(\text{-CH}_3)$, s and as
2923	
2853	
1744	$\nu(\text{-C=O})$ in esters or carboxylic acid
1641	amide I oraz $\delta(\text{-OH})$
1629	$(\nu(\text{C=O}) + \nu(\text{N-H}))$ in-plane and $\delta(\text{C-N})$
1540	amide II, fatty acids, proteins, and polisacharides $(\nu(\text{C=C}))$ of nonconjugated trans and cis-lipids etc.)
1416	
1378	
1314	
1240	
1151	polisacharides $(\nu(\text{C-O-C})$ and $\nu(\text{C-O}))$
1115	
1070	
1034	
892	α -glycosidic bond
876	
782	
700	
537	

N—stretching vibrations; δ —deformation vibrations; s—symmetric, as—asymmetric.

4. Discussion

4.1. Basic Physico-Chemical Parameters in Milk

According to *Codex Alimentarius* [60], the titratable acidity, microbiological, and chemical criteria should be used to detect unacceptable conditions in milk products. Titrimetric acidity expresses the degree of buffering of milk and indicates any changes taking place in the concentration of acidic compounds in the milk, even if the pH remains unchanged [61]. In the present study, the active acidity (pH value) and potential acidity (titrimetric, in $^{\circ}\text{SH}$) were found to meet the requirements of Polish standard PN-A-86003/A1:1998 [62] for drinking milk, according to which the pH value of heat-treated milk should be 6.6–6.8 and titrimetric acidity should be 6.0–7.5 $^{\circ}\text{SH}$. The content of basic ingredients (dry matter, fat, protein, and lactose) was typical for processing milk, consistent with the results of other authors [63].

4.2. Yoghurts

4.2.1. Acidity

The acidity of the experimental yoghurts was within the normal range [33], according to which titrimetric acidity expressed as lactic acid content should be at least 0.6%. Comparable results were obtained by Selvamuthukumar and Farhath [28], who found a higher level of lactic acid in sea buckthorn fruit syrup yoghurt (15% added to milk) when compared with plain yoghurt. The initial lactic acid content was 0.86%, and rose to 1.15% at 21 days, when stored at 4 $^{\circ}\text{C}$. Kudelka [64] observed that, during 21-day storage of natural yoghurt in refrigerated conditions, the amount of lactic acid generated steadily increased. It should be emphasized that the increase in acidity of fruit yoghurt is a normal phenomenon. According to Selvamuthukumar and Farhath [28], the addition of sea buckthorn pulp positively influences the enrichment of lactic acid content from yoghurt with probiotic culture. Increases in lactic acid content may be due to a small extent of sea buckthorn pulp due to the addition of organic acids. Moreover, the decrease in the pH value during the

storage was indicated by Gunenc et al. [25], who investigated sea buckthorn whole fruit and sea buckthorn purified mucilage as additives into yoghurt (28-day storage at 4 °C).

4.2.2. Nutritional Value

The obtained results are partially consistent (only in terms of the direction of the changes) with the results of other authors [28]. Generally, there is one publication in the literature devoted to the nutritional value of yoghurts with the addition of sea buckthorn in the form of fruit syrup. Selvamuthukumaran and Farhath [28] obtained a higher content of total solids, fat, and protein in relation to our results. Their result shows that the total solids of fruit yoghurt decreased from 28.9% to 28.4% during 21-day storage at 4 °C, protein from 7.90% to 7.34%, and fat from 5.40% to 5.15%, respectively. Therefore, the direction and magnitude of the changes were comparable. The obtained significant decrease ($p \leq 0.05$) in dry matter content could be due to syneresis, i.e., leakage of whey, which contains whey proteins, lactose, and minerals. Lee and Lucey [65] indicated that dry matter content and the ratio of casein to whey proteins are important factors influencing the water-holding capacity of yoghurt curd. Other of our studies showed a decrease in the dry matter content in natural yoghurts during storage [66].

4.2.3. Sensory Evaluation

Sensory evaluation, taking into account colour, consistency, flavor, and aroma, is a key tool enabling the assessment of consumer acceptance of a new product. The factors playing the most important role in determining the market behaviour of consumers, including in the case of yoghurt, include freshness, flavor, and aroma. These characteristics directly depend on the acidity, including the lactic acid content, free fatty acids, and volatile compounds content [67,68]. In our research, the decrease in acidity was not very high in the initial day and during storage; therefore, the general acceptance of yoghurts was satisfactory (above 4.54 points/5 max points for the yoghurts with the addition). As reported by Selvamuthukumaran and Farhath [28], the fresh sea buckthorn fruit yoghurt had an overall acceptability score of 8.0 (on nine-point hedonic scale). A score of 6.1 was taken as the lowest limit, implying the end-of-shelf-life of yoghurts. Sensory acceptance of fruit yoghurts remained up to 18 days during storage at 4 °C. Moreover, Dabija et al. [29], optimizing the fruit yoghurt production, stated that yoghurt with the best physico-chemical and sensory properties was obtained by adding 1.75% sea buckthorn powder combined with 0.25% grape seed extract (4.22 points for the taste, 4.14 for the mouth feel, 4.58 for the texture, and 4.27 for the overall acceptability). However, research by Grega et al. [69] showed that the type of bacterial culture used also determines the sensory quality during storage. ABT-1 is generally a very well-accepted yoghurt culture, with mild aroma and flavour.

4.2.4. Microbiological Evaluation

The activity of the appropriate groups of microorganisms is the basic indicator of the microbiological quality of yoghurt. Apart from the presence of typical flora, its survival is important during the entire shelf-life of the product [70]. The conducted microbiological analysis showed that the dominant species of the characteristic microflora of the tested yoghurts were lactic acid bacteria and bacteria of the genus *Lactobacillus* and *Bifidobacterium* derived from the starter culture. An appropriately high concentration of these microorganisms allows to obtain a product with the expected flavour and aroma. According to the guidelines of the *Codex Alimentarius* [71], the number in one gram of product should be at least 10^7 cfu [72]. In the current study, this value was obtained and maintained throughout the storage period of material batches. Maintaining high concentrations of yoghurt bacteria in refrigerated storage is difficult. Many studies have shown that the number of yoghurt bacteria is then reduced to a level lower than 10^7 cfu/g [73]. According to Augustyniak's research [74], only a few percent of yoghurts had the desired number of live lactobacilli during the final storage period. The addition of fruit filling, as research

showed, could reduce the number of yoghurt bacteria [75]. In the present study, such an effect of sea buckthorn mousse on lactic acid bacteria, *Lactobacillus*, was not observed, and a temporary decrease in the concentration of *Bifidobacterium* was recorded only in the first week of storage. A high concentration of yoghurt bacteria determines their functional and probiotic features. Research has shown that the application of lactic acid microorganisms in the diet has a positive effect on the stimulation of the body's immune processes by increasing lymphocyte activity, stabilizing intestinal microbiota, and improving nutrient digestibility and assimilation [76]. Hygienic quality is an important aspect of the shelf life and quality of yoghurt during storage. No growth of saprophytic and pathogenic flora of fecal origin, anaerobic microorganisms, or pathogenic *Salmonella* and *Campylobacter* bacilli was observed in the conducted experiments. This confirms the effectiveness of the pasteurization process and good quality of the raw material. These results were consistent with those obtained by other authors, who indicated a small percentage of the tested batches of marketed yoghurts [75,77]. The presence of yeast-like fungi at the level of 10^2 , noted in yoghurt enriched with sea buckthorn mousse, and their persistence throughout the storage period, should be attributed to the contamination of the additive. Their presence could indicate that the yoghurt batch was deteriorating quickly [78]. The lack of fungal flora development in the tested yoghurt batch was the result of the antagonistic effect of lactic acid against yeast cells. At the same time, an increase in the number of yeasts could stimulate the development of lactic acid bacteria and lower pH of the product, while protecting it against the development of unfavourable flora. This synergism has not been fully elucidated and requires further research on the flora of fermented milk products [79]. This is a result indicating that, despite the high quality of the raw material and the application of thermal disinfection treatments of milk batches, additives of natural origin can be a source of contamination of the final product. Simultaneously, the presence of yeast does not always have to be a negative phenomenon, and with appropriate cooling conditions, preventing their uncontrolled growth, it can stimulate a favourable yoghurt flora.

4.2.5. Microstructure Analysis

The higher number of bonds reported in our research probably contributed to the increase in yoghurt cohesiveness and elasticity. The recorded microstructure of cow's milk yoghurt was characteristic, similar to that recorded by Akalin [80]. For this reason, yoghurt without the addition of sea buckthorn was characterized by higher hardness and cohesiveness. The yoghurt microstructure obtained by adding sea buckthorn is more desirable from a health and nutritional point of view. More empty spaces as well as thinner chains will contribute to a higher digestibility of the product because of the larger surface area available for acid action and gastrointestinal enzymes, as well as potentially faster degradation of the thus formed gel network. The loss of the initial structure by yoghurt with the addition of sea buckthorn after 21 days of refrigerated storage compared with yoghurt on day "0" and yoghurt without the addition of sea buckthorn is the result of the degradation changes taking place.

4.2.6. FTIR Measurements

All panels in Figure 5 showed that the samples selected for testing had a fairly clear infrared spectrum, which was consistent with the literature data, and additionally proved the good quality of the samples selected for testing. Regardless of the yoghurt type, it should be noted that the positions of the bands related to protein structures and fat content assumed the same location, which was an additional argument for the above thesis.

Firstly, Panel A, which shows spectra from the initial phase of the experiment, is very interesting. As can be observed, the spectra of plain, natural yoghurt (black line) and yoghurt with sea buckthorn mousse (red line) differ significantly in some areas. A band with a maximum at 1744 cm^{-1} had a much greater intensity in the sample of yoghurt with mousse. This confirmed the higher protein content of this sample, as shown by previous results obtained using other methods. In addition, the higher intensity of the bands from

the 1000–1700 cm^{-1} region for yoghurt with the addition of sea buckthorn indicated a much higher content of polysaccharide additives. In addition, there was also a clearly greater intensity of bands corresponding to the vibrations from the 2970–2840 cm^{-1} area related to fat content. A greater intensity of vibrations in the range from 1460 to 1230 cm^{-1} and greater band intensity in this range was also visible in the entire region. However, it was the part of the region that consisted of vibrations from fatty acids, proteins, and polysaccharides. However, it was more a consequence/connection of the aforementioned regions and confirmed the above discussion.

The second research aspect in this paper was the observation of sample stability and aging effects occurring in them. For this purpose, the samples were measured at weekly intervals. During the experiment, degradation changes occurred in the structure of amino acids and fats present in the tested yoghurts.

Preliminary analysis of FTIR spectra showed that the applied additives did not have a detrimental effect on the position of the main bands in the spectra, thus they did not change the internal structure of the samples in any way. A very interesting effect could be observed in the next panels in Figure 5, where the measurements of the spectra from the following measurement days are presented. As can be seen, the greatest changes in samples for individual components occurred in the initial phase of the experiment. However, along with the duration of the experiment, changes between individual bands practically completely disappeared in the fourth week. This very interesting result demonstrated that, regardless of the addition to the yoghurt, individual components degrade over time in the same or similar manner. This meant that the additive from the molecular aspect did not significantly affect the shelf life of the product. It should also be noted that, during the experiment, it is clearly visible that the content of the protein and fat fractions is decreasing, which is also reflected in other studies.

5. Conclusions

In Poland, the application of new, plant-based additives in dairy production is still at the stage of development. Gradually, consumers are moving away from their usual habits and increasingly turning to new dairy products. The present study showed the possibility of using sea buckthorn fruit mousse in the production of fermented milk beverages, specifically yoghurts. Fruit filling is an added value to the product as it is a valuable source of nutrients in itself. The produced yoghurts were characterized by an adequate acidity and high nutritional value. Yoghurt with sea buckthorn addition was generally characterized by a higher active acidity and lactic acid content throughout the entire study period (21 days of storage). It was also fully sensory accepted by the evaluation panel. The conducted microbiological tests confirmed the high sanitary and hygienic quality of the analysed yoghurts at all test time points. No growth of fecal coliform bacteria, *Escherichia coli*, spores of anaerobic flora of the species *Clostridium perfringens* as well as *Salmonella* and *Campylobacter* species was recorded in any yoghurts during refrigerated storage. The obtained results indicated that the obtained chemical composition was related to the structural image. Moreover, the applied yoghurt additive was visible at the spectral level (FTIR infrared spectra) as an increase in the intensity of the characteristic bands for vibrations related to protein, fat, and polysaccharide structures. The greatest changes were observed for vibrations ranging from 2960 to 2840, from 1160 to 1000 cm^{-1} , and in the band with the maximum at about 1744 cm^{-1} . However, these changes require further and more detailed research, which will be carried out in subsequent research stages.

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