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Inhibition of Galactooligosaccharide (GOS) Degradation in High-Heat-Treated Goat's Milk as a Raw Material for Functional Dairy Products

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Abstract: The aim of this study was to analyze the inhibition of galactooligosaccharide (GOS) degradation in heat-treated milk with permeate obtained by microfiltration and concentrated by ultrafiltration. An attempt was made to obtain raw material with a designed composition and stability in terms of GOS content for the production of fermented milk beverages. This study is important due to the versatile possibility of using milk and permeate with GOS in further processing and food production. During the heat treatment of goat's milk, GOS degradation was approx. 16%. However, no changes in GOS content were observed in goat's milk with permeate after 30 and 60 min of heating between 72 and 92 °C. Therefore, goat's milk with permeate in terms of GOS content was stable for up to 60 min, regardless of the temperature. The addition of permeate effectively inhibits GOS degradation in milk. It has been shown that the produced raw material with a stable GOS content during heating can be further used for the production of kefir.

Keywords: enzymatic conversion of lactose; stability; galactooligosaccharides; β-galactosidase; dairy products; heating; fermentation

1. Introduction

Modern consumers are becoming more demanding regarding food and dairy products. On the one hand, these demands are associated with the quality, attractiveness, and packaging of dairy products. On the other hand, these demands are based on the quality characteristics of the functional content of bioactive compounds and new sensory experiences. The desire of producers to satisfy these expectations is the result of innovations within the dairy industry, which consist of the introduction of new or improved versions of existing technology, e.g., membrane techniques (e.g., microfiltration and ultrafiltration), for the enzymatic conversion of lactose [1]. The microfiltration (MF) of milk is already well known [2]. Currently, the production of casein concentrate (the retentate after MF) and the rest of the components of milk (the MF filtrate) are generally directed to the production of whey protein concentrates (WPCs) and other dairy products with increased pro-health value. The base material can be composed of cow's, goat's, sheep's, or buffalo's milk, among others.

Goat's milk products are well established in the dairy industry [3]. The typical composition of goat's milk is 87% water, with the remaining being a dry substance consisting of 3.8% fat, 4.3% lactose, and 3.5% protein. Additionally, goat's milk protein consists of 70% casein, 25% water-soluble whey protein, and 5% fat globule membrane proteins. Compared to cow's milk, goat's milk contains more β-casein fractions (0–64 g/100 mL) and less αs1-casein (0–28 g/100 mL) [4,5]. Hence, it is a valuable raw material in cheese making. Goat's milk cheese production technology involves milk being increasingly fractionated by microfiltration [6]. Then, when the casein content increases, some of the native whey

proteins (serum proteins) are eliminated. The protein fraction of the serum is the permeate, which can be further concentrated by ultrafiltration (UF) [7]. In addition, the use of enzymatic processes, e.g., lactose hydrolysis and transgalactosylation, may lead to changes in lactose content [8–10]. Thus, it is possible to not only increase the protein content but also reduce or eliminate the lactose content, which leads to the formation of prebiotics, e.g., galactooligosaccharides (GOS).

GOS are a mixture of galactose and glucose with the molecular structure (Gal)n–Glu. They are produced by the transgalactosylation of lactose by the enzyme β-galactosidase from lactose-rich products, particularly milk and whey. These stimulate the growth and development of the intestinal microflora and are prebiotics [11]. GOS are resistant to high temperatures and low pH. As reported by Sangwan et al. [12], GOS alone, due to the presence of β-type bonds, are stable at 160 °C for 10 min at neutral pH or 120 °C for the same period at pH 3. At pH 2, the stability of GOS at 100 °C was 10 min. Therefore, an important issue that must be considered is GOS production in the raw material and maintaining it at a constant level during subsequent technological activities, e.g., heating in various temperature–time systems. The majority of reports have described the scope of GOS synthesis in the food matrix [13,14] or the thermal stability of GOS formulations [15].

However, there are limited reports available describing the stability of GOS in goat's milk or in goat's milk with permeate, where the protein and salt system changes as compared to milk. Goat's milk in many countries is not as widespread as cow's milk. The industrial processing of goat's milk is therefore not performed. These are often the conditions of a farm and a small processing plant. However, the demand for goat's milk products is continuously increasing. This applies to the quantity and types of products. One very popular product is kefir. Kefir has a high nutritional value and has a health-promoting effect on the body [16,17]. Many properties of kefir result from the type and amount of LAB and yeasts used. The typical composition of the microflora participating in lacto-alcoholic fermentation includes heterofermentative and homofermentative *Lactobacillus* and *Streptococcus*, thermophilic lactic *Streptococcus*, acetic bacteria, and lactose-fermenting and non-fermenting yeasts [18]. Within a few years, the interest in kefir consumption increased, and in 2021, it was recognized as one of the food trends [19]. In addition to the traditional assortment (milk, kefir, yogurt, and cheese), other products (e.g., desserts, cake mixes, creams, foams, and cookies) are being prepared using goat's milk. The technology of their production uses milk previously subjected to thermal treatment. Therefore, goat's milk preserved by heating can be used in another place and at any time. This is why it is so important to further control the composition and quality of heated goat's milk during processing, especially when its value is increased by GOS content.

The aim of our research was to evaluate the stability of GOS in goat's milk and in milk enriched with concentrated permeate after MF during heating under various temperature–time conditions. Does the use of permeate (generated during cheese production) proposed in this experiment lead to a dairy product with a designed composition and stability in terms of GOS content? Can the obtained product be a raw material for the production of fermented milk beverages? Therefore, kefir was produced from the sample with the most stable GOS content (before its degradation) using lacto-alcoholic fermentation. The obtained kefir was characterized on the basis of its physical characteristics, the number of mesophilic lactic acid bacteria (LAB) and yeast cells, and the activity of the lactase enzyme.

2. Materials and Methods

2.1. Goat's Milk with Permeate and the Hydrolysis and Transgalactosylation of Lactose

The method of obtaining the permeate was described in detail by Kaczyński and Cais-Sokolińska [7] and Kaczyński et al. [20]. In terms of composition and quality, the milk met the requirements for raw goat's milk for processing [21]. The goat's milk was of very good microbiological (182.3×10^3 cfu/mL) and cytological quality (somatic cell number 462.5×10^3 in 1 mL). The limit of the total number of microorganisms in 1 mL of raw goat's milk, according to the directive, is 1,500,000. Neither in the country nor in Europe

is there a fixed limit on the content of somatic cells in goat's milk. Only the Food and Drug Administration standardization documents in the United States have specified the maximum limit of somatic cells, which is 1,000,000 per 1 mL [22]. Goat's milk before and after the enzymatic conversion of lactose was also analyzed in terms of acidity and other physicochemical characteristics. Goat's milk with permeate was combined in a ratio of 60:40 (%, *v/v*). The enzymatic conversion of lactose was carried out at 37 °C for 20 min [20]. Goat's milk (0.7 L) with permeate was heated at 72, 85, and 92 °C for 30 and 60 min in a laboratory thermostat (TIP200, WSL, Świętochłowice, Poland). Silicone oil was used for heat transfer. The temperatures used were selected on the basis of the manufacturing technology for products that use heated goat's milk. Goat's milk was one of the ingredients. The temperature of 71 °C is related to the production of cream/confectionery mass. A mixture of skimmed rennet cheese, cream, pasteurized milk, and powdered milk is heated at this temperature for 20–30 min. The temperature of 85 °C is related to the production of powdered cheeses, in which the ground cheese mass together with milk (1:1 ratio; 30% dry matter content) is pasteurized for 30 min. The temperature of 85 °C is also applied to the production of fried ripened curd cheese. Milk is used in this technology to compensate for the loss of water during the peptonization of casein from the curd and the frying of the cheese mass. The temperature of 92 °C is related to the heating of the cheese mass in the production of processed cheese (milk instead of the partial addition of water).

2.2. Basic Composition of Milk and Determination of GOS

The measurement of the basic components of milk was performed using a DairySpec FT analyzer (Bentley Instruments, Inc., Chaska, MN, USA). The GOS content was determined according to AOAC method 2001.02 using LC–MS [23,24] and as described by Kaczyński et al. [20].

2.3. Physicochemical Analysis

The pH was measured using a CP-502 pH meter (Elmetron, Zabrze, Poland) with ES AgP-301W (Eurosensor, Gliwice, Poland) [20]. The titratable acidity, freezing point, density, and viscosity were determined according to Teichert et al. [25]. The measurement of conductivity (EC) was performed with the use of a conductivity meter (CC 401) along with an EC-60 sensor produced by Elmetron (Zabrze, Poland). The water activity was measured using an AquaLab Series 4TE instrument (Decagon Devices Inc., Pullman, WA, USA). Samples with v = 15 mL were placed in a DE 501 measurement vessel (DE 501 vessels; Decagon Devices Inc., Pullman, WA, USA) and tested at 15 °C.

2.4. Fermentation—Kefir Production

Samples heated at 92 °C for 30 min were used for the production of kefir because no GOS degradation was found in them. Lacto-alcoholic fermentation was performed at 22 °C and pH 4.4 using starter cultures with the code 75106 (Abiasa Inc., Quebec, QC, Canada). The compositions of the starter cultures were as follows: mesophilic strains of lactic acid bacteria (LAB) were *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis*, *Leuconostoc mesenteroides* subsp. *cremoris*, *Lactiplantibacillus plantarum*, and *Lacticaseibacillus casei*; the yeast was *Kluyveromyces fragilis*. The dose was equal to 30 u.a. per 100 L. After 48 h of fermentation, the products were evaluated. Four types of kefir samples were produced: from pasteurized goat's milk (KM), from pasteurized goat's milk after the enzymatic conversion of lactose with the participation of the enzyme β-galactosidase (KM/GOS), from goat's milk with permeate (KM + P), and from goat's milk with permeate after the enzymatic conversion of lactose by the enzyme β-galactosidase (KM + P/GOS).

2.5. Sensory Analysis of Kefir

The taste and smell profiles were assessed. The conditions and the course of the evaluation were as described by Kaczyński and Cais-Sokolińska [7]. A glossary of these descriptors was described by Wróblewska et al. [26].

2.6. Determination of the Number of Mesophilic Lactic Acid Bacteria (LAB) and Yeast Cells and Lactase Activity of Kefir

The determination of the number of lactic acid bacteria of the genera *Lactococcus*, *Lactobacillus*, and *Leuconostoc* was carried out after 14 days of refrigerated storage (in 4 °C) on MRS agar according to de Man, Rogosa, and Sharpe (no. 110660 from Merck KgA (Darmstadt, Germany)) [27]. The microbiological assay conditions were as described by Cais et al. [28]. Lactase activity was determined by the method provided by Passerat and Desmaison [29].

2.7. Statistical Analysis

In order to verify the statistical hypotheses, a level of significance of $\alpha = 0.05$ was used. The choice of statistical significance was based on the analysis of univariate data (ANOVA) for multiple comparisons, and the post hoc Tukey HSD test was used. Statistical analyses were carried out using dedicated analytical software, version 13.3 (TIBCO Software Inc., Palo Alto, CA, USA).

3. Results and Discussion

3.1. Components and Properties of Goat's Milk with Permeate after Enzymatic Conversion of Lactose

The basic composition of goat's milk and goat's milk with permeate is presented in Table 1. The GOS content in goat's milk (6.3% of total sugars) was lower than that in the mixture of milk and permeate (6.9% of total sugars; $p < 0.05$). However, the addition of permeate changed the casein–whey protein proportion from 4.2 to 1.1 and increased the ash content by 4%. The composition of the mixture was determined by the composition of the milk and permeate. The permeate (also called native whey), added to milk in this experiment in the amount of 40% (*v/v*), had the following composition: solid non-fat—84.3 g/kg; fat—0.01 g/kg; lactose—43.2 g/kg; total protein—27.5 g/kg; casein—0.1 g/kg; whey protein—26.8 g/kg. The proportion in casein–whey protein permeate was <0.004. The proportion of whey protein to total protein was thus 97.5%. It should be noted that it was concentrated permeate (a volume concentration factor of 4.5). As demonstrated by Song et al. [30], the type and protein content of permeate can be controlled by using different membrane pore sizes (0.10, 0.14, and 0.20 μm) and different transmembrane pressures (100, 120, and 150 kPa). Jørgensen et al. [31] investigated the effect of the pore size of a ceramic membrane (0.05, 0.10, and 0.20 μm) and temperature on the fractionation of skim milk proteins. Microfiltration was carried out with a uniform transmembrane pressure, a continuous flow of permeate, and a volume concentration factor of 2.5. They showed that protein transmission increased with increasing pore size. The obtained permeate had a significantly higher concentration of native whey proteins at $\varnothing = 0.20$ μm (0.50%) compared to the permeability through $\varnothing = 0.05$ μm (0.24%) and $\varnothing = 0.10$ μm (0 39%). Significant amounts of casein penetrated the 0.20 μm (1.4%) membrane, giving a permeate with a whitish appearance and the decomposition of casein: αS2-CN: αS1-CN: κ-CN: β-CN. The research of Svanborg et al. [32] additionally showed that both the nitrogen and mineral distributions were altered by the initial pasteurization.

Companies related to milk processing want to maintain a leading position in the market, using milk to the maximum extent. This is evidenced, on the one hand, by the deliveries of produced raw milk to plants, which are increasing every year, and on the other hand, by the management of all by-products generated during milk processing. Such products include not only whey after the production of curd and rennet cheese but also permeates containing serum proteins obtained from milk by membrane techniques. Such

a situation takes place during the preparation of processing milk during cheese making, which is a retentate with an increased content of casein [33]. This is an activity used especially in the processing of goat's milk due to its lower thermal stability. The best direction for the management of permeates, such as native whey, may be technologies based on devices that already exist in plants and that will contribute to the creation of new products. An additional advantage is the fact that microfiltered native whey protein has many beneficial metabolic effects [34]. This is important due to the global demand for dietary protein [35]. Therefore, native whey is a functional food ingredient. Beverages made with milk permeate may serve as an efficacious source for the purpose of hydration [36]. The research of Muuronen et al. [37] showed that even the production of powdered products from native whey caused no further changes in protein distribution and only a minimal decline in overall protein nativity.

Table 1. Contents and amounts of components in goat's milk and goat's milk with permeate after enzymatic conversion of lactose.

Parameters	M	M/GOS	M + P	M + P/GOS
Solid non-fat (g/kg)	85.2 ± 0.7 [a]	85.3 ± 0.6 [a]	85.1 ± 0.5 [a]	85.2 ± 0.6 [a]
Fat (g/kg)	0.04 ± 0.01 [a]	0.03 ± 0.01 [a]	0.02 ± 0.01 [a]	0.03 ± 0.01 [a]
Total protein (g/kg)	32.0 ± 0.4 [a]	32.1 ± 0.5 [a]	30.3 ± 0.10 [a]	30.3 ± 0.60 [a]
Casein (g/kg)	25.8 ± 0.2 [b]	25.6 ± 0.2 [b]	15.4 ± 0.3 [a]	15.6 ± 0.3 [a]
Whey protein (g/kg)	6.20 ± 0.1 [a]	6.1 ± 0.1 [a]	14.0 ± 0.1 [b]	14.1 ± 0.2 [b]
Casein–whey protein (g/kg)	4.2	4.2	1.1	1.1
Lactose (g/kg)	41.4 ± 0.7 [b]	36.4 ± 0.2 [a]	42.2 ± 0.2 [b]	36.6 ± 0.2 [a]
Lactose–total protein	1.30	1.1	1.4	1.2
Ash (g/kg)	7.7 ± 0.1 [a]	7.7 ± 0.1 [a]	8.0 ± 0.1 [a]	7.9 ± 0.1 [a]
Σ Glucose and galactose (g/kg)	-	2.4 ± 0.1 [a]	-	2.7 ± 0.2 [b]
Σ Glucose and galactose (% of total sugars)	-	5.8	-	6.4
Degree of lactose hydrolysis (%)	-	12.1 [a]	-	13.3 [b]
GOS (g/kg)		2.6 ± 0.1 [a]		2.9 ± 0.1 [b]
GOS (% of total sugars)	-	6.3 [a]	-	6.9 [b]

M—goat's milk; M/GOS—goat's milk after enzymatic conversion of lactose; M + P—goat's milk with permeate; M + P/GOS—goat's milk with permeate after enzymatic conversion of lactose. Values represent mean ± SD (n = 7); SD—standard deviation; [a,b], different letters with mean values in a row indicate statistically significant differences at the level α = 0.05.

The analysis of the acidity of goat's milk and goat's milk with permeate before and after the enzymatic conversion of lactose is presented in Table 2. No differences were found ($p > 0.05$) in pH, acidity, or titratable acidity in any of the samples. Acidity was in the range of 6.70–6.73 °SH, and pH was in the range of 6.63–6.65.

Table 2. Acidity of goat's milk and goat's milk with permeate after enzymatic conversion of lactose.

Parameters	M	M/GOS	M + P	M + P/GOS
pH	6.64 ± 0.01 [a]	6.63 ± 0.01 [a]	6.65 ± 0.02 [a]	6.64 ± 0.01 [a]
Acidity (°SH)	6.70 ± 0.01 [a]	6.72 ± 0.01 [a]	6.73 ± 0.01 [a]	6.71 ± 0.02 [a]
Titratable acidity (% lactic acid)	0.05 ± 0.01 [a]	0.05 ± 0.01 [a]	0.06 ± 0.01 [a]	0.06 ± 0.01 [a]

M—goat's milk; M/GOS—goat's milk after enzymatic conversion of lactose; M + P—mixture of goat's milk with permeate; M + P/GOS—goat's milk with permeate after enzymatic conversion of lactose. Values represent mean ± SD (n = 7); SD—standard deviation; [a], different letters with mean values in a row indicate statistically significant differences at the level α = 0.05.

The parameters that determine the suitability for processing in dairies are also presented (Table 3). There were no differences among the samples ($p > 0.05$). Neither the addition of permeate to milk nor the enzymatic conversion of lactose changed the physico-chemical properties.

Table 3. Physicochemical properties of goat's milk and goat's milk with permeate after enzymatic conversion of lactose.

Parameters	M	M/GOS	M + P	M + P/GOS
Freezing point (°C)	-0.5610 ± 0.001 [a]	-0.5580 ± 0.002 [a]	-0.5593 ± 0.001 [a]	-0.5586 ± 0.003 [a]
Density, in 20 °C (kg/m^3)	1.031 ± 0.001 [a]	1.030 ± 0.000 [a]	1.032 ± 0.000 [a]	1.031 ± 0.001 [a]
Viscosity (mPas)	3.12 ± 0.03 [a]	3.13 ± 0.02 [a]	3.15 ± 0.01 [a]	3.14 ± 0.02 [a]
Conductivity (Ω^{-3}/cm)	6.19 ± 0.02 [a]	6.15 ± 0.03 [a]	6.17 ± 0.02 [a]	6.18 ± 0.01 [a]
Water activity (-)	0.9839 ± 0.003 [a]	0.9830 ± 0.002 [a]	0.9829 ± 0.004 [a]	0.9832 ± 0.002 [a]

M—goat's milk; M/GOS—goat's milk after enzymatic conversion of lactose; M + P—goat's milk with permeate; M + P/GOS—goat's milk with permeate after enzymatic conversion of lactose. Values represent mean $\pm$ SD (n = 7); SD—standard deviation; [a], different letters with mean values in a row indicate statistically significant differences at the level α = 0.05.

3.2. Inhibition of Galactooligosaccharide (GOS) Degradation in Heated Goat's Milk and Goat's Milk with Permeate

The GOS content in goat's milk depended on the temperature and time of heating (Table 4). Temperatures of 72 °C and 85 °C had no effect on the GOS content in total sugars in goat's milk ($p > 0.05$). A reduction in GOS content was observed at 92 °C after 60 min. After heating at 92 °C for 60 min, the proportion of GOS in total sugars decreased from 6.3% to 5.3% ($p < 0.05$). Greater GOS stability was observed in goat's milk with permeate as compared to goat's milk alone. The GOS content remained consistent in the mixture before and after heating (6.9%; $p > 0.05$). Hence, goat's milk with permeate in terms of the GOS content was stable for 60 min at 72, 85, and 92 °C.

Table 4. Galactooligosaccharide (GOS) content in total sugars (%) in goat's milk and goat's milk with permeate after enzymatic conversion of lactose.

Temperature (°C)	Time (min)	M/GOS	M + P/GOS
72	0	6.3 ± 0.1 [aB]	6.9 ± 0.1 [bA]
	30	6.3 ± 0.2 [aB]	6.9 ± 0.3 [bA]
	60	6.3 ± 0.1 [aB]	6.9 ± 0.1 [bA]
85	0	6.3 ± 0.1 [aB]	6.9 ± 0.1 [bA]
	30	6.3 ± 0.1 [aB]	6.9 ± 0.0 [bA]
	60	6.0 ± 0.2 [aB]	6.9 ± 0.2 [bA]
92	0	6.3 ± 0.1 [aB]	6.9 ± 0.1 [bA]
	30	6.0 ± 0.5 [aB]	6.9 ± 0.2 [bA]
	60	5.3 ± 0.2 [aA]	6.9 ± 0.1 [bA]

M/GOS—goat's milk after enzymatic conversion of lactose; M + P/GOS—goat's milk with permeate after enzymatic conversion of lactose. Values represent mean $\pm$ SD (n = 7); SD—standard deviation; [a,b]; [A,B], different lowercase letters in the superscript in a row and capital letters in a column for each parameter indicate statistically significant differences at the level α = 0.05.

Pruksasri and Supee [38] reported the heat stability of GOS in goat's milk. Their results showed that at 85 °C and 121 °C for 60 min, the GOS content remained stable throughout the heating and storage period. The GOS content in goat's milk heated at 85 °C for 0, 15, 30, 45, and 60 min was 13.2%, 13.4%, 13.6%, 13.2%, and 12.6% of total sugars, respectively, which confirmed that GOS in goat's milk was stable at high temperatures. In contrast, after heat treatment at 121 °C for 60 min, the GOS content was 13.3% of total sugars. Thus, no significant changes were observed compared to the initial GOS content (13.2% GOS). These results inspired us to conduct this research. However, our research is enriched with the results of GOS stability in goat's milk with permeate. Forgo et al. [15] studied the thermal stability and decomposition of samples of different carbohydrate families, such as fructo-oligosaccharides, cyclodextrins, and resistant starches at 150 °C, 170 °C, 190 °C, 210 °C, and 220 °C for 10 min. They showed that most samples retained their composition and structure up to 170 °C, and degradation began to occur at 190 °C. Intense degradation occurred at 210 °C and 220 °C. The degradation processes mainly involved chain degradation and the

formation of low-molecular-weight components. The advantage of oligomers was observed at higher temperatures. The formation of oligomers, dimers, and monomers significantly changed the taste and properties of the food product. Additionally, heat treatment changed the prebiotic effect.

Presumably, the greater stability of GOS in the mixture with permeate results from the presence of the ionic system associated with salts, such as phosphorus, calcium, sodium, and potassium. Their presence in amounts different from those in milk may stimulate the process of GOS degradation. Permeate is a source of minerals such as sodium and potassium and also contains calcium, phosphorus, and magnesium [36]. Song et al. [39] showed that permeate with a protein content of 0.2 g/100 g contains 0.3–0.4 g/kg phosphorus, 0.2 g/kg calcium, and 0.1 g/kg sodium. These authors determined these minerals in milk with protein contents of 3.4 g/100 g, 1.6 g/kg, 1.2 g/kg, and 0.4 g/kg. On the other hand, Jørgensen et al. [31] determined that permeate contained twice the protein content (0.39%): 0.44 g/kg, 0.32 g/kg, and 0.38 g/kg, respectively. Permeate with a protein content of 0.56%, tested by Hurt et al. [40], contained 0.401 g/kg phosphorus and 0.254 g/kg calcium.

However, as Jørgensen et al. [31] showed, the separation of minerals in the permeate depends, among other things, on the diameter of the pores in the membranes. Svanborg et al. [32] also reported a significant difference in the potassium content. There was more of it in the permeate (2639.3 mg, calculated on a total solids basis) than in milk (1845.1 mg, calculated on a total solids basis). Performing a partial lactose hydrolysis reaction causes the accumulation of glucose and galactose. Fischer and Kleinschmidt [14], when analyzing GOS synthesis, did not notice a significant reduction in the total GOS efficiency in acid whey solutions compared with the reaction in buffered lactose solution, and the GOS produced had a greater structural diversity.

The thermally induced decomposition of the studied carbohydrates was explained by a proton catalytic process. Protons could originate from the moisture of the sample. The addition of water to the double bond reformed the glycosidic protonated hydroxyl group, and the neutral form of the second fragment was produced by proton elimination [13].

3.3. Goat's Milk and Goat's Milk with Permeate with GOS as a Raw Material for the Production of Kefir

The kefir taste and odor evaluation values are presented as multidimensional exploration lines (Figure 1). This allowed the identification of the interaction between components of the kefir flavor profile of milk and the permeate mixture. Three categorical ranges were used for the values of the descriptors being evaluated. The majority of samples contained a kefir-like odor and kefir-like taste. A smaller but statistically significant number of samples were evaluated as having a sour odor, sour taste, and aftertaste. Other flavors and odors had the least effect on the sample characteristics of kefir.

However, the enzymatic hydrolysis and transgalactosylation of lactose made kefirs made from this raw material sweeter in taste and have an aftertaste. The intensity of the bitter taste decreased. This was noticeable in both kefir with goat's milk and its mixture. Rutkowska et al. [41] showed, using kefir as an example, that the more sour and bitter it was, the less sweet it was. The taste of lactose-free kefir was sweeter and milkier compared to kefir with typical lactose content. The sweetness of kefir could be due to the presence of the monosaccharides glucose and galactose, which are 80% and 35% sweet, respectively. It should also be mentioned that the sweetness of GOS is 35% [41].

The addition of permeate to milk intensified the cream odor in kefir, but after hydrolysis and transgalactosylation, this odor was almost imperceptible. Quite the opposite was found in the case of milk kefir. In addition, the presence of permeate in kefir increased the buttermilk taste, and the presence of GOS intensified it even more. On the other hand, the presence of GOS in kefir from the mixture of milk and permeate increased the sour odor and weakened the kefir-like odor.
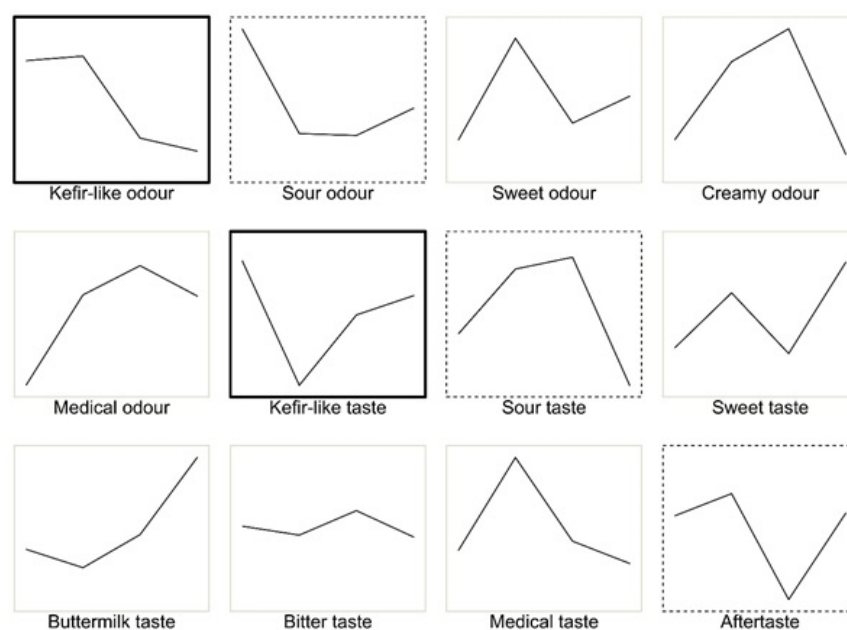
Figure 1. Categorizing the kefir flavor profile of goat's milk and goat's milk with permeate after enzymatic conversion of lactose. From left to right: KM—kefir from goat's milk; KM/GOS—kefir from goat's milk after enzymatic conversion of lactose; KM + P—kefir from goat's milk with permeate; KM + P/GOS—kefir from goat's milk with permeate after enzymatic conversion of lactose.

It was also observed that GOS formed as a result of the enzymatic conversion of lactose, and the change in the casein-to-serum-protein ratio from 4.2 in goat's milk to 1.1 in goat's milk after microfiltration may have a positive effect on the composition of microorganisms characteristic of kefir obtained from goat's milk with permeate (Table 5). It was shown that after 14 days of refrigerated storage, the mesophilic LAB and yeast counts were 8.2-fold and 2.1-fold higher, respectively, in the sample of milk subjected to the enzymatic conversion of lactose (KM/GOS) than in the non-reacted milk (KM). Additionally, a greater number of mesophilic LAB were observed in milk with permeate. The process of the enzymatic conversion of lactose resulting in the formation of GOS in goat's milk with permeate (KM + P/GOS) resulted in about 6.6-fold more mesophilic LAB than in the kefir sample without the (KM + P) reaction. These parameters may change with the further expiration of the refrigerated storage time. The mesophilic LAB count is similar to those in other studies. Delgado-Fernández et al. [42] reported that the number of *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis*, *Streptococcus thermophilus*, *Lactobacillus kefir*, and *Lactobacillus acidophilus* was stable during refrigeration and amounted to approx. 9.3 log cfu/mL. In our research, the number of yeast cells in all kefir samples (on average, approx. 3.66 cfu/mL) was higher compared to the result reported by Delgado-Fernández et al. [42], where it was approx. 2.3 log cfu/mL. As reported by the Codex Standard for fermented milk, the number of yeast cells in kefir should be at least 4 log cfu/mL [43].

Table 5. The number of characteristic bacteria and yeast cells (log cfu/mL) in kefir from goat's milk and goat's milk with permeate after enzymatic conversion of lactose.

Bacteria/Yeast	KM	KM/GOS	KM + P	KM + P/GOS
Mesophilic LAB	7.34 ± 0.02 [a]	8.25 ± 0.01 [b]	7.55 ± 0.01 [a]	8.34 ± 0.02 [b]
Yeast	3.45 ± 0.01 [a]	3.78 ± 0.03 [b]	3.51 ± 0.02 [a]	3.81 ± 0.01 [b]

KM—goat's milk; KM/GOS—goat's milk after enzymatic conversion of lactose; KM + P—goat's milk with permeate; KM + P/GOS—goat's milk with permeate after enzymatic conversion of lactose. Values represent mean ± SD (*n* = 7); SD—standard deviation; [a,b], different letters with mean values in a row indicate statistically significant differences at the level α = 0.05.

The activity of lactase produced by microorganisms present in goat's milk kefirs was similar regardless of the GOS content ($p > 0.05$) and amounted to 0.31 μkat per 100 g of sample (Table 6). Kefir from goat's milk with permeate after the enzymatic conversion of lactose had the highest activity of lactase (0.39 μkat/100 g; $p < 0.05$). In fermented products, the activity of lactase is directly proportional to the number of lactic acid bacteria and the number of yeasts [28]. Studies on the activity of lactase produced by LAB in kefir were carried out by Jeong et al. [44]. They showed that bacterial lactase takes galactosyl moieties from lactose molecules and transfers them, forming new oligosaccharides. There are varied intra- and extracellular lactase activities derived from the bacteria present in kefir. As demonstrated by Zhang et al. [45], the higher the acidity of kefir, the lower the enzyme activity, which is related to the change in the structure of the enzyme and the contents of ions in the environment. The more potassium ions, the greater the activity of the enzyme. On the other hand, calcium ions inhibit the activity of the enzyme [45]. As acidity increases during storage, the activity of the lactase enzyme decreases. This was demonstrated by Teichert and Chudy [46] by storing fermented milk with LAB 4 and yeast for 3 weeks. The lactase activity then decreased from 0.79 to 0.12 μkat/100 g [46].

Table 6. Lactase activity (μkat/100 g) in kefir.

	KM	KM/GOS	KM + P	KM + P/GOS
Lactase activity	0.31 ± 0.02 [a]	0.31 ± 0.01 [a]	0.32 ± 0.02 [a]	0.39 ± 0.02 [b]

KM—goat's milk; KM/GOS—goat's milk after enzymatic conversion of lactose; KM + P—goat's milk with permeate; KM + P/GOS—goat's milk with permeate after enzymatic conversion of lactose. Values represent mean $\pm$ SD ($n = 7$); SD—standard deviation; [a,b], different letters with mean values in a row indicate statistically significant differences at the level $\alpha = 0.05$.

Milk containing varying amounts of ingredients with proven health benefits in the literature can be used to produce functional fermented milk beverages. The functional features of these products are controlled by controlling the proportions of ingredients contained in milk, i.e., increasing the amount of whey proteins, which are the source of many bioactive peptides, and mixing milk with permeate, which supplement each other with their bioactive ingredients. The application of the above technological activities allows raw materials to be obtained based on milk components with increased biological activity compared to milk. The use of the lactic-alcoholic fermentation of milk allows for the acidification of the raw materials and thus the development of new properties. However, in order for products to meet specific consumer expectations, in addition to their biological activity, they must generally be sensory-acceptable. The accurate characterization of the physical and biochemical properties of products allows for defining their role in the process of creating sensory features.

4. Conclusions

The addition of goat's milk permeate after microfiltration and ultrafiltration to goat's milk can inhibit galactooligosaccharide (GOS) degradation during heating. The GOS content in goat's milk is stable at 72 °C and 85 °C for 1 h. GOS degradation only occurred at higher temperatures of 92 °C after 60 min. In contrast, the mixture of goat's milk permeate shows that the initial GOS content is stable after 1 h in the range of 72–92 °C. Our research has shown that further investigations of the GOS degradation inhibition mechanism should be conducted, e.g., when heated to higher temperatures. The effect of protein attraction should be considered due to water binding by GOS, hydrophobic affinity regions in the protein, covalent Maillard reactions between amino acid residues, especially in β-lactoglobulin, and the reduction of GOS groups. The sensory analysis of heated milk with permeate containing GOS showed that, in kefir, the majority of samples had a kefir-like odor and kefir-like taste. Additionally, the presence of GOS resulting from the enzymatic conversion of lactose may have a positive effect on the composition of microorganisms characteristic of kefir. Therefore, goat's milk with permeate after the enzymatic conversion

of lactose with GOS heated at high temperatures (92 °C for 30 min) may be a suitable raw material for the production of innovative and pro-health kefirs. The enzymatic conversion of lactose to GOS does not limit lacto-alcoholic fermentation. Kefir obtained from a mixture of goat's milk and its permeate may be an innovative product in the dairy market. Thus, the diversity of the functional food assortment may increase as a result of the presence of galactooligosaccharides belonging to the group of prebiotics in the produced kefir.

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