

## Article

# The Effect of Storage on Potentially Synbiotic Emulsion Spread Based on Milk Fat and Inulin

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**Abstract:** The effect of four-week storage of milk fat–inulin emulsion as a product designed for spreading on bread was analysed. The emulsion contained 20% inulin, 20% milk fat, and 2% whey protein concentrate as an emulsifier. Salt (0.2%),  $\beta$ -carotene (0.04%), *Lactobacillus acidophilus* La-5, *Streptococcus thermophilus*, as well as *Bifidobacterium animalis* BB-12 were also added. Rheological and textural analysis showed either no significant ( $p \leq 0.05$ ) or no substantial effects of storage on apparent viscosity, storage and loss modulus, hardness, cohesiveness, adhesiveness, and spreadability. The applied probiotic bacteria stayed alive at a level above  $10^7$  cfu/g during four-week storage, which is expected from a probiotic product. The whole time period of storage did not affect the chemical composition of the applied milk fat in the product. Sensory analysis showed that milk fat–inulin spread is acceptable and usually no different than commercial products, however, some off-taste and off-flavours were detected by panellists. In summary, a potentially pro-healthy product for spreading on bread was designed and studied. Besides the presence of fibre and health-promoting bacteria, the studied emulsion characterized a stable chemical composition and rheological as well as textural properties similar to commercial spreads.

**Keywords:** milk fat–inulin emulsion; probiotic; spread; textural properties; rheological properties; sensory analysis; storage



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## 1. Introduction

Among products designed for spreading, there is butter, various types of margarines, and several other products available on the market, the offer of which is changing quite dynamically. For many years, the use of butter has been controversial due to the content of cholesterol and saturated fatty acids. Until the 1990s, margarines seemed to be an alternative; however, usually they contained high levels of trans fatty acids, which turned out to be no less harmful than saturated fatty acids. Currently, margarines in most countries are free from trans fatty acids. Changes in technology in margarine production resulted in the use of palm oil. The use of palm oil is controversial because the cultivation of palm trees is often carried out at the expense of tropical forests, which contributes to an increase in the greenhouse effect. Due to the recent reports about relatively small or neutral associations of milk fat consumption with mortality, diabetes, and cardiovascular disease [1,2], the idea was born [3] of obtaining emulsions for spreading containing milk fat and inulin—indigestible carbohydrates which have fat-mimetic properties [4]. Dairy production still takes place, and there is a surplus of milk fat on the market. Production of the milk fat–inulin emulsion will, on the one hand, use up some of the milk fat, and, on the other hand, reduce the use of palm oil. In addition, when compared with butter or traditional margarine, replacing part of the fat with inulin allows producers to reduce the caloric value of such a product and decrease the amount of saturated fatty acids in the diet, simultaneously increasing the amount of fibre.

Inulin, from a chemical perspective is a group of fructans with a 2–60 degree of polymerization (DP) [5]. Hundreds of thousands of tons of inulin, in the form of white powder, are produced in the world every year. As a fat substitute (high polymerized inulin with a DP of at least 23), sugar substitute (native inulin with a DP of 12, or short chain inulin with DP of 4), and being a fibre and bifidogenic agent, inulin is widely applied in many food products present in the market. Many of these products were previously studied in laboratories, such as dairy products [6–9], meat products [10–12], bakery products [13,14], mayonnaise [15,16], as well as prebiotic beverages [17].

So far, several attempts have been made to obtain spreads based on inulin. After preliminary studies showing that inulin gels have the potential to be used as fat mimetics [18], Glibowski et al. [19] conducted research demonstrating the possibility of obtaining a spread based on inulin (20% *w/w*) and rapeseed oil (20% *w/w*) with the addition of polyglycerol polyricinoleate (1% *w/w*) or whey protein isolate (3% *w/w*) as an emulsifier. Although the analysis of rheological properties showed the stability of the tested emulsion during storage and in the sensory and instrumental analysis, the spreadability did not differ significantly from the margarines available on the market; however, the assessment of flavour, meltability in mouth, and general acceptance recorded significantly worse results than commercial margarines. Another attempt showed that it is possible to manufacture emulsions based on milk fat (15%, 20%) and inulin (15%, 18%, 21%, and 24%) with soya lecithin (2%) as an emulsifier; however, under the condition that during production the temperature will not exceed 65 °C to keep the predictable textural and rheological properties of the final product [3]. Recently, Toczek et al. [20] optimised the composition of emulsion spreads, based on milk fat and inulin with lecithin or whey protein concentrate (WPC) as emulsifiers, with the addition of probiotics: *Bifidobacterium animalis* BB-12, *Streptococcus thermophilus*, and *Lactobacillus acidophilus* La-5. The optimal concentration of milk fat and of inulin was 20%, and only the emulsion spreads with WPC contained live probiotic bacteria. In addition, a critical factor which caused the rheological and textural properties of the obtained emulsions to be predictable was the addition of crystal seeds, helping inulin to gel. The application of probiotic bacteria in emulsions was confirmed by other authors. De Souza et al. [21], analysing margarine with 60% fat (palm and canola oil in a 3:2 ratio), demonstrated that production of probiotic margarine was possible due to the 3% inulin addition.

To the best of our knowledge, there is still a need to analyse how storage time affects the physical properties of the milk fat–inulin emulsion and the survivability of probiotic bacteria. For these reasons, the aim of this study was to analyse the effect of storage of spreads, based on milk fat and inulin, on textural and rheological properties, as well as microbiological and chemical stability. In addition, a sensory analysis of this emulsion was performed.

## 2. Materials and Methods

### 2.1. Materials

Inulin Frutafit® TEX! (DP  $\geq$  23) was donated by Sensus Operations C.V. (Roosendaal, The Netherlands). Anhydrous milk fat (AMF) was purchased from Mlekovita (Wysokie Mazowieckie, Poland), whey protein concentrate (WPC), with 80% protein content (manufacturer's data), was purchased from Ostrowia (Ostrów Mazowiecka, Poland), and  $\beta$ -carotene was purchased from JAR Jaskulski (Warszawa, Poland). FD-DVS ABT-1-Probio-Tec™ thermophilic lactic acid culture, containing *Streptococcus thermophilus*, *Bifidobacterium animalis* BB-12, and *Lactobacillus acidophilus* La-5, was kindly provided by Chr. Hansen Sp. z.o.o. (Czosnów, Poland). TOS, M-MRS and M-17 agars were obtained from Merck (Darmstadt, Germany) or BTL (Lodz, Poland), and all supplements were from Oxoid (Cambridge, UK) or Merck (Darmstadt, Germany). Table salt, reduced-fat margarine (20% fat), butter (82% fat), white toast bread, and ham were purchased in a local supermarket.

## 2.2. Preparation of Samples

The procedure was previously described by Toczek et al. [20]. The final emulsion contained inulin (20%), milk fat (20%), salt (0.2%),  $\beta$ -carotene (0.04%), WPC (2%), and FD-DVS ABT-1-Probio-Tec™ thermophilic lactic acid culture (0.015%). The emulsions were poured into female cones for spreadability analysis and cylindrical plastic containers with a 35 mm inner diameter. In order to prevent evaporation, the female cones were covered with aluminium foil and put into a bigger hermetic plastic container, while cylindrical plastic containers were sealed with lids. The containers were kept at 42 °C for 12 h and then cooled and stored at 5 °C in a thermostatic cabinet. The samples were analysed within 24 h from the production and after 7 days, 14 days, and 28 days of storage at 5 °C. Sensory analysis was carried out after 48 h of storage after the production. The emulsion was manufactured in three independent trials.

## 2.3. Rheological Measurements

Rheological measurements were carried out according to the method previously described by Toczek et al. [20]. Briefly, all measurements were conducted using a Kinexus lab + rheometer (Malvern Panalytical, Cambridge, United Kingdom). All rheological data were collected and calculated using Kinexus Malvern software—rSpace. The apparent viscosity was measured at a 20 s<sup>-1</sup> shear rate for 120 s. Dynamic oscillatory rheological measurements were performed using parallel plate geometry (serrated plates—PU40X SW1382 SS and PLS40X S2222 SS, plate–plate configuration). The measurements were carried out at a 1 mm gap. For analytical purposes, values of  $G'$  and  $G''$  obtained at 1 Hz were taken [3].

## 2.4. Texture Analysis

Texture analyses were performed according to the method previously described by Glibowski et al. [22]. Briefly, samples were punched with a cylindrical probe (1 cm diameter) with a crosshead speed of 1 mm·s<sup>-1</sup> at 15 mm depth, using a TA-XT2i texture analyser (Stable Microsystems, Godalming, England). The analyses were performed immediately after storage at 5 °C without removing the samples from the containers. Hardness, adhesiveness, and cohesiveness were analysed for the purpose of the study [23]. Spreadability was measured with a TTC Spreadability Rig (HDP/SR) attachment, using a method previously described by Glibowski et al. [22].

## 2.5. Microbiological Analyses

Microbiological analyses were carried out according to the method previously described by Gustaw et al. [24] with minor modifications described by Toczek et al. [20]. The plate count method was carried out in duplicate for samples from each trial.

## 2.6. pH

pH measurement was carried out with a pH meter CP-401 (Elmetron Sp. J., Zabrze, Poland).

## 2.7. Sensory Evaluation

To carry out sensory evaluation, we prepared sandwiches based on white toasted bread spread with the tested emulsion, margarine, and butter, on which the ham was placed. When testing sandwich samples, the panellists were said to pay particular attention to the taste and smell of the spread used. Sensory evaluation was performed using 25 semi-trained panellists (graduate students of our University). They examined aroma (buttery, margarine, and off-flavours) and taste (buttery, margarine, salty, acidic, and off-taste) giving points, where 1 was undetectable, 5 was medium detectable, and 10 was strongly detectable.

## 2.8. Fatty Acids Analysis

The fatty acids (FAs) profile of lipids in the sample was determined following fat extraction according to the Rose–Gottlieb method. Methyl esters of FAs (FAME) were prepared by

the transmethylation of fat samples (50 mg) using a mixture of concentrated  $H_2SO_4$  (95%) and methanol, according to the AOCS Official Method Ce 2-66 [25]. Gas chromatographic (GC) analyses were performed in detail according to Domaradzki et al. [26]. The fatty acid composition was expressed as a percentage of total identified FAs. The analyses were carried out in triplicate. The following groups of fatty acids were identified: SFA—saturated FAs (SFA: sum of C4:0, C6:0, C8:0, C10:0, C12:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C22:0, and C24:0); monounsaturated FAs (MUFA: sum of C10:1, C14:1c9, C16:1c13, C16:1c7, C16:1t9, C16:1c9, C18:1t6/7, C18:1t9, C18:1t10, C18:1t11, C18:1n9, C18:1t15, C18:1c11, C18:1c12, C18:1c13, C18:1t16, C20:1 n-11, C20:1 n-13, C20:1trans n-11, and C24:1 n-9), polyunsaturated FAs (PUFA: sum of C18:3 n-3, C20:3 n-3, C20:5 n-3, C22:5 n-3, C22:6 n-3, C18:2 n-6, C18:3 n-6, C20:2 n-6, C20:3 n-6, C20:4 n-6, C22:4 n-6, and C20:2 n-9), and trans FAs (TFA: sum of MUFA trans,  $\Sigma$ 18:2 trans, and  $\Sigma$ C18:3 trans;  $\Sigma$ CLA—conjugated linoleic acid: sum of 18:2 c9,t11; 18:2 t9,c11;  $\Sigma$ C18:2 trans—sum of non-conjugated 18:2 t,c/c,t/t,t isomers; and n-6/n-3 ratios). In Results and Discussion, the most important groups of FAs are presented.

### 2.9. Statistical Analysis

The data were analysed by means of the Statistical Analysis System using the ANOVA procedure for analysis of variance and the Student–Newman–Keuls *t*-test for ranking the means.

## 3. Results and Discussion

### 3.1. Rheological and Textural Properties

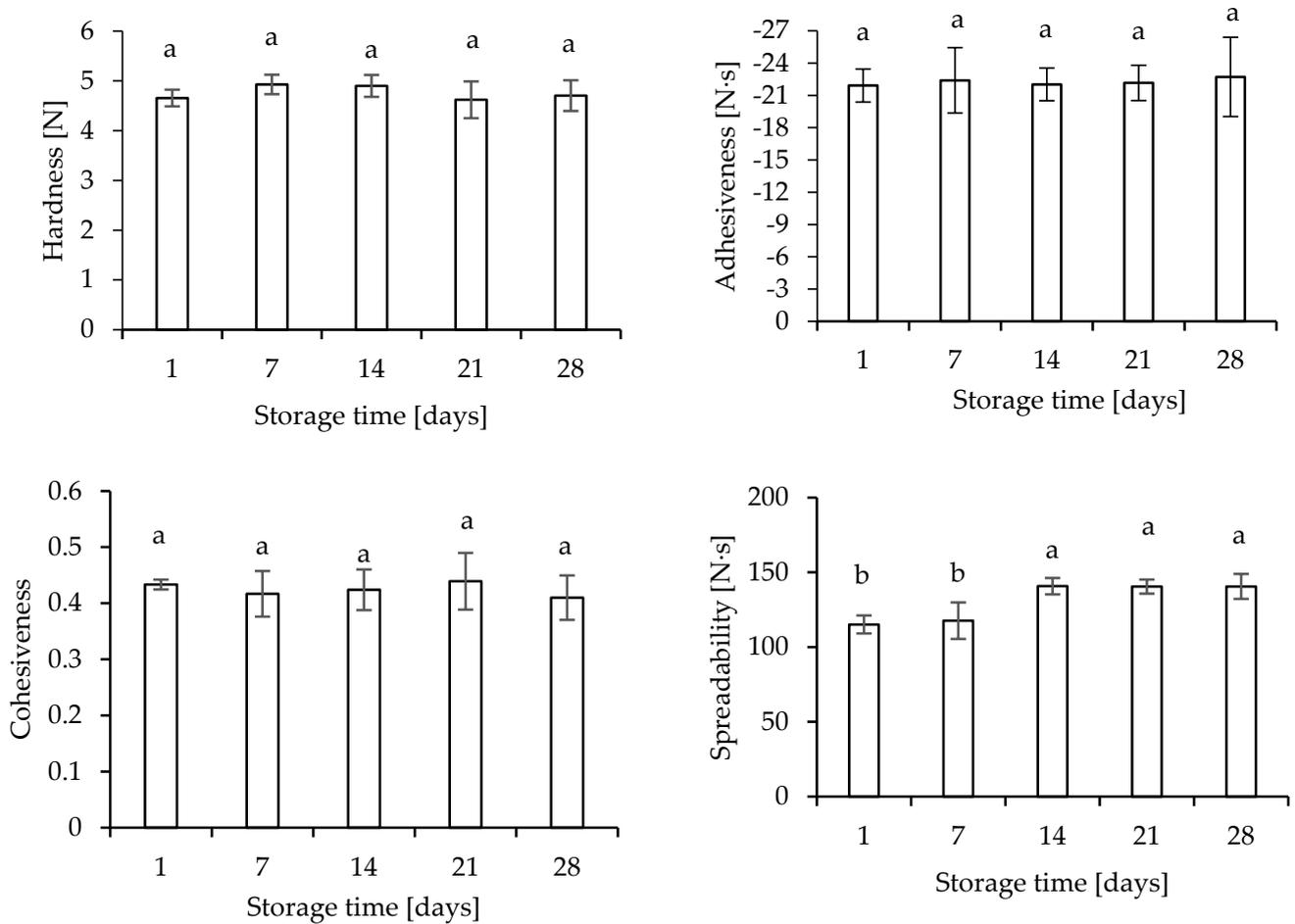
Four weeks of storage did not significantly affect most of the rheological and textural properties of the tested emulsions. Hardness, cohesiveness, and adhesiveness (Figure 1) did not change significantly ( $p \leq 0.05$ ). Only in the case of spreadability (Figure 1) was a slight increase in the obtained values noted. This was probably due to the way the samples were prepared and stored, as the emulsions were poured into the target containers as soon as they were obtained. While the containers for measuring hardness, adhesiveness, and cohesiveness were tightly closed with lids, in the case of spreadability, the female cones were only covered with aluminium foil and, despite being placed additionally in a sealed box, some of the water from the samples may have evaporated. This made the spreadability values with longer storage higher, which means the samples were more difficult to spread. Previous studies [19] concerning the effect of storage time on inulin–rapeseed oil emulsions also showed a lack of change in texture during storage. The presence of milk fat does not change the texture properties which is expected from the product designed for spreading on bread. The results obtained during textural analysis confirmed that the studied milk fat–inulin emulsion was easily spreadable immediately after being taken out of the refrigerator because the hardness values were similar to those recorded for soft margarines containing 20–40% fat, and spreadability values were similar to 40–60% fat margarines [22].

Rheological properties of the analysed samples showed that during storage milk fat–inulin emulsions were quite stable (Figure 2). No significant ( $p \leq 0.05$ ) differences were recorded for elastic ( $G'$ ) modulus: however, some fluctuations of storage ( $G''$ ) modulus were observed. A slight but significant decrease in apparent viscosity in the 4th week of storage was also recorded. In summary, we can suppose that there were no noticeable changes in the structure of the studied emulsion affecting the texture and rheological properties during storage, even though the probiotic bacteria were present in the matrix.

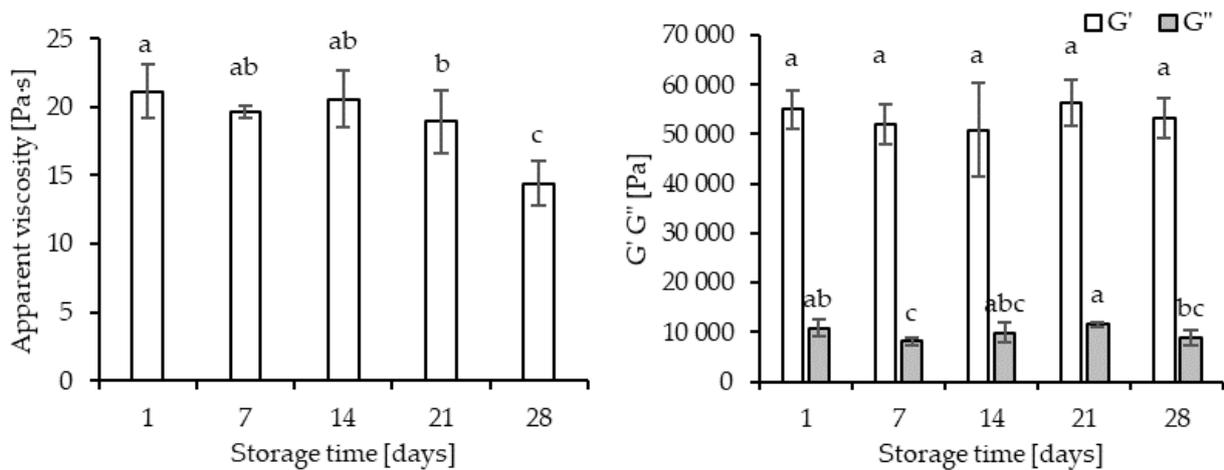
### 3.2. Stability of Probiotic Bacteria

The storage time did not adversely affect the values on the number of bacterial colonies of *Lactobacillus acidophilus* La-5 (Table 1). Although lower values ( $p \leq 0.05$ ) were recorded after four weeks of storage in the case of *Streptococcus thermophilus* and *Bifidobacterium animalis* BB-12 bacteria, the number of bacteria still corresponded to the requirements that should characterize a probiotic product. Consumption of 10 g of this emulsion, which is

a typical amount of spread on a slice of bread, is enough to achieve therapeutic effects because in such products a minimum level of probiotic bacteria is  $10^6$  cfu/g, and daily intake should be about  $10^8$  cfu/g [27,28].



**Figure 1.** Texture properties of the milk fat-inulin spread. Values are means. Means with different letters for the same texture feature are significantly different ( $p \leq 0.05$ ).



**Figure 2.** Apparent viscosity. Elastic ( $G'$ ) and loss ( $G''$ ) moduli of the milk fat-inulin spread. Values are means. Means with different letters for the same rheological feature are significantly different ( $p \leq 0.05$ ).

**Table 1.** Number of bacteria and pH of the milk fat–inulin spread as an effect of storage at 5 °C.

Storage Time [day]	pH	Number of Bacteria [cfu */g]		
		ST	LA-5	BB-12
1	4.78 <sup>a</sup>	$1.6 \times 10^7$ <sup>a</sup>	$1.3 \times 10^7$ <sup>a</sup>	$1.4 \times 10^7$ <sup>a</sup>
7	4.77 <sup>a</sup>	$1.7 \times 10^7$ <sup>a</sup>	$1.4 \times 10^7$ <sup>a</sup>	$1.4 \times 10^7$ <sup>a</sup>
14	4.60 <sup>b</sup>	$1.7 \times 10^7$ <sup>a</sup>	$1.4 \times 10^7$ <sup>a</sup>	$1.3 \times 10^7$ <sup>a</sup>
28	4.61 <sup>b</sup>	$1.4 \times 10^7$ <sup>b</sup>	$1.2 \times 10^7$ <sup>a</sup>	$1.0 \times 10^7$ <sup>b</sup>

\* Colony-forming unit; values are means. Means with different letters for the same bacteria strain or pH are significantly different ( $p \leq 0.05$ ).

The emulsion environment was appropriate for the applied bacteria over a longer period. Analysis of pH showed a little, however significant ( $p \leq 0.05$ ), decrease after two weeks of storage. This can be an effect of the production of organic acids, mainly lactic acid, by the bacteria [29]. Although, to the best of our knowledge, there are no earlier studies on the effect of storage on the survivability of probiotic bacteria in milk fat–inulin emulsions, other research confirmed that probiotic bacteria can survive in emulsions [21,30].

Since there is inulin, a prebiotic compound, in the analysed product, and there are live probiotic bacteria, we can assume that this product can be synbiotic. Additional studies are necessary to show how this product affects consumers in improving the survival and implantation of live health-promoting bacteria [31].

### 3.3. Sensory Analysis

Sensory analysis of emulsions showed in previous studies that the presence of inulin negatively affects the general acceptance of the final product [19]. Since, in the above-mentioned studies, the analysed emulsion was assessed in its pure form, this time it was decided to conduct a sensory analysis by making the tested samples similar to the kitchen reality by preparing sandwiches made of toasted bread with ham because usually no one eats spread alone.

The obtained results showed (Table 2) that a buttery aroma was significantly ( $p \leq 0.05$ ) easier to detect in the case of sandwiches with butter, but there were no differences between sandwiches with milk fat–inulin spread and margarine. A 20% content of milk fat is not enough to give a noticeable aroma sensation. Perhaps, since anhydrous milk fat in the tested emulsion was applied, aromatic compounds were not present there at the level usually in butter [32].

**Table 2.** Sensory evaluation of the milk fat–inulin spread, margarine and butter.

	Milk Fat–Inulin Spread	Margarine	Butter
Buttery aroma	$3.76^b \pm 2.39$	$3.92^b \pm 2.36$	$7.12^a \pm 2.05$
Margarine aroma	$2.68^b \pm 1.89$	$6.60^a \pm 2.59$	$2.20^b \pm 1.29$
Off-flavours	$3.60^a \pm 2.94$	$2.20^b \pm 1.68$	$1.44^b \pm 0.77$
Buttery taste	$3.56^b \pm 2.45$	$4.04^b \pm 2.49$	$8.60^a \pm 1.50$
Margarine taste	$3.68^b \pm 2.53$	$6.92^a \pm 3.15$	$2.16^c \pm 1.80$
Salty taste	$5.00^a \pm 2.16$	$3.08^b \pm 1.10$	$2.64^b \pm 1.80$
Acidic taste	$2.76^a \pm 1.81$	$1.60^b \pm 1.35$	$1.52^b \pm 1.19$
Off-taste	$5.56^a \pm 3.56$	$2.00^b \pm 1.81$	$1.88^b \pm 1.80$

Values are means  $\pm$  standard deviation ( $n = 25$ ). Means with different letters for the same row are significantly different ( $p \leq 0.05$ ).

Consequently, margarine aroma was easily detected by the panellists only in the samples containing margarine. Some off-flavours and off-tastes in the case of the analysed milk fat–inulin emulsion were recorded. This was probably caused by anhydrous milk fat, where different off-flavours had increased, especially during storage [33]. As for the buttery taste, no differences were recorded between sandwiches with analysed spread and

margarine, as opposed to sandwiches with butter, where this taste was clearly perceived. Although margarine taste was clearly noticed in sandwiches with margarine, some of the panellists tasted it in milk fat–inulin emulsion samples but not in sandwiches with butter. The saltier taste was probably caused by too high concentration of salt in the recipe; although it was lower than declared on the label of the margarine, we took it for sensory tests. As for the acidic taste, this was more noticeable for the panellists due to the acidity (Table 2), which was similar to fermented probiotic milk products [34].

### 3.4. Fatty Acids Composition

Analysis of fatty acid composition in fat extracted from the milk fat–inulin spread stored at 5 °C for four weeks showed some significant changes in half of the analysed groups of fatty acids (Table 3). Statistically significant but practically small changes were recorded for saturated fatty acids, polyunsaturated fatty acids, and n-3 fatty acids. This analysis showed that the product is chemically stable, which we expected because milk fat composition does not change in a short time [35]. The analysed fat contained about 65% of saturated fatty acids, which is of course a lot from a nutritional perspective; however, since the milk fat content in the final product is 20%, that means the saturated fatty acids content is 13%. This is less than in full-fat margarine, where the contents of fatty saturated fatty acids can reach 28% [20].

**Table 3.** Fatty acid composition in fat extracted from the milk fat–inulin spread stored at 5 °C for four weeks.

	Storage Time [Day]			
	1	7	14	28
SFA	64.45 <sup>b</sup> ± 0.60	65.32 <sup>a</sup> ± 0.29	65.17 <sup>a</sup> ± 0.27	65.60 <sup>a</sup> ± 0.60
MUFA	26.42 <sup>a</sup> ± 0.23	26.56 <sup>a</sup> ± 0.25	26.69 <sup>a</sup> ± 0.12	26.31 <sup>a</sup> ± 0.40
PUFA	3.78 <sup>a</sup> ± 0.38	3.25 <sup>b</sup> ± 0.08	3.25 <sup>b</sup> ± 0.18	3.24 <sup>b</sup> ± 0.18
TFA	2.38 <sup>a</sup> ± 0.08	2.40 <sup>a</sup> ± 0.08	2.67 <sup>a</sup> ± 0.38	2.40 <sup>a</sup> ± 0.04
n-3	0.63 <sup>a</sup> ± 0.02	0.62 <sup>ab</sup> ± 0.05	0.56 <sup>b</sup> ± 0.03	0.60 <sup>ab</sup> ± 0.06
n-6	1.49 <sup>a</sup> ± 0.10	1.42 <sup>a</sup> ± 0.10	1.54 <sup>a</sup> ± 0.14	1.46 <sup>a</sup> ± 0.16
n-6/n-3	2.36 <sup>a</sup> ± 0.21	2.32 <sup>a</sup> ± 0.26	2.74 <sup>a</sup> ± 0.28	2.45 <sup>a</sup> ± 0.37

Values are means ± standard deviation. Means with different letters for the same row are significantly different ( $p \leq 0.05$ ). SFA—sum of saturated fatty acids; MUFA—sum of monounsaturated fatty acids; PUFA—sum of polyunsaturated fatty acids; and TFA—sum of trans fatty acids.

## 4. Conclusions

Storage did not affect the chemical stability of the milk fat–inulin emulsion. The fatty acid composition did not change or changed, but not profoundly, during the four-week storage at 5 °C. The emulsion was shown to be a potential carrier of probiotic bacteria because until the end of the analysed storage period, all three strains remained viable above a minimum level which should characterize the probiotic product. The storage time did not substantially affect the rheological and textural properties of the tested spread, and the analysis confirmed its easy spreadability right after taking the product out of the refrigerator. Although some off-taste and off-flavours were detected by panellists, sensory analysis showed that the emulsion did not deviate from commercially available products and, bearing in mind its potentially pro-healthy properties, as a product increasing fibre in diet and containing health-promoting bacteria, it probably can find a place on the market of functional food. Further human studies are needed to confirm the functional properties of the studied product.

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