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Comparison of the Spreadability of Butter and Butter Substitutes

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Abstract: There are many types of butter, soft margarine, and blends, e.g., a mixture of butter and vegetable fats, on the market as bread spreads. Among these, butter and blends of butter with vegetable fats are very popular. The consumer's choice of product is often determined by functional properties, such as texture, and the physicochemical composition of butter and butter substitutes. The aim of this study was to compare sixteen market samples of butter and butter substitutes in terms of spreadability and other selected structural (spreadability, hardness, adhesive force, and adhesiveness) and physicochemical parameters (water content, water distribution, plasma pH, color, acid value, peroxide number, saponification number, and instrumentally measured fatty acid profile) to investigate their correlation with spreadability. The parameters determined here were correlated with factors such as the type of sample, measuring temperature, and physicochemical composition. The statistical analysis revealed a very strong positive correlation between hardness and spreadability for all samples tested at 4 °C, as well as between hardness and spreadability for all samples tested 30 min after removal from the refrigerator; however, the interpretation of the results was different if the butter and butter substitute samples were subjected to a multivariate analysis separately.

Keywords: spreadable fats; texture; hardness; adhesive force; adhesiveness; fatty acid profile; multivariate analysis; functional food

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1. Introduction

Butter is a high-fat product with at least 80%, but no more than a 90% fat content, and it is obtained from cow's milk by whipping previously obtained cream or sour cream. However, the assortment of spreads for bread on the market consists of different types of butter, soft margarine, and blends such as a mixture of butter and vegetable fats. Among the wide range of edible fats, in addition to butter and margarine, products called butter mixtures with vegetable fats are of great interest. All of these edible fats are water-in-oil emulsions [1]. The decision as to which fat to consume in spreads can therefore be somewhat difficult for some consumers, since these fats differ not only in their calorific value and therefore in their chemical composition but also in their functional properties, such as spreadability [2–5].

The study comparing the spreadability of butter and butter substitutes makes a significant scientific contribution to the field of food science and technology. Understanding the spreadability of food products is essential, as it plays a critical role in consumer acceptance and product functionality. In order to improve the spreadability of butter, several technological steps are taken, starting with the selection of raw materials with a fixed

fatty acid composition, followed by technological treatments, including parameters for the physical ripening of the cream, enrichment with a low-melting-point milk fat fraction or repeatedly churning the butter [6–10]. The spreadability of butter and blends of butter and vegetable fats can be assessed based on the solid fat content (SFC) in the temperature range of 4–10 °C. In order to achieve good spreadability at a refrigeration temperature, the SFC of butter should be below 32% (by comparison, pure butterfat has an SFC of 38.8–32.4% at 5–10 °C) [6]. In addition, the textural properties of butter are determined by a number of interrelated parameters, such as the concentration, size, shape, and distribution of structural elements: fat crystals, fat globules, air bubbles, and water phase droplets [3,10–19].

Butter and butter substitutes have different physical and chemical properties, and it is important to understand how these differences affect the spreadability of these products. This study can help identify the factors that influence spreadability such as fat content, viscosity, and solid fat content. The scientific contribution and specific significance of the study comparing the spreadability of butter and butter substitutes lies in its potential to improve our understanding of the properties that affect the spreadability of food products and to inform the development of new and improved products for the food industry. A real downside to butter is its hard-to-spread texture. Many authors have attempted to study the spreadability of butter, soft margarine, mixtures of butter and vegetable fats, and shortenings using various instruments such as a penetrometer and texture analyzer (textrometer) with a penetration test, a shear test, a compression test, a texture profile analysis (TPA), DSC methods, EPR spectroscopic methods and the back extrusion test [2,9,11,16,20–31]. The results of such studies can be used to model the relationship between the structure, rheological properties, and textural properties of fat products, taking into account the morphology of the fat crystal network, the solid fat content of the product, and the properties of the fat crystal networks [10].

The aim of this study was to compare market samples of butter and butter substitutes in terms of spreadability and other selected structural, physicochemical, and chemical parameters to investigate their correlation with spreadability. The results of the study can be used to develop new and improved butter and butter substitutes that have similar spreadability characteristics without sacrificing other important properties such as flavor, texture, and nutritional value. In addition, the results of this study can have practical applications in the food industry, helping manufacturers to improve the quality and performance of their products. It can also provide valuable information to consumers to help them make informed choices when buying food.

2. Materials and Methods

2.1. Materials

The study material consisted of eight butter samples coded as follows—LMK, LaME, LoME, MEG, MEH, MMP, PME, and PrME—and eight samples of butter substitutes (spreadable fats, consisting of blends of butter with vegetable oils) coded as follows: FM, LuPM, LaM, PaEM, RMTM, SSO, ZaM, and BGP (Table 1).

These products were available in the Polish food market at the time of the analysis. All butter samples were declared by the manufacturers as products with an 82% fat content, and they were unsalted. After purchase, the product samples, packed in insulating bags, were transported to the laboratory within 0.5 h, where they were stored in a refrigerator at 4 °C until the analyses were carried out. Three independent purchases of each butter and butter substitute sample were made, representing independent replicates from three different suppliers.

Table 1. Declared composition and nutritional value of tested butter and butter substitute samples.

Sample Code	Declared Ingredients	Energy Value (in 100 g)	Fat [g]	Of Which Saturated Fatty Acids [g]	Carbohydrates [g]	Of Which Sugars [g]	Protein [g]	Salt [g]
butter samples								
LMK	pasteurized cream, lactic acid bacteria cultures	3071 kJ/747 kcal	82	53	0.7	0.7	0.6	0.00
LaME	pasteurized cream	3095 kJ/753 kcal	83	54	0.8	0.8	0.6	0.00
LoME	pasteurized cream	3058 kJ/744 kcal	82	55	0.7	0.7	0.7	0.00
MEG	pasteurized cream, lactic acid concentrate, natural flavoring	3061 kJ/744 kcal	82	57	0.6	0.6	1.0	0.02
MEH	pasteurized cream	3068 kJ/746 kcal	82	54	1.0	1.0	1.0	0.02
MMP	pasteurized cream	3095 kJ/753 kcal	83	54	0.8	0.8	0.6	0.00
PME	pasteurized cream	3097 kJ/753 kcal	83	55	0.7	0.5	0.8	0.02
PrME	pasteurized cream	3063 kJ/745 kcal	82	57	1.0	1.0	0.7	0.03
butter substitute samples								
FM	milk butter, vegetable oils (rapeseed, linseed), milk buttermilk, vitamins (A, D)	2807 kJ/683 kcal	75	29	1.1	1.1	0.8	0.00
LuPM	butter, rapeseed oil, water, lactic acid bacteria cultures	2905 kJ/706 kcal	78	35	0.6	0.6	<0.05	<0.01
LaM	cream, rapeseed vegetable oil, annatto bixin color, flavoring	2559 kJ/622 kcal	68	34	1.4	1.4	1.1	0.00
PaEM	vegetable fat: non-hydrogenated palm oil, sunflower oil, cream, cereal fat, emulsifiers: E471, E472c, E322, acidity regulator: lactic acid, beta-carotene, flavors	2822 kJ/686 kcal	75	33	1.6	0.9	0.6	0.04
RMTM	rapeseed and palm oils, butter, reconstituted butter, water, sea salt, lecithin, natural flavoring, lactic acid, vitamins A, D, carotenes	2994 kJ/717 kcal	80	30	0.6	0.6	<0.05	0.32
SSO	pasteurized cream, rapeseed vegetable oil, lactic acid cultures	2523 kJ/613 kcal	67	37	1.4	0.8	1.2	0.03
ZaM	cream, rapeseed oil, annatto, flavoring	2559 kJ/622 kcal	68	34	1.4	1.4	1.1	0.00
BGP	Palm, rapeseed and sunflower oils, water, anhydrous milk fat, E471, E472c, E322, salt, flavorings, E160a, E330, vitamins A, D	3034 kJ/738 kcal	82	36	0.0	0.0	0.0	0.30

2.2. Texture Characteristics of Butter and Butter Substitutes

Spreadability. The tests were performed with a TA.HD.Plus Texture Analyzer (Stable Micro Systems, Toruń, Poland). This was measured using penetration analysis with a "spreadability ring" spreadability test unit [31]. During the analysis, the upper cone was inserted into the lower container (in the form of an inverted cone) at a speed of 3 mm/s until a gap of one millimeter was obtained between the two elements of this fixture. All samples were analyzed in triplicate. Samples at 20 °C were placed in the bottom reservoir of the attachment without bubbles.

Hardness, adhesive force, and adhesiveness. The tests were performed with a TA.HD. Plus Texture Analyzer (Stable Micro Systems, Toruń, Poland). The procedure consisted of testing the penetration force at a depth of 14 mm applied to a given sample at a speed of 2 mm/s using a P/5 cylinder probe [31]. The samples were analyzed at given time intervals: straight from the fridge (at 4 °C), 30 min after removal from the refrigerator, and at 20 °C for each type of butter sample and butter substitute. All samples were analyzed in triplicate. Hardness was expressed as the maximum force necessary to obtain accurate probe deformation (N). The adhesion force was expressed as the force necessary to overcome the forces of attraction between the surface of the sample and the surface of other materials with which the food came into contact (N). Adhesiveness was expressed as the product of the force required to pull the probe from the sample and the pull time (N × s).

2.3. Physicochemical Properties of Butter and Butter Substitutes

Water content. The weight loss was calculated by determining the weight of the sample after it was dried with appropriately prepared sea sand and calculating the percentage of water content in the product [32,33]. A sample was dried in a laboratory oven at 102 ± 2 °C until a constant mass was obtained. All samples were analyzed in triplicate. Formula (1) used for the calculation was:

$$W = 100 - \frac{B \times 100\%}{A} \quad (1)$$

where W represents the water content of the sample [%]; A represents the sample weight before drying (g); and B represents the sample weight after drying (g).

Water distribution. The main principle of the method of water distribution in samples is to apply indicator paper soaked with an indicator to the freshly cut sample surface [32,34]. The indicator paper turns dark blue where it meets water droplets. All the samples were analyzed in triplicate. A point scale of 0–3 was used to determine the degree of water distribution, after which the samples were classified using the criteria given in Table 2. The analysis was carried out using commercially available indicator paper (Dysperwod, LABLACTA, Olsztyn, Poland) according to the manufacturer's instructions. This method enables the determination of whether the butter and butter substitutes had been properly kneaded and the water droplets properly dispersed in the butter and butter substitute matrix.

Table 2. Classification of butter and butter substitutes according to the degree of water distribution [32,34].

Verbal Definition of Water Distribution in Sample	The Size (Diameter) and Density of the Spots on the Indicator Paper	Class [Points]
Very bad	Diameter 3 mm 8 mm densely occurring (takes up approx. 20% of the paper surface)	0
Bad	Diameter 1 mm 3 mm moderately dense (takes up approx. 10% of the paper surface)	1
Sufficient	Diameter 0.3 mm 1 mm rare (occupies approx. 5% of the paper area)	2
Good	No spots	3

Plasma pH. The determination consisted of melting a 40.0 g weight of the butter or butter substitute sample in a water bath at 50 °C, followed by centrifugation in a laboratory

centrifuge at 1100× *g* at 20 °C for 10 min to separate the aqueous phase (plasma) [32]. The pH of the separated aqueous phase of the butter and butter substitutes was measured with a CPO-505 pH meter (Elmetron, Zabrze, Poland) with a conventional electrode at 25 °C. All samples were analyzed in triplicate.

Color. Color components were measured at 3 or 4 locations for each sample using the reflectance method, using a Minolta CR-200 camera (Konica Minolta, Tokyo, Japan) with a D65 illuminant, 2° observer angle, and 8 mm aperture size. The parameters measured were lightness (*L**), taking values from 0 (black) to 100 (perfect white); redness (*a**), taking negative values for green color shades and positive values for red color shades; and yellowness (*b**), the proportion of blue or yellow color in the sample, taking negative values for blue and positive values for yellow. All samples were analyzed in triplicate. To determine the color differences between the butter and butter substitutes, they were compared to a predetermined standard. The standard was compared to the average results of the *a**, *b**, and *L** components obtained in the color test. The standard table (Table 3) for comparing the test samples was taken from the publication of Chudy et al. [35].

Table 3. The standard table for comparing the test samples was taken from the publication of Chudy et al. [35].

	L*	***a****	***b****
Standard butter	91.6	5.5	24.7

To assess the changes in CIE*Lab* color, a delta E (Δ*E*) calculated according to Formula (2) was used to describe the difference between the two sample colors as follows:

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \quad (2)$$

where Δ*L* is the difference in *L** components between the butter standard (Table 2) and the test sample; Δ*a* is the difference in *a** components between the butter standard (Table 2) and the test sample; and Δ*b* is the difference in *b** components between the butter standard (Table 2) and the test sample.

2.4. Additional Characteristics of Butter Milk Fat

Determination of the acid value. The tests were only carried out on butter fat samples according to [36]. The procedure consisted of neutralizing the free fatty acids present in the fat sample to be analyzed with a standard potassium hydroxide solution. The 10 g butter sample was weighed to the nearest 0.01 g in a 200–300 mL Erlenmeyer flask. The sample was then melted using a water bath (at 40 °C). Then, 50 mL of neutralized alcohol–ether mixture was added to the flask (it was neutralized to phenolphthalein with a KOH solution before use, to a pale pink color that did not disappear within 30 s) and mixed thoroughly. It was further titrated from the buret with a 0.1 M KOH versus phenolphthalein to give a pale pink color which persisted for 30 s. All samples were analyzed in triplicate. The acid number was calculated using Formula (3):

$$LK = \frac{(a \times 5.611)}{m} \quad (3)$$

where LK is acid value (mg KOH/g fat); a is the amount of 0.1 M KOH solution used for the titration (mL); m is the sample fat weight (g); and 5.611 is the amount of KOH contained in 1 mL of 0.1 M KOH solution (mg).

Determination of the peroxide number. The tests were only carried out on butter fat samples according to [37]. The method consisted of the quantitative determination of iodine released from potassium iodide by the action of peroxides present in the fat under study. The released iodine was titrated with a standard solution of sodium thiosulphate (VI). Approximately 1 g of butter was weighed to the nearest 0.001 g into an Erlenmeyer flask

with a ground-glass stopper. Immediately, 25 mL of the chloroform–acetic acid mixture and 1 mL of saturated potassium iodide solution were added. The flask was quickly stoppered and mixed thoroughly. It was left in the dark for 5 min. After this time, 75 mL of distilled water was added using a cylinder, the stopper was rinsed thoroughly and a few drops of starch were added; after mixing, the solution was immediately titrated with 0.002 M sodium thiosulfate standard solution until the solution remained discolored for at least 30 s. At the same time, a reagent test was carried out. All samples were analyzed in triplicate. The peroxide number was calculated using Formula (4):

$$\mathrm{LOO} = 1000 \times \frac{(\mathrm{a} - \mathrm{b}) \times \mathrm{T}}{\mathrm{m}} \quad (4)$$

where a is the volume of sodium thiosulphate solution used to titrate the sample (mL); b is the volume of sodium thiosulphate solution used to titrate the reagent sample (mL); m is the sample fat weight (g); T is the molality of the sodium thiosulphate solution used; and 1000 is the conversion factor for the volume used (mL) of sodium thiosulphate per milliequivalent of oxygen in 1 kg of fat.

Determination of the saponification number. Tests were carried out only in butter fat samples according to [38]. The method involved saponification of esterified and neutralized free fatty acids with an excess of potassium hydroxide solution, followed by quantification of unbound KOH by titration with HCl solution. A total of 2 g of butter was weighed to the nearest 0.001 g into a 100 mL ground-glass Erlenmeyer flask. The sample was then melted using a water bath. A total of 25 mL of alcoholic potassium hydroxide solution was pipetted into the sample. An aftercooler was fitted to the flask, and the flask was placed in the water bath for 30 min with occasional stirring. After this time, the aftercooler was removed and the hot solution was titrated against phenolphthalein with 0.5 M hydrochloric acid solution until the indicator color disappeared. If the volume of the solution decreased during heating, it was made up of the original volume with ethanol before titration. At the same time, a reagent test was carried out under the same conditions. All the samples were analyzed in triplicate. The saponification number was calculated using Formula (5):

$$\mathrm{LZ} = \frac{(\mathrm{a} - \mathrm{b}) \times 28.055}{\mathrm{m}} \quad (5)$$

where LZ is the saponification number (mg KOH/g fat); a is the volume of standard HCl solution used to titrate the reagent sample (mL); b is the volume of standard HCl solution used to titrate the sample (mL); m is the sample weight (g); and 28.055 is the amount of KOH present in 1 mL of 0,5 M KOH solution (mg).

Chromatographic determination of the fatty acid profile. Tests were carried out only in butter fat samples according to [39]. The process consisted of separating the components, which were divided into two phases: one was stationary (stationary phase) and the other was moving in a given direction (mobile phase). The different distribution of the mixture components between the two phases leads to different migration and separation speeds of the components. The effect of the chromatographic separation was plotted in the form of a chromatogram, showing a graph of the signal obtained at the detector as a function of time [39].

The evaporated samples were weighed to the nearest 0.001 g and then dissolved in 2 mL of hexane. A total of 0.5 mL of 2 M KOH in methanol was added to the samples and was then shaken and left for a period of 60 min, with stirring every 10 min for transesterification. Using a syringe, 1 mL of the upper hexane layer containing fatty acid methyl esters was carefully collected and transferred to a glass vial. The sample was then evaporated in a stream of nitrogen, and 0.5 mL of hexane was added.

Determination of fatty acids was carried out using a Shimadzu gas chromatograph coupled to a mass spectrometer GC–MS QP-2010S (SHIM-POL, Warsaw, Poland), using a ZB FFAP column (30 m × 0.25 mm × 0.25 µm; Phenomenex, Torrance, CA, USA). The column operating temperature was initially 40 °C for 3 min, with a programmed temperature rise

at 4 °C/min to 230 °C, and finally, isotherm for 5 min. The injection chamber and ion source temperatures were 230 °C and 240 °C, respectively. The carrier gas was helium and the flow rate was 1.14 mL/min. The GC–MS coupler temperature was 225 °C. Fatty acid methyl ester analyses were carried out at an ionization energy of 70 eV. The qualitative analysis (of the obtained fatty acid methyl esters) was carried out based on a comparison of the retention times of available standards and spectra. All samples were analyzed in triplicate.

2.5. Statistical Analysis

Data were analyzed using a one-way or two-way analysis of variance (ANOVA method). Mean differences between the statistical groups were tested at a significance level of $\alpha = 0.05$. Tukey's test was used for multiple comparisons (statistical ranking) of mean responses to the sample groups (for $\alpha = 0.05$). Multivariate analysis was used to describe the relationship of multiple variables for each sample (for $\alpha = 0.05$). The statistical software Statgraphics Centurion XVII (Kraków, Poland) was used to test the data.

3. Results and Discussion

3.1. Texture Characteristics of Butter and Butter Substitutes

Research into the texture characteristics of butter and blends of butter with vegetable fats is relevant to consumers because it can provide information about the performance of these products. The texture is an important attribute for many consumers as it affects the ease of use and enjoyment of the product. By understanding the texture characteristics of butter and butter substitutes, consumers can make informed decisions about which products meet their needs and preferences. Research into the texture characteristics of butter and vegetable fat blends can play an important role in helping manufacturers to create high-quality products that meet consumer needs and preferences. By providing valuable information on the texture and performance of these products, manufacturers can ensure that their products are competitive in the marketplace and are well received by consumers.

Spreadability. The ability to spread the bread spread easily is one of its most important properties [22,40]. It is worth noting that the higher spreadability value of the butter and butter substitutes tested, as shown in Table 4, indicated poorer spreadability of the product on the bread. The highest spreadability value was obtained for the butter samples at 4 °C. If the butter was left at ambient temperature for 30 min after removal from the refrigerator, these values hardly approached the parameters obtained for butter substitute samples at 4 °C; however, for most butter substitute samples, the spreadability value was still statistically significantly better than for the butter samples. The butter samples coded as MEH and PrME were the exceptions. In their case, the spreadability value at 4 °C was the lowest of the results obtained for the butter samples, and 30 min after removal from the refrigerator, the spreadability value reached the same level as the butter substitute samples at 4 °C. By bringing the butter samples to 20 °C, the spreadability value measured reached the value originally obtained for the butter substitute samples at 4 °C.

The texture of spreadable fats, and more importantly their spreadability, is one of the most important differentiators when assessing their quality. The spreadability of butter and butter blends containing vegetable fats is determined by their chemical composition—the type of fat used in their manufacture, as well as the ratio of the aqueous phase to the fat phase and the balance between the liquid and crystalline phases [2,17]. The higher the degree of crystallization of the fat, the poorer the spreadability of the butter [17]. The spreadability of butter can be improved, among other things, by changing the fat composition (e.g., changing the diet of the animals from which the milk is obtained) [41]. Bobe et al. [30] found that butter samples from the milk of cows that had a more unsaturated composition of milk fats due to a special diet had better spreadability.

Hardness. Butter samples at 4 °C were characterized by a high hardness that was statistically significantly higher than butter substitutes at the same temperature (Table 4). Increasing the temperature of the butter samples resulted in a decrease in their hardness. Thirty minutes after removing the butter samples from the refrigerator, their hardness was already on a similar level to that of the butter substitute samples at a temperature of 4 °C. On the other hand, heating the butter samples to 20 °C led to hardness parameters comparable to those of the butter substitute samples 30 min after removal from the refrigerator.

Table 4. Texture characteristics of butter and butter substitutes.

Butter Samples	Spreadability [N × s]		
	at 4 °C	**30 min after Removing it from the Refrigerator**	**at 20 °C**
LMK	91.49 ± 3.54 ^{i}	34.95 ± 5.66 ^{f}	15.31 ± 0.79 ^{d}
LaME	64.76 ± 2.03 ^{g}	31.22 ± 2.99 ^{f}	4.79 ± 1.45 ^{c}
LoME	70.72 ± 6.04 ^{g,h}	30.51 ± 6.31 ^{f}	8.70 ± 1.22 ^{d,e}
MEG	94.38 ± 6.56 ^{i}	38.85 ± 5.24 ^{f}	16.40 ± 4.34 ^{d,e}
MEH	78.05 ± 1.11 ^{h}	27.30 ± 8.33 ^{e,f}	20.86 ± 2.46 ^{e}
MMP	69.69 ± 4.51 ^{g}	37.84 ± 3.48 ^{f}	5.71 ± 1.14 ^{b,c}
PME	94.62 ± 5.18 ^{i}	45.39 ± 7.50 ^{f,g}	17.31 ± 7.11 ^{d,e}
PrME	56.64 ± 4.65 ^{g}	28.03 ± 1.40 ^{e,f}	8.14 ± 1.03 ^{c}
Butter substitutes samples			
FM	20.38 ± 1.41 ^{e}	3.35 ± 1.27 ^{b}	n.d. ^{1}
LuPM	22.39 ± 0.63 ^{e}	8.29 ± 1.01 ^{c}	1.75 ± 0.40 ^{a}
LaM	23.18 ± 1.67 ^{e}	7.51 ± 1.37 ^{c}	0.53 ± 0.02 ^{a}
PaEM	30.94 ± 0.78 ^{f}	16.51 ± 1.10 ^{d,e}	14.46 ± 1.20 ^{d}
RMTM	13.27 ± 0.80 ^{d}	7.90 ± 1.86 ^{c}	2.12 ± 0.29 ^{a}
SSO	26.58 ± 0.96 ^{e}	16.26 ± 1.68 ^{d,e}	2.21 ± 0.05 ^{a,b}
ZaM	26.76 ± 1.10 ^{e}	9.35 ± 1.28 ^{c,d}	1.57 ± 0.55 ^{a}
BGP	13.21 ± 0.49 ^{d}	7.80 ± 1.29 ^{c}	2.17 ± 0.14 ^{a}
Butter Samples	**Hardness [N]**		
	at 4 °C	**30 min after Removing it from the Refrigerator**	**at 20 °C**
LMK	17.01 ± 0.55 ^{h}	6.13 ± 0.65 ^{d}	2.04 ± 0.17 ^{b,c}
LaME	12.54 ± 0.17 ^{f}	5.58 ± 0.48 ^{d}	1.02 ± 0.29 ^{b}
LoME	13.67 ± 0.34 ^{f}	5.85 ± 0.59 ^{d}	1.61 ± 0.22 ^{b}
MEG	19.28 ± 0.78 ^{i}	7.49 ± 0.72 ^{e}	3.27 ± 0.81 ^{c}
MEH	15.69 ± 0.07 ^{g}	6.14 ± 0.93 ^{d}	3.41 ± 0.34 ^{c}
MMP	14.15 ± 0.69 ^{f,g}	7.07 ± 0.79 ^{e}	1.23 ± 0.19 ^{b}
PME	17.56 ± 0.37 ^{h}	7.92 ± 0.63 ^{e}	3.92 ± 0.57 ^{c}
PrME	10.12 ± 0.82 ^{f}	5.21 ± 0.21 ^{d}	1.64 ± 0.18 ^{b}
Butter substitutes samples			
FM	3.85 ± 0.15 ^{c,d}	0.65 ± 0.20 ^{a}	n.d. ^{1}
LuPM	4.02 ± 0.06 ^{d}	1.46 ± 0.16 ^{b}	0.33 ± 0.06 ^{a}
LaM	4.42 ± 0.29 ^{d}	1.40 ± 0.21 ^{b}	0.12 ± 0.01 ^{a}
PaEM	6.91 ± 0.11 ^{d,e}	3.15 ± 0.13 ^{c}	2.58 ± 0.28 ^{c}
RMTM	2.61 ± 0.06 ^{c}	1.57 ± 0.35 ^{b}	0.46 ± 0.06 ^{a}
SSO	5.01 ± 0.15 ^{d}	2.85 ± 0.37 ^{c}	0.47 ± 0.02 ^{a}
ZaM	4.96 ± 0.15 ^{d}	1.66 ± 0.35 ^{b}	0.30 ± 0.09 ^{a}
BGP	3.79 ± 0.05 ^{c,d}	1.61 ± 0.33 ^{b}	0.38 ± 0.07 ^{a}

Table 4. *Cont.*

Butter Samples	Adhesive force [N]		
	at 4 °C	30 min after Removing it from the Refrigerator	at 20 °C
LMK	−5.03 ± 0.76 ^{a}	−2.48 ± 0.33 ^{c}	−0.84 ± 0.06 ^{d,e}
LaME	−3.82 ± 0.05 ^{b}	−2.16 ± 0.27 ^{c}	−0.50 ± 0.11 ^{e}
LoME	−3.79 ± 0.19 ^{b}	−1.92 ± 0.11 ^{c,d}	−1.39 ± 0.09 ^{d}
MEG	−3.16 ± 0.40 ^{b}	−2.23 ± 0.12 ^{c}	−1.24 ± 0.20 ^{d}
MEH	−3.79 ± 0.19 ^{b}	−1.81 ± 0.23 ^{d}	−1.71 ± 0.73 ^{d}
MMP	−3.44 ± 0.16 ^{b}	−2.68 ± 0.26 ^{c}	−0.59 ± 0.05 ^{f}
PME	−3.68 ± 0.45 ^{b}	−2.29 ± 0.07 ^{c}	−1.15 ± 0.45 ^{d}
PrME	−3.46 ± 0.14 ^{b}	−2.28 ± 0.07 ^{c}	−0.77 ± 0.09 ^{e}
Butter substitutes samples			
FM	−1.49 ± 0.11 ^{d}	−0.26 ± 0.05 ^{g}	n.d. ^{1}
LuPM	−1.42 ± 0.07 ^{d}	−0.59 ± 0.06 ^{e}	−0.16 ± 0.03 ^{g}
LaM	−1.46 ± 0.05 ^{d}	−0.58 ± 0.07 ^{e}	−0.08 ± 0.01 ^{g}
PaEM	−2.08 ± 0.13 ^{c}	−1.31 ± 0.05 ^{d}	−1.16 ± 0.10 ^{d}
RMTM	−1.01 ± 0.05 ^{d,e}	−0.66 ± 0.10 ^{e}	−0.24 ± 0.03 ^{g}
SSO	−1.47 ± 0.11 ^{d}	−0.95 ± 0.09 ^{e}	−0.24 ± 0.02 ^{g}
ZaM	−1.50 ± 0.06 ^{d}	−0.63 ± 0.08 ^{e}	−0.16 ± 0.03 ^{g}
BGP	−1.26 ± 0.04 ^{d}	−0.64 ± 0.08 ^{e}	−0.20 ± 0.03 ^{g}
Butter Samples	**Adhesiveness [N × s]**		
	at 4 °C	**30 min after Removing it from the Refrigerator**	**at 20 °C**
LMK	−18.46 ± 0.98 ^{a}	−12.16 ± 1.67 ^{b}	−4.12 ± 0.34 ^{e,f}
LaME	−17.26 ± 0.29 ^{a}	−10.60 ± 1.23 ^{b,c}	−2.53 ± 0.50 ^{f,g}
LoME	−9.43 ± 0.35 ^{c}	−8.59 ± 1.03 ^{c,d}	−3.55 ± 0.61 ^{f}
MEG	−10.82 ± 1.97 ^{c}	−8.81 ± 1.20 ^{c}	−5.64 ± 0.83 ^{e}
MEH	−14.10 ± 0.80 ^{b}	−7.93 ± 1.02 ^{d}	−6.93 ± 1.30 ^{d}
MMP	−16.59 ± 0.44 ^{a}	−12.68 ± 1.23 ^{b}	−2.93 ± 0.27 ^{f,g}
PME	−17.32 ± 1.57 ^{a}	−10.45 ± 0.70 ^{b,c}	−6.97 ± 1.32 ^{d}
PrME	−16.03 ± 0.34 ^{a}	−11.40 ± 0.34 ^{b}	−3.84 ± 0.51 ^{f}
Butter substitutes samples			
FM	−6.35 ± 0.32 ^{d}	−1.15 ± 0.17 ^{h}	n.d. ^{1}
LuPM	−6.13 ± 0.22 ^{d,e}	−2.61 ± 0.25 ^{g}	−0.86 ± 0.14 ^{h,i}
LaM	−5.97 ± 0.87 ^{d,e}	−2.65 ± 0.38 ^{g}	−0.52 ± 0.00 ^{i}
PaEM	−3.30 ± 0.83 ^{f}	−3.70 ± 0.76 ^{f}	−3.98 ± 0.45 ^{f}
RMTM	−3.99 ± 0.13 ^{f}	−3.13 ± 0.45 ^{f}	−1.22 ± 0.13 ^{h}
SSO	−3.86 ± 0.31 ^{f}	−3.16 ± 1.10 ^{f}	−1.19 ± 0.07 ^{h}
ZaM	−6.50 ± 0.75 ^{d}	−2.83 ± 0.35 ^{g}	−0.77 ± 0.12 ^{h,i}
BGP	−3.92 ± 0.12 ^{f}	−3.14 ± 0.70 ^{f}	−1.20 ± 0.05 ^{h}

^{a,b,c,d,e,f,g,h,i}—within a given parameter that is labeled with the same letters do not differ statistically significantly at the level of $\alpha = 0.05$. ^{1} The analysis of samples under these conditions was not possible.

The high hardness of the butter samples at 4 °C can be explained by the higher proportion of saturated fatty acids, which contribute to the hardness and poor spreadability of butter at refrigerator temperatures, which has been confirmed by several studies [2,8,23,30,31,42–46]. Lower temperatures increase the fat solidity; however, it should be noted that both the butter samples and the butter substitute samples differed in hardness, which could indicate that the technological process parameters also determine this product quality parameter [23,44,46–48]. The results of Glibowski et al. [31] highlighted that samples with a high content of milk fat showed a stronger increase in hardness when changing the temperature conditions from room to cooling temperatures compared with samples that were predominantly vegetable fats. The authors concluded that the presence of milk fat promoted an increase in hardness. Queirs et al. [44] found that the hardness of butter depended on the crystallization of the butter at the manufacturing stage and not only on the storage temperature. Rønholt [48]

found that the ratio between solid and liquid fats and the water content strongly influenced the hardness and spreadability of the product. The presence of unsaturated and liquid fats in the composition of butter and vegetable fat mixtures reduces the hardness of these products. The higher the water content, the more the ratio of solid-to-liquid fat shifts in the direction of the liquid phase, so that less fat contributes to crystal formation and thus influences product hardness and water droplet stability. It should therefore be noted that the butter substitutes included in this study were characterized by a higher water content than the butter samples, as is discussed later in this manuscript. With increasing temperature, the firmness and spreadability of the fat products analyzed decreased, i.e., the spreadability improved. The higher the temperature of the product, the more the product structure approaches that of a liquid. This can be caused, among other things, by the water content of the product [48]. As the water percentage increases, the total fat content decreases, affecting the hardness parameter [48]. As has been noted, the higher water content of the butter/vegetable fat blends allowed for a smoother and therefore more spreadable product.

Adhesive force. Adhesive force is the force between the surfaces of two different bodies to hold them together (a food product is perceived as being sticky when the adhesive force is high) [26]. In the case of butter and butter substitutes, this parameter expresses the force that allows the butter or butter substitute to spread evenly over the surface of the bread. Small statistically significant differences in the adhesive force values were found between the butter samples at 4 °C. The same observation was applied to the samples of the butter substitutes at 4 °C (Table 4). The study showed that market samples of butter had statistically significantly higher adhesion values than samples of the butter substitutes, which could be related to differences in the fatty acid composition of the butter samples and butter substitute samples [31]. As the temperature of the samples of the tested products was increased, changes in the adhesive force values towards a value close to zero were observed. These changes were statistically significant for both butter and butter substitutes as early as 30 min after removing the samples from the refrigerator.

Adhesiveness. Adhesiveness is the work required to separate a product from the surface being tested; its measurement serves to express the adhesive properties by measuring the force needed to separate them. The greater the force required to separate the two, the stickier the product. The data presented in Table 4 show that the adhesiveness of both butter and butter substitute samples was statistically significantly higher, but it was dependent on the product's temperature. The higher the temperature of the butter or butter substitute, the lower the adhesiveness, i.e., the samples were less sticky. For one of the butter substitute samples (coded as FM) raising the product temperature to 20 °C made measurement impossible as the sample had already become liquid rather than sticky. It is also worth noting that the butter and butter substitute samples differed in their adhesiveness, and this was statistically significant.

For the butter and butter substitute samples examined in this study, it can be seen that hardness, adhesive force, and adhesiveness were parameters that were partially correlated with spreadability. If their status changed, the spreadability status would also change. Some correlations between the results of measurements of the rheological properties of edible fats were also found by Glibowski et al. [31]. In their study, spreadability and cohesiveness measured at 5 °C correlated very well, but spreadability and cohesiveness at 5 °C were not very well correlated. The researchers showed a low correlation coefficient between spreadability at 20 °C and spreadability at 5 °C, and between hardness at 20 °C and hardness at 5 °C, which very clearly indicates differences in the rheological properties of edible fats at different temperatures. This was also confirmed by the low correlation coefficients between spreadability at 5 °C and apparent viscosity at 20 °C, and hardness at 5 °C and apparent viscosity at 20 °C [31].

The statistical analysis performed in this study of the spreadability, hardness, adhesive force, and adhesiveness of the butter and butter substitute samples (Figure 1a,b) revealed a completely different relationship pattern than those found by Glibowski et al. [31]. Figure 1a,b show the corrgrams (i.e., correlation plots) of the correlation matrix, with the colored cells representing the magnitude of the correlation. Correlation coefficients range from −1 to +1 and measure the strength of the linear relationship between variables (statistically significant correlations occur at the 95.0% confidence level). The colors ranged from blue for strong negative correlations to red for strong positive correlations. The interpretation of the results for butter samples and butter substitutes differed when the samples were subjected to separate multivariate analyses. Few strong correlations (whether positive or negative) were observed for the butter samples (Figure 1a) between hardness measurements at specific temperature conditions (correlation coefficients 0.96–0.86); between adhesiveness and adhesive force for samples tested 30 min after removal from the refrigerator (correlation coefficient 0.87); and between adhesiveness and spreadability or hardness for the samples tested at 20 °C (correlation coefficients −0.81 and −0.94, respectively).

Significantly stronger correlations (both positive and negative) were recorded for the butter substitute samples. This plot of correlations revealed the following strong positive correlations (Figure 1b): between spreadability and hardness at specific temperature conditions (correlation coefficients 0.89–1.00); between adhesiveness for samples tested at 20 °C and adhesive force for samples tested 30 min after removal from the refrigerator or for samples tested at 20 °C (correlation coefficients 0.90 and 0.99, respectively); and between adhesive force for samples tested at 20 °C and adhesive force for samples tested 30 min after removal from the refrigerator (correlation coefficient 0.87). Strongly negative correlations were no less important and were observed between spreadability and adhesive force for samples tested 30 min after removal from the refrigerator (correlation coefficient −0.93); between spreadability and adhesive force for samples tested at 4 °C (correlation coefficient −0.81); between spreadability for samples tested at 20 °C and adhesive force for samples tested 30 min after removal from the refrigerator or adhesive force for samples tested at 20 °C or adhesiveness for samples tested at 20 °C (correlation coefficients −0.85; −1.00 and −0.98, respectively); between hardness and adhesive force for samples tested at 4 °C (correlation coefficient −0.92); between hardness and adhesive force for samples tested 30 min after removal from the refrigerator (correlation coefficient −0.97); and between hardness for samples tested at 20 °C and adhesive force for samples tested 30 min after removal from the refrigerator or for samples tested at 20 °C or adhesiveness for samples tested at 20 °C (correlation coefficients −0.86; −1.00 and −0.98, respectively). However, it is important to remember that a high correlation coefficient does not necessarily indicate causality. It simply indicates that the two variables are related in some way. Further investigation and analysis, such as regression analysis, may be required to determine the nature of the relationship and to establish causality.

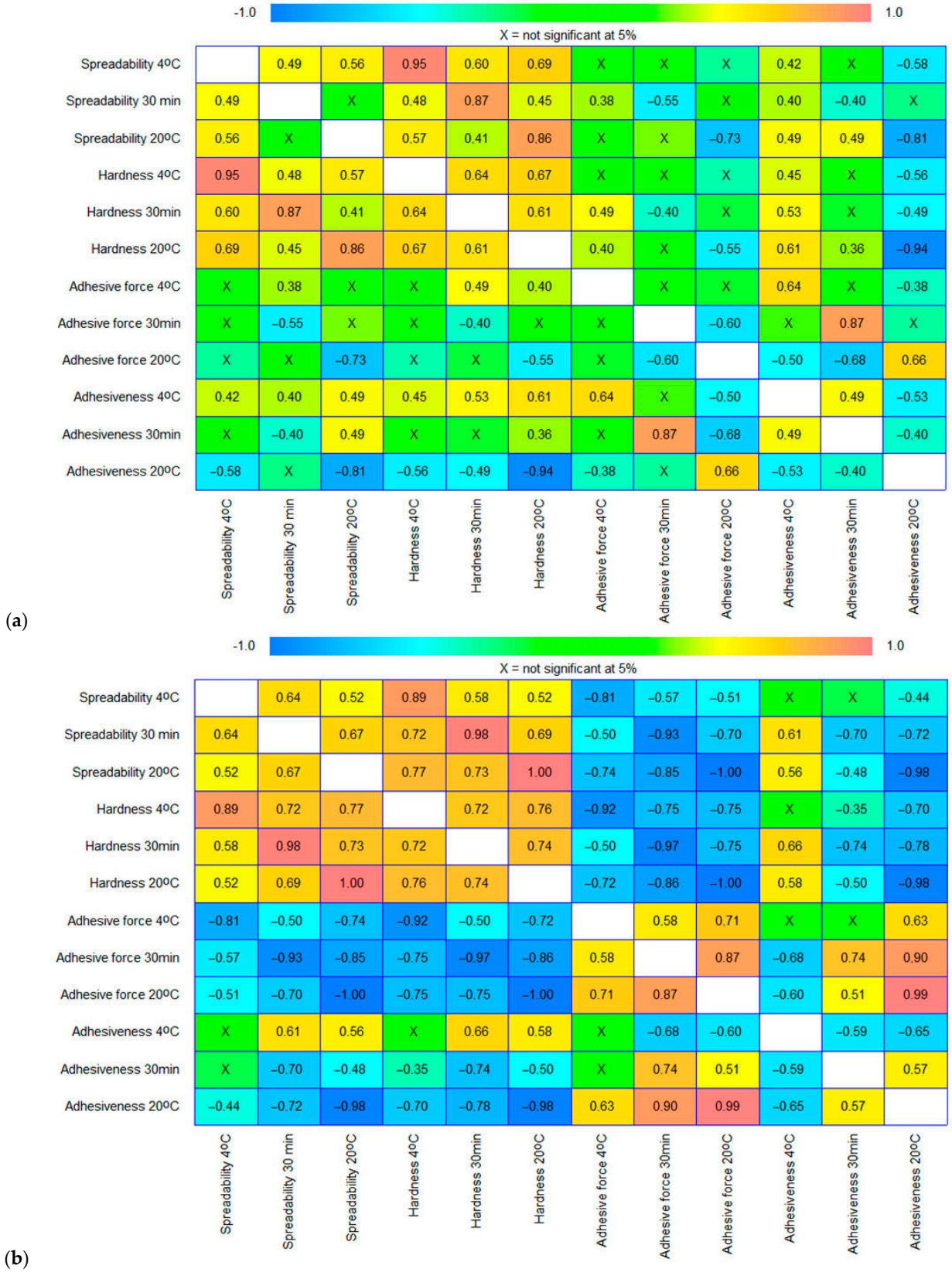


Figure 1. Graphs of correlation matrix showing the relationship between the spreadability, hardness, adhesive force and adhesiveness of butter and butter substitute samples (**a**) and butter substitute samples alone (**b**) measured at different temperatures (with a confidence level of 95.0%).

3.2. Physicochemical Properties of Butter and Butter Substitutes

Water content. The water contents of the tested butter (Table 5) did not exceed the set limit of 16 ± 0.5% [49,50], were in line with producers' declarations (Table 1), and, importantly, these values were not statistically significantly different from each other at the significance level $\alpha = 0.05$. Butter substitutes, on the other hand, were characterized by significantly higher water content values (in the studies presented here, the water content of the butter substitutes samples ranged from 17.93 ± 0.35% to 32.97 ± 0.33%), whereby these samples were divided into three different homogeneous groups with a significance level of $\alpha = 0.05$.

Table 5. Physicochemical properties of butter and butter substitutes.

Parameter / Butter Samples	Water Content [%]	Degree of Dispersion of Water [Points]	Plasma pH
LMK	16.12 ± 0.16 ^{a}	3.0 ± 0.0 ^{a}	5.45 ± 0.43 ^{b,c}
LaME	15.39 ± 0.49 ^{a}	3.0 ± 0.0 ^{a}	5.94 ± 0.18 ^{c}
LoME	15.99 ± 0.44 ^{a}	3.0 ± 0.0 ^{a}	6.38 ± 0.36 ^{c}
MEG	16.08 ± 0.35 ^{a}	3.0 ± 0.0 ^{a}	5.17 ± 0.18 ^{b}
MEH	15.59 ± 0.36 ^{a}	3.0 ± 0.0 ^{a}	6.32 ± 0.11 ^{c}
MMP	15.24 ± 0.18 ^{a}	3.0 ± 0.0 ^{a}	6.38 ± 0.30 ^{c}
PME	15.61 ± 0.27 ^{a}	3.0 ± 0.0 ^{a}	6.27 ± 0.27 ^{c}
PrME	16.08 ± 0.24 ^{a}	3.0 ± 0.0 ^{a}	6.77 ± 0.16 ^{c}
Butter substitutes Samples			
FM	24.88 ± 0.40 ^{d}	3.0 ± 0.0 ^{a}	4.59 ± 0.28 ^{a}
LuPM	22.04 ± 0.40 ^{c}	3.0 ± 0.0 ^{a}	4.56 ± 0.24 ^{a}
LaM	32.09 ± 0.29 ^{e}	3.0 ± 0.0 ^{a}	4.54 ± 0.34 ^{a}
PaEM	24.95 ± 0.44 ^{d}	3.0 ± 0.0 ^{a}	4.05 ± 0.32 ^{a}
RMTM	19.93 ± 0.47 ^{c}	3.0 ± 0.0 ^{a}	4.50 ± 0.17 ^{a}
SSO	32.97 ± 0.33 ^{e}	3.0 ± 0.0 ^{a}	3.98 ± 0.08 ^{a}
ZaM	32.10 ± 0.42 ^{e}	3.0 ± 0.0 ^{a}	4.45 ± 0.28 ^{a}
BGP	17.93 ± 0.35 ^{b}	3.0 ± 0.0 ^{a}	4.43 ± 0.09 ^{a}

^{a,b,c,d,e}—values in the same column and marked with the same letters are not statistically significantly different at the $\alpha = 0.05$ level.

Butter and butter substitutes are physically composed of fat globules, fat crystals, air bubbles, and water droplets, all of which play a role in the physical properties of these products [51,52]. The physical and chemical properties of butter and butter substitutes (including water content and water droplet size, and textural and rheological properties, such as hardness and spreadability) are of great importance as they determine the functionality of these products [17,46,53]. Water content is closely linked to the quality of the end product, such as butter or its vegetable substitutes and blends. As studies [48,54,55] have shown, the water content of butter is influenced by the technological parameters of the creamer process and the kneading of the butter, which aims for an even distribution of water droplets that are as small as possible, in order for the butter to have the right consistency. Rønholt [48] showed that the water content is also decisive for the smear value. The water content influences the crystallization of the fat phase, and thus, also the structure of the butter [56]. The strength of the crystals formed depends on the size of the water droplets and the amount of fat crystallized. As the water content of the product increases, interactions between the water droplets can occur and the textural stability of the butter is consequently lost [57]. Similar effects are observed with butter substitutes [57].

Water distribution. Test samples of butter and butter substitutes received the maximum score in determining the degree of water dispersion (Table 5). The physical composition of butter and its vegetable substitutes varies as the different manufacturing processes result in different microstructures of these products. In addition, butter is less homogeneous and has a more complex chemical composition than its plant substitutes or blends such as

margarine, which requires the use of sophisticated analytical techniques in instrumental analysis to determine water droplet size distribution [58].

The degree of water dispersion is of microbiological importance, as well as being important for the sensory properties of fat products such as butter [59]. The greater the degree of water dispersion, the more difficult it is for unwanted microflora to grow. The water content and degree of distribution can influence the course of fat crystallization, which in turn can influence the texture of the product, and thus, its spreadability [16,48,51,56,57,60].

Plasma pH. The results of the plasma pH measurements of the analyzed samples of butter and butter substitutes are summarized in Table 5. The pH of butter plasma ranged from 5.94 ± 0.18 to 6.77 ± 0.16 and was not statistically significantly different but was dependent on the butter sample. The statistical analysis allowed the butter samples to be distinguished into two groups: (a) MEG and LMK; (b) LMK, LaME, LoME, MEH, MMP, PME, and PrME (Table 5). The plasma acidity of the butter substitutes was statistically significantly different from the pH of the butter plasma and was the same for all butter substitute samples tested.

The plasma pH of butter and its vegetable substitutes (blends with other fats) is a result of the production and storage parameters of the product [61]. An important step in the production of butter, which later influences the pH value of the milk plasma, is the biological maturation of the cream, i.e., its fermentation. The lactose contained therein is converted into lactic acid, which subsequently causes the plasma of the aqueous phase to acidify and thus improves the shelf life of the product. As can be seen from the analysis of the butter samples tested in this work, the pH value indicates that the cream had not undergone biological maturation, i.e., the butter samples were made from sweet cream. The situation is different with butter substitutes, the production of which usually involves regulating the plasma pH value by adding chemical acidity regulators such as citric and lactic acids (what was claimed by some manufacturers, Table 1). It should be noted that the acidity of butter and butter substitutes is a poorly understood parameter in terms of its significant relationship to lubricity values. No available literature data were found on this topic.

Color. It was found that the butter samples tested were different from the chosen standard (Table 6). The mean values of the L^* and b^* color components for the test samples were higher than the corresponding values of the standard, while the mean value of the a^* color component was lower than that of the standard. The measured color of the butter samples according to the standard tended towards slightly greenish and lighter tones. The color component a^* did not statistically differentiate the butter and butter substitute samples.

The parameter b^* in the color analysis is often used as an indicator of the yellow-blue color bias in a sample. When discussing color results, the color tendency of parameter b^* is usually described as the amount of yellow or blue present in the sample. The b^* component divided the butter and butter substitutes into seven homogeneous groups at the 0.05 level, with the majority of butter samples ranking above the majority of butter substitutes on the CIELab scale. The magnitude of the b^* value would provide a measure of the intensity or saturation of the yellow or blue color. A positive b^* value would indicate a yellow color in the butter sample, while a negative b^* value would indicate a blue color. A high positive b^* value would indicate a strong yellow sample, while a low positive b^* value would indicate a lighter yellow color. The samples studied in this work obtained high positive values for the parameter b^*, which in most cases were statistically significantly higher for butter than for its substitutes.

Regarding the color component L^*, all butter samples and the four butter substitutes (LuPM, LaM, SSO, and ZaM) showed the same value for this parameter, which was statistically significant, while the other three butter substitutes (FM, PaEM, and RMTM) were significantly darker. It is worth noting that the butter substitutes compared to the standard for butter color components a^*, b^*, and L^* gave surprisingly similar results for each component, despite the differences in chemical composition (e.g., different fats used or

water content), in textural parameters (e.g., spreadability or hardness) and due to different technological processes.

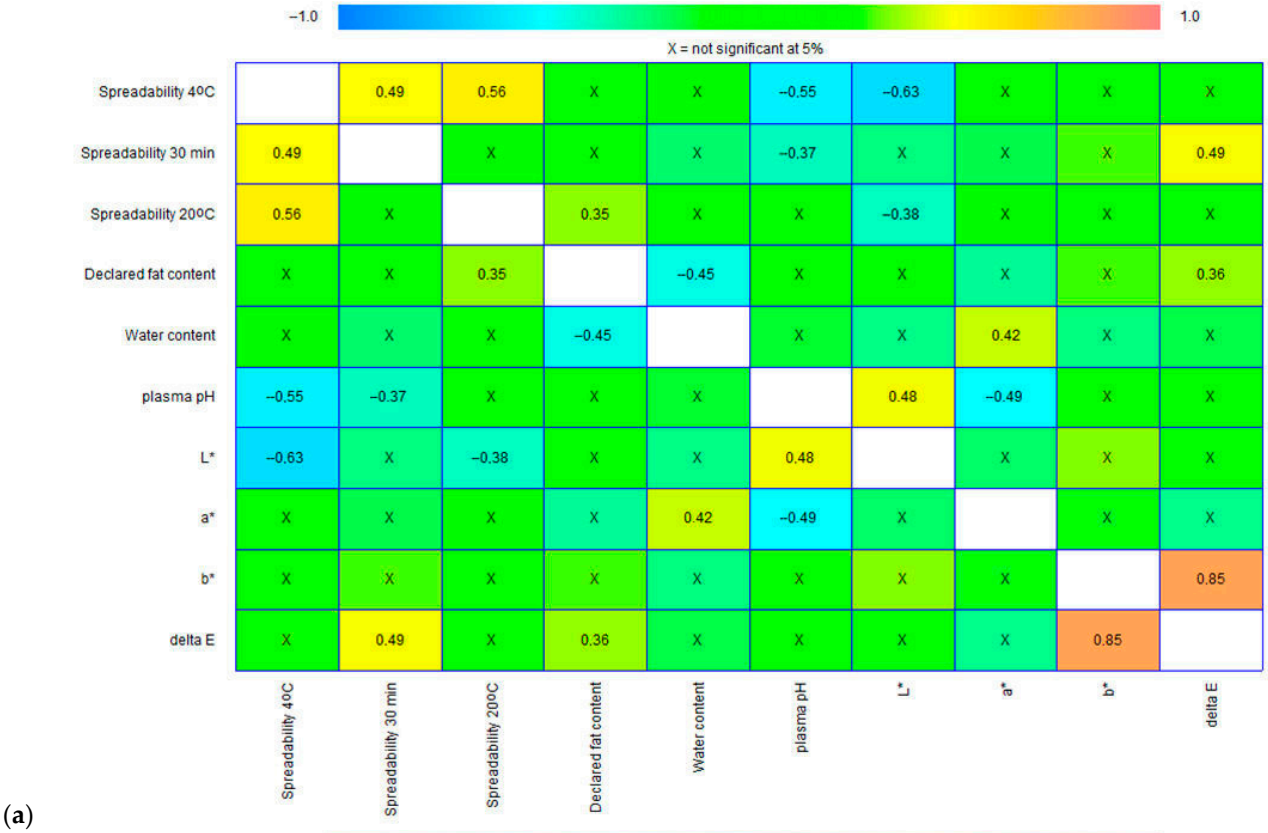
Table 6. Color components of butter and butter substitutes.

Parameter / Butter Samples	*L**	*a**	*b**	Δ*E*
LMK	85.72 ± 0.78 ^{a,b,c,d}	−6.98 ± 0.10 ^{a}	30.25 ± 0.40 ^{c,d,e}	14.89 ± 0.11 ^{b,c}
LaME	88.44 ± 0.39 ^{c,d}	−7.24 ± 0.07 ^{a}	31.82 ± 0.26 ^{e,f,g}	14.94 ± 0.10 ^{b,c}
LoME	88.13 ± 0.59 ^{c,d}	−7.48 ± 0.11 ^{a}	29.04 ± 0.37 ^{c}	14.13 ± 0.12 ^{b}
MEG	87.67 ± 1.41 ^{b,c,d}	−6.93 ± 0.07 ^{a}	31.57 ± 0.94 ^{e,f}	14.81 ± 0.17 ^{b,c}
MEH	87.50 ± 0.57 ^{b,c,d}	−7.16 ± 0.05 ^{a}	30.67 ± 0.37 ^{c,d,e}	14.59 ± 0.09 ^{b,c}
MMP	88.69 ± 0.19 ^{c,d}	−7.32 ± 0.14 ^{a}	32.08 ± 0.05 ^{e,f,g}	15.08 ± 0.16 ^{b,c}
PME	87.12 ± 0.49 ^{a,b,c,d}	−7.49 ± 0.12 ^{a}	33.84 ± 0.63 ^{f,g}	16.51 ± 0.44 ^{c}
PrME	89.30 ± 0.16 ^{d}	−7.13 ± 0.02 ^{a}	33.06 ± 0.14 ^{f,g}	15.32 ± 0.06 ^{b,c}
Butter Substitutes Samples				
FM	83.70 ± 1.71 ^{a}	−7.01 ± 0.26 ^{a}	25.34 ± 0.70 ^{b}	14.88 ± 0.68 ^{b,c}
LuPM	86.58 ± 2.26 ^{a,b,c,d}	−7.24 ± 0.16 ^{a}	25.55 ± 1.26 ^{b}	13.90 ± 0.48 ^{b}
LaM	87.98 ± 0.65 ^{c,d}	−5.97 ± 0.04 ^{a}	30.81 ± 0.48 ^{c,d,e}	13.50 ± 0.04 ^{b}
PaEM	85.40 ± 1.37 ^{a,b,c}	−6.74 ± 0.04 ^{a}	31.18 ± 0.78 ^{d,e,f}	15.23 ± 0.17 ^{b,c}
RMTM	84.11 ± 4.17 ^{a,b}	−0.63 ± 0.09 ^{a}	20.92 ± 2.22 ^{a}	15.67 ± 3.15 ^{c}
SSO	86.82 ± 0.87 ^{a,b,c,d}	−7.56 ± 0.13 ^{a}	26.76 ± 0.33 ^{b}	14.09 ± 0.14 ^{b}
ZaM	86.63 ± 0.20 ^{a,b,c,d}	−6.22 ± 0.15 ^{a}	29.31 ± 0.23 ^{c,d}	13.55 ± 0.06 ^{b}
BGP	86.06 ± 0.45 ^{a,b,c,d}	−5.04 ± 2.98 ^{a}	28.04 ± 0.31 ^{c}	12.37 ± 2.40 ^{a}

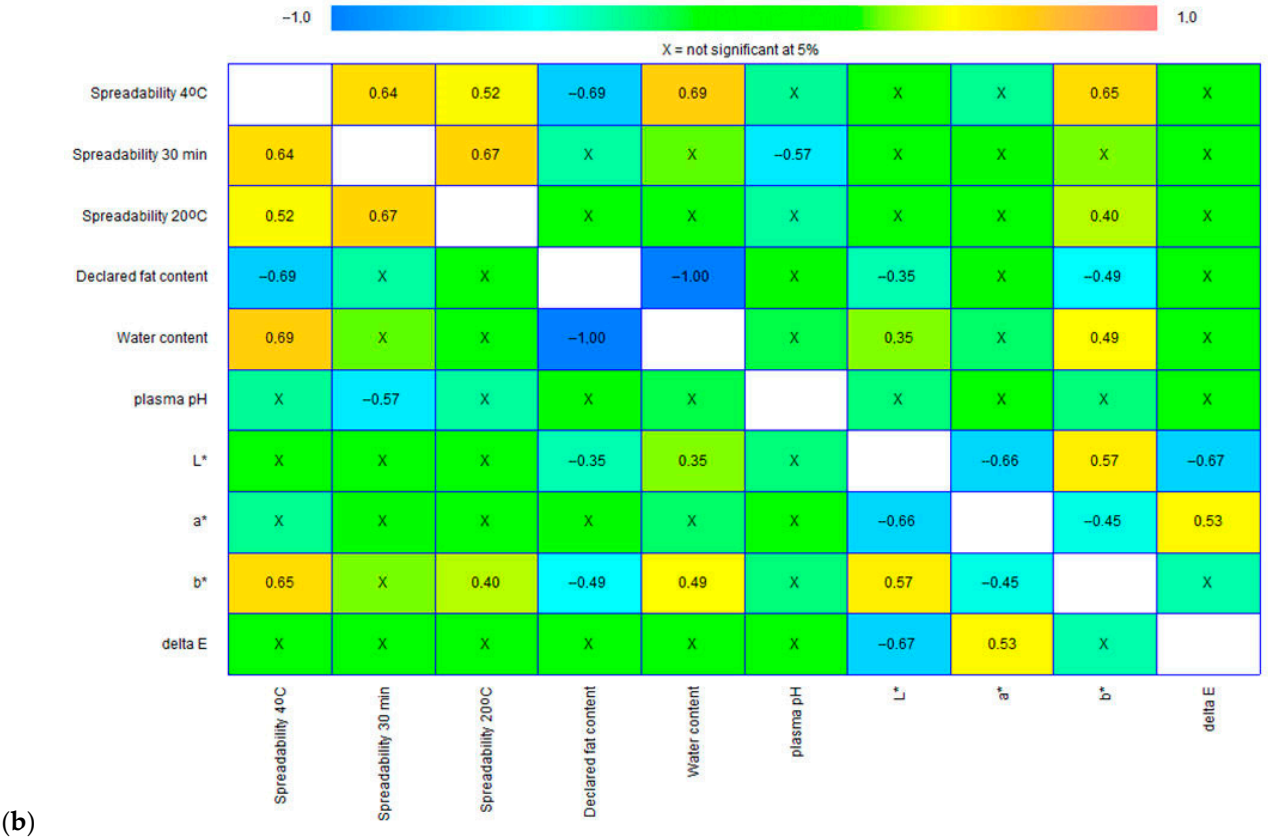
^{a,b,c,d,e,f,g}—values in the same column and marked with the same letters are not statistically significantly different at the $\alpha = 0.05$ level.

The calculated ΔE* values represented the difference between the color of the test sample and the color of the standard in CIELab space and therefore expressed the magnitude of the color change but not its direction. With regard to the expression of this parameter, the samples of butter and butter substitutes were statistically significantly different in two homogeneous groups at the $\alpha = 0.05$ level. The calculated ΔE^* values for the butter and butter substitute samples ranged in excess of 5, indicating large color differences to the unaided eye of an unexperienced observer between the test butter and butter substitutes and the standard color [35,62].

A multivariate analysis of the spreadability measurements, selected physicochemical properties, and the color components of the butter and butter substitute samples did not reveal any significant strong relationships between these parameters (Figure 2a,b). The only significant correlation found was between the ΔE* value and b* color compound for the butter samples (correlation coefficient 0.85). Lapčíková et al. [46] also found no general relationship between the content and composition of total fat in the samples and the values of textural parameters (i.e., springiness, cohesiveness, and stringiness). Furthermore, no correlation was to be expected for the color components, since both butter and butter substitutes can be colored (while carotenes annatto, bixin, norbixin, and curcumin are permitted in butter in the EU, as are other fat- and oil emulsions) [63].



(**a**)



(**b**)

Figure 2. Graphs of correlation matrix showing the relationship between the spreadability, selected physicochemical properties, and the color components of butter and butter substitute samples (**a**) and butter substitute samples alone (**b**) measured at different temperatures (with a confidence level of 95.0%).

3.3. Additional Characteristics of Butter Milk Fat

Determination of the acid value. The acid number is defined as the number of milligrams of potassium hydroxide required to neutralize the free fatty acids in a gram of fat sample. The acid number values of the butter samples tested ranged from 1.14 to 1.54 mg KOH/g fat and did not exceed the maximum value of 2 mg KOH/g fat permitted for butter (Table 7). The acid numbers of the butter tested in this study were statistically significantly different (at a significance level of $\alpha = 0.05$). This may be due to different production dates or because the comparison involves samples from different manufacturers and technologies.

Table 7. Characteristics of butter milk fat.

Butter Samples	Acid Value [mg KOH/g fat]	Saponification Number [mg KOH/g fat]
LMK	1.27 ± 0.03^{b}	227.58 ± 0.43^{b}
LaME	1.20 ± 0.02^{a}	$228.03 \pm 0.58^{b,c}$
LoME	1.16 ± 0.03^{a}	226.19 ± 0.80^{a}
MEG	1.31 ± 0.02^{b}	230.35 ± 0.32^{d}
MEH	1.54 ± 0.03^{d}	$228.18 \pm 0.36^{b,c}$
MMP	1.14 ± 0.03^{a}	229.02 ± 0.11^{c}
PME	1.16 ± 0.03^{a}	230.30 ± 0.6^{d}
PrME	1.37 ± 0.02^{c}	231.18 ± 0.55^{d}

^{a,b,c,d}—values in the same column and marked with the same letters are not statistically significantly different at the $\alpha = 0.05$ level.

These data are consistent with those of other scientists [64,65]. Similar results were obtained by Bellinazo et al. [64], who examined the properties of butter during storage and obtained an acid number value of 1.08 mg KOH/1 g fat just after production. The acid value increased with the storage time and was 2.74 mg KOH/g fat after storage for 90 days.

Determination of the peroxide number. No peroxides were found at detectable levels in any of the tested samples, which was due to the good quality of the tested products. These results were consistent with the findings of other researchers [66]. This number is a measure of the peroxide content and is considered an indicator of the rancidity of the fat. The butter samples tested were products derived from sweet cream; meanwhile, Khaskheli et al. [67] showed that the peroxide number of market sweet butter (1.56 ± 0.17 mEq O2/kg fat) was significantly higher than the peroxide number of butter derived from fermented cream (1.00 ± 0.08 mEq O2/kg fat), which was determined by changes that were reported to have occurred during the storage of the tested butter samples. In comparison, the peroxide number of butter samples freshly prepared from sweet cream or fermented cream under the laboratory conditions by Khaskheli et al. [67] was 1.00 ± 0.10 mEq O2/kg fat and 1.04 ± 0.11 mEq O2/kg fat, respectively. The observed fluctuations in the peroxide number values of market butter (0.35 ± 0.24 to 1.80 ± 0.36 mEq O2/kg fat) were explained by Gonçalves and Baggio [68] by differences in the way the products were packaged, and thus, their exposure to atmospheric oxygen.

Determination of the saponification number. The saponification number values of the butter tested were in the range of 226.2–231.2 mg KOH/g fat and did not exceed the usual range specified for butter, i.e., 220–236 mg KOH/g fat (Table 7). Although the differences between the values obtained for the different butter samples were small, the values were significantly different (at a significance level of $\alpha = 0.05$). Similar results were obtained by Kahyaoğlu and Çakmakçı [69], who studied butter and obtained a saponification number of 228.1 mg KOH/g fat. Another study by Kahyaoğlu and Çakmakçı [70] showed that the saponification number increased with storage time. As the studies mentioned above have shown, the saponification number (such as the acid number) can be an indicator of the degree of freshness of the fat and, above all, of its shelf life. Determination of the

saponification number in fats enables the average molecular weight of the fatty acids to be determined. Its high levels in butter are due to the presence of palmitic acid.

Chromatographic determination of the fatty acid profile. Table 8 provides a summary of the percentage of individual fatty acids found in the butter samples tested. Types of butter, which are products of animal origin, are characterized by a high percentage share of saturated fatty acids (SFA) and a low content of unsaturated fatty acids: monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). The analyzed kinds of butter contained the following fatty acids in the highest proportion: palmitic acid (C16:0), oleic acid (C18:1 cis 9), stearic acid (C18:0), and myristic acid (C14:0).

Table 8. Percentage of fatty acids present in the fat fraction of butter samples.

Fatty Acids	Percentage of Fatty Acids Present in the Fat Fraction [%]							
Butter Samples	**LMK**	**LaME**	**LoME**	**MEG**	**MEH**	**MMP**	**PME**	**PrME**
C 4:0	$0.24 \pm 0.13^{a,b}$	$0.20 \pm 0.04^{a,b}$	0.17 ± 0.20^{a}	0.71 ± 0.04^{c}	$0.29 \pm 0.07^{a,b}$	$0.5 \pm 0.11^{b,c}$	0.69 ± 0.17^{c}	0.78 ± 0.08^{c}
C 6:0	$0.52 \pm 0.15^{a,b}$	0.36 ± 0.04^{a}	$0.43 \pm 0.06^{a,b}$	0.81 ± 0.04^{c}	$0.54 \pm 0.09^{a,b}$	$0.65 \pm 0.04^{b,c}$	0.85 ± 0.09^{c}	0.89 ± 0.06^{c}
C 8:0	$0.5 \pm 0.12^{b,c}$	0.32 ± 0.06^{a}	$0.38 \pm 0.07^{a,b}$	$0.65 \pm 0.04^{c,d}$	$0.48 \pm 0.04^{a,b,c}$	$0.57 \pm 0.01^{b,c,d}$	$0.62 \pm 0.07^{c,d}$	0.69 ± 0.04^{d}
C 10:0	$1.89 \pm 0.21^{a,c}$	1.25 ± 0.41^{a}	$1.46 \pm 0.2^{a,b}$	2.07 ± 0.10^{c}	$1.71 \pm 0.11^{a,b,c}$	$1.96 \pm 0.08^{a,c}$	2.15 ± 0.09^{c}	2.15 ± 0.04^{c}
C 12:0	$2.67 \pm 0.18^{a,b}$	1.89 ± 0.73^{a}	$2.15 \pm 0.31^{a,b}$	2.9 ± 0.11^{b}	$2.55 \pm 0.12^{a,b}$	$2.83 \pm 0.06^{a,b}$	2.96 ± 0.04^{b}	2.93 ± 0.06^{b}
C 14:0	$9.44 \pm 0.74^{a,b}$	8.03 ± 2.79^{a}	$9.32 \pm 0.37^{a,b}$	$11.01 \pm 0.14^{a,b}$	$10.44 \pm 0.23^{a,b}$	$10.82 \pm 0.19^{a,b}$	11.41 ± 0.18^{b}	$10.96 \pm 0.12^{a,b}$
C 14:1	0.13 ± 0.02^{a}	0.13 ± 0.05^{a}	$0.15 \pm 0.02^{a,b}$	$0.18 \pm 0.01^{a,b,c}$	$0.20 \pm 0.01^{b,c}$	$0.20 \pm 0.01^{b,c}$	0.22 ± 0.01^{c}	0.23 ± 0.01^{c}
C 15:0	0.90 ± 0.09^{a}	0.74 ± 0.29^{a}	0.93 ± 0.19^{a}	1.03 ± 0.01^{a}	0.98 ± 0.03^{a}	1.14 ± 0.05^{a}	1.12 ± 0.08^{a}	1.05 ± 0.03^{a}
C 16:0	32.49 ± 1.19^{a}	33.13 ± 2.47^{a}	37.15 ± 2.31^{b}	$34.61 \pm 0.22^{a,b}$	$34.56 \pm 1.02^{a,b}$	$34.85 \pm 0.38^{a,b}$	$35.11 \pm 0.74^{a,b}$	31.37 ± 0.39^{a}
C 17:0	0.54 ± 0.03^{a}	0.52 ± 0.17^{a}	0.58 ± 0.09^{a}	0.60 ± 0.01^{a}	0.65 ± 0.04^{a}	0.69 ± 0.07^{a}	0.68 ± 0.08^{a}	0.69 ± 0.02^{a}
C 18:0	12.10 ± 0.34^{a}	12.73 ± 1.21^{a}	11.39 ± 0.60^{a}	11.59 ± 0.11^{a}	11.34 ± 0.46^{a}	11.06 ± 0.11^{a}	11.17 ± 0.27^{a}	11.73 ± 0.06^{a}
C 20:0	0.13 ± 0.01^{a}	0.15 ± 0.01^{a}	0.14 ± 0.03^{a}	0.13 ± 0.00^{a}	0.17 ± 0.03^{a}	0.14 ± 0.02^{a}	0.13 ± 0.02^{a}	0.12 ± 0.01^{a}
C 14:1 cis	$1.02 \pm 0.10^{a,b}$	0.90 ± 0.33^{a}	$1.09 \pm 0.05^{a,b,c}$	$1.27 \pm 0.05^{a,b,c}$	$1.25 \pm 0.04^{a,b,c}$	$1.37 \pm 0.02^{b,c}$	1.39 ± 0.06^{c}	1.39 ± 0.04^{c}
C 15:1	0.12 ± 0.02^{a}	$0.14 \pm 0.06^{a,b}$	$0.19 \pm 0.03^{a,b}$	$0.20 \pm 0.01^{a,b}$	0.21 ± 0.02^{b}	0.23 ± 0.02^{b}	0.22 ± 0.03^{b}	0.22 ± 0.01^{b}
C 16:1 trans	$0.14 \pm 0.02^{a,b}$	$0.13 \pm 0.01^{a,b}$	0.13 ± 0.02^{a}	$0.14 \pm 0^{a,b}$	$0.15 \pm 0.02^{a,b}$	$0.15 \pm 0.01^{a,b}$	$0.14 \pm 0.01^{a,b}$	0.17 ± 0.01^{b}
C 16:1 cis9	1.9 ± 0.05^{a}	2.04 ± 0.13^{a}	1.98 ± 0.36^{a}	1.91 ± 0.01^{a}	1.86 ± 0.13^{a}	2.01 ± 0.07^{a}	1.91 ± 0.07^{a}	1.84 ± 0.03^{a}
∑C 16:1 cis	0.48 ± 0.05^{a}	$0.41 \pm 0.16^{a,b}$	$0.59 \pm 0.11^{a,b}$	$0.58 \pm 0.01^{a,b}$	$0.55 \pm 0.05^{a,b}$	$0.63 \pm 0.05^{a,b}$	$0.60 \pm 0.03^{a,b}$	0.64 ± 0.01^{b}
C 17:1 cis	0.14 ± 0.00^{a}	0.16 ± 0.02^{a}	0.19 ± 0.07^{a}	0.19 ± 0.03^{a}	0.20 ± 0.04^{a}	0.23 ± 0.01^{a}	0.20 ± 0.03^{a}	0.21 ± 0.02^{a}
C 17:1 cis izo	0.02 ± 0.01^{a}	0.05 ± 0.02^{a}	0.05 ± 0.03^{a}	0.04 ± 0.00^{a}	0.06 ± 0.01^{a}	0.05 ± 0.00^{a}	0.05 ± 0.01^{a}	0.04 ± 0.00^{a}
∑C 18:1 trans	$2.13 \pm 0.22^{b,c}$	1.12 ± 0.46^{a}	1.32 ± 0.40^{a}	$1.65 \pm 0.02^{a,b}$	1.30 ± 0.14^{a}	$1.73 \pm 0.05^{a,b}$	$1.49 \pm 0.18^{a,b}$	2.84 ± 0.06^{c}
C 18:1 cis9	$25.83 \pm 1.88^{a,b}$	28.80 ± 5.12^{b}	$25.18 \pm 1.73^{a,b}$	$22.90 \pm 0.21^{a,b}$	$25.34 \pm 1.82^{a,b}$	$23.07 \pm 0.17^{a,b}$	22.27 ± 0.25^{a}	$23.17 \pm 0.12^{a,b}$
C 18 1 trans9	$1.58 \pm 0.25^{a,b}$	1.76 ± 0.68^{b}	$1.13 \pm 0.26^{a,b}$	$0.98 \pm 0.01^{a,b}$	$1.01 \pm 0.09^{a,b}$	$1.01 \pm 0.0^{a,b}$	0.89 ± 0.04^{a}	$1.01 \pm 0.01^{a,b}$
∑C 18:1 cis	1.18 ± 0.09^{b}	0.81 ± 0.19^{a}	$0.89 \pm 0.20^{a,b}$	$1.10 \pm 0.01^{a,b}$	0.79 ± 0.06^{a}	1.11 ± 0.02^{a}	$0.99 \pm 0.06^{a,b}$	1.19 ± 0.06^{b}
C 19:1 cis	0.07 ± 0.02^{a}	0.06 ± 0.02^{a}	0.08 ± 0.02^{a}	0.10 ± 0.01^{a}	0.11 ± 0.02^{a}	0.12 ± 0.00^{a}	0.10 ± 0.01^{a}	0.11 ± 0.01^{a}
C 20:1	$0.10 \pm 0.01^{a,b}$	0.07 ± 0.04^{a}	0.08 ± 0.02^{a}	$0.08 \pm 0.01^{a,b}$	0.13 ± 0.01^{b}	$0.12 \pm 0.02^{a,b}$	$0.10 \pm 0.01^{a,b}$	$0.09 \pm 0.01^{a,b}$
C 20:1 cis	0.14 ± 0.07^{a}	0.20 ± 0.15^{a}	0.07 ± 0.07^{a}	0.03 ± 0.01^{a}	0.09 ± 0.04^{a}	0.04 ± 0.01^{a}	0.02 ± 0.01^{a}	0.02 ± 0.01^{a}
C 18:2 trans	0.12 ± 0.01^{b}	0.07 ± 0.02^{a}	$0.08 \pm 0.02^{a,b}$	$0.10 \pm 0.00^{a,b}$	$0.08 \pm 0.02^{a,b}$	$0.11 \pm 0.01^{a,b}$	$0.10 \pm 0.02^{a,b}$	0.11 ± 0.01^{b}
C 18:2 cis9,cis12	$2.46 \pm 0.34^{a,b}$	2.94 ± 1.32^{b}	$1.89 \pm 0.21^{a,b}$	$1.62 \pm 0.02^{a,b}$	$1.74 \pm 0.15^{a,b}$	$1.73 \pm 0.09^{a,b}$	1.40 ± 0.09^{a}	$1.86 \pm 0.02^{a,b}$
C 18:3 cis9,cis12,cis15	$0.61 \pm 0.05^{a,b}$	$0.59 \pm 0.13^{a,b}$	0.52 ± 0.10^{a}	0.49 ± 0.02^{a}	0.83 ± 0.19^{b}	$0.54 \pm 0.05^{a,b}$	0.52 ± 0.05^{a}	$0.69 \pm 0.06^{a,b}$
C 18:2 trans9,trans11	0.42 ± 0.02^{c}	0.29 ± 0.08^{a}	$0.29 \pm 0.05^{a,b}$	$0.37 \pm 0.01^{a,b,c}$	$0.39 \pm 0.02^{a,b,c}$	0.43 ± 0.04^{c}	$0.41 \pm 0.04^{b,c}$	0.81 ± 0.03^{d}

^{a,b,c,d}—values in the same row and marked with the same letters are not statistically significantly different at the $\alpha = 0.05$ level.

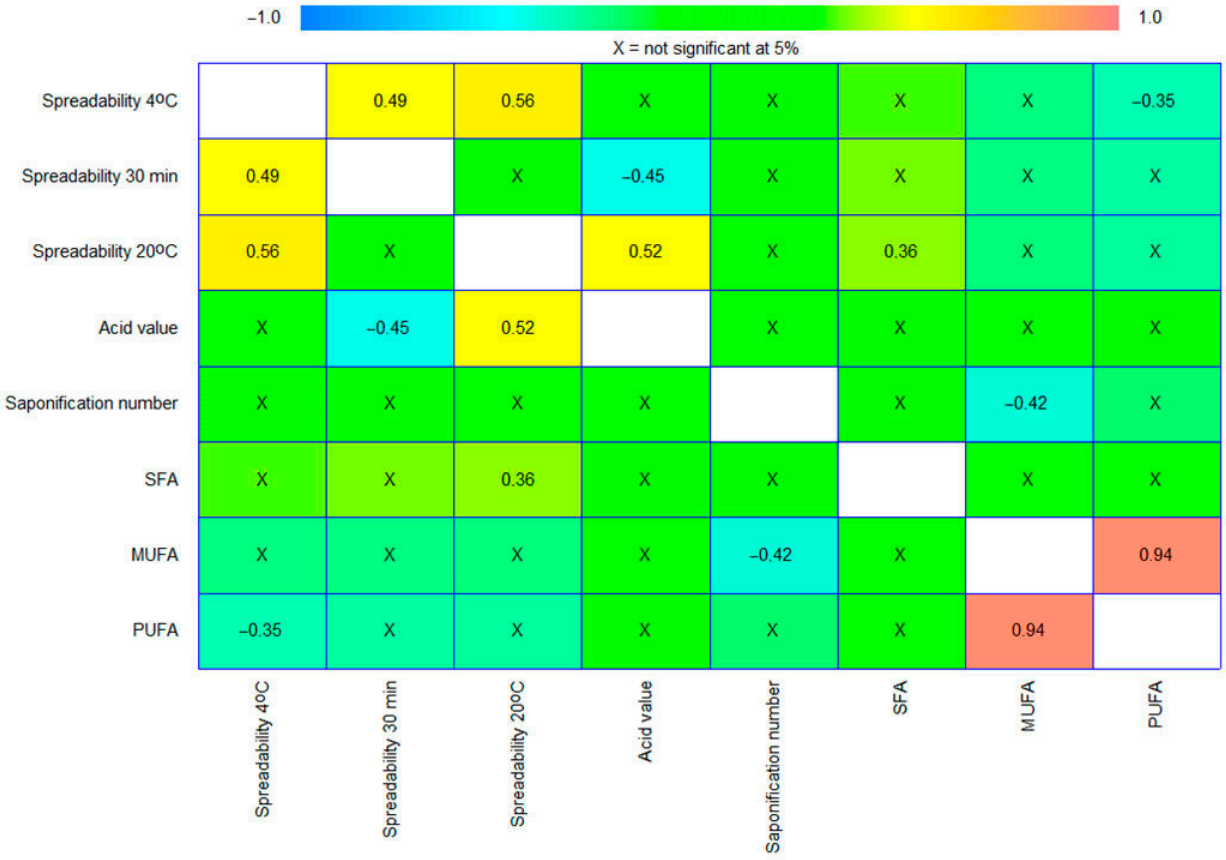
Among the saturated fatty acids (SFA) found in the butter analyzed in this study, one can distinguish between short-chain fatty acids (SCFAs) and medium-chain fatty acids, characteristic of milk fat [71]. Five fatty acids classified as SCFAs were detected in all butter samples analyzed: butyric acid (C4:0), caproic acid (C6:0), caprylic acid (C8:0), capric acid (C10:0) and lauric acid (C12:0). SCFAs are also an important component of milk fat due to their biological properties and health-promoting effects [72,73]. The total saturated fat percentage share present in the butter tested differed significantly between the samples at a significance level of $\alpha = 0.05$.

In all the kinds of butter tested, among the identified MUFA were myristoleic acid (C14:1), isomers of palmitoleic acid (C16:1), and isomers of margaric acid (C17:1). However, oleic acid (C18:1 cis 9) was the predominant fatty acid. The total monounsaturated fatty acid percentage share present in the kinds of butter tested also differed significantly between the butter samples at a significance level of $\alpha = 0.05$.

The predominant polyunsaturated fatty acid (PUFA) in the butter samples of this study was linoleic acid (C18:2: cis 9, cis 12). The total polyunsaturated fatty acid percentage

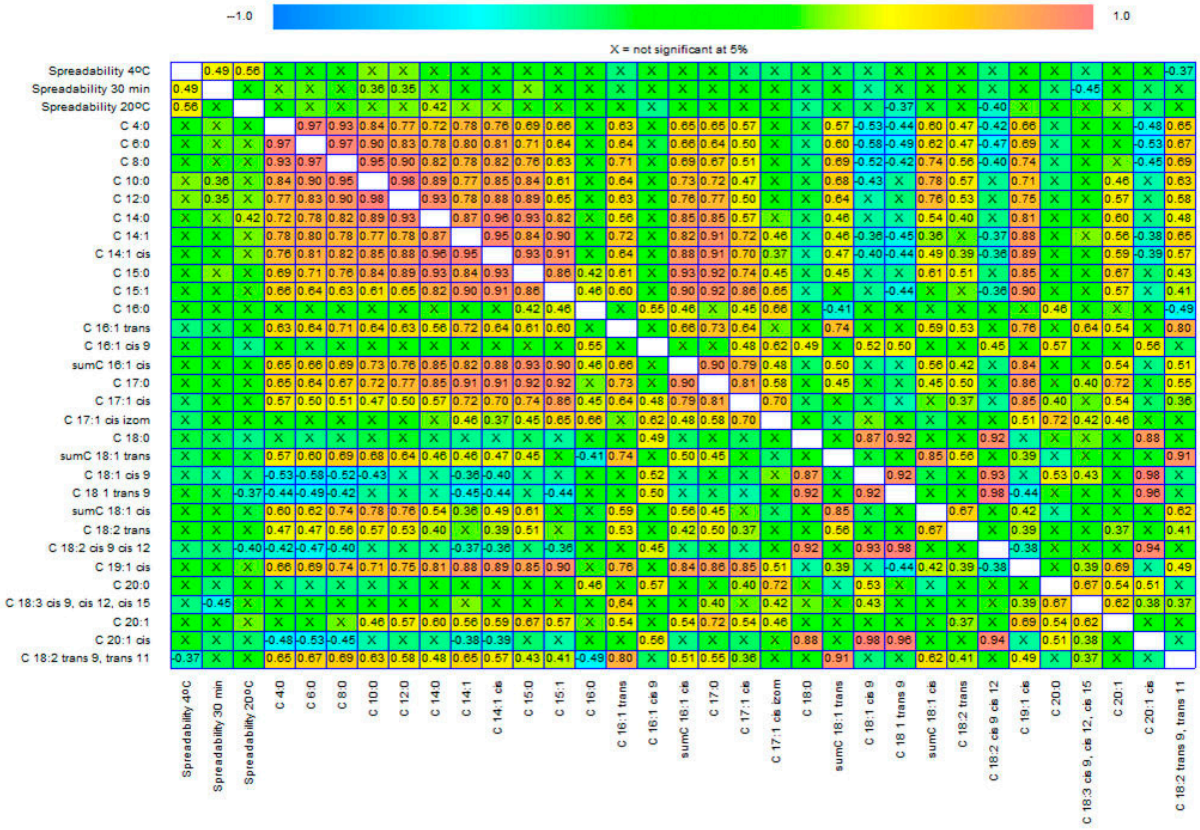
share in the kinds of butter studied also differed significantly between the samples, at a significance level of $\alpha = 0.05$. The fatty acid composition of butter is primarily influenced by the raw material selection, and thus, by the genetics (breed), feeding, and environmental factors (season and region) of the dairy cows that the butter comes from [72,74,75].

The rheological results obtained for the butter samples in this study did not correspond with other chemical data obtained exclusively for the butter samples and were determined by the techniques used. A multivariate analysis of spreadability measurements with acid value, saponification number, or percentage fatty acid content (percentage of saturated fatty acids, MUFA, and PUFA) of the butter samples showed no significant strong relationships between these parameters (Figure 3a). In addition, a multivariate analysis was performed to analyze the correlation between the percentage of each fatty acid identified in the butter samples and the spreadability for the butter samples measured at different temperatures (Figure 3b). In this analysis, no correlation was found between the spreadability of the butter samples and the fatty acid profile.



(**a**)

Figure 3. *Cont.*



(**b**)

Figure 3. Graphs of correlation matrix showing the relationship between spreadability, acid value, saponification number and SFA, MUFA, and PUFA fatty acid profile (**a**), as well as between spreadability, and the fatty acid percentage share (the percentage for each fatty acid determined) of butter samples (**b**) measured at different temperatures (with a confidence level of 95.0%).

Meanwhile, Brunner [76] found that 80% of the differences in butter texture could be explained by differences in the composition of milk fatty acids. However, Jaeck and Pabst [77] found differences in butter texture in herds of cows fed similar diets. Meanwhile, some researchers [43,78,79] have found sufficient variability between cows fed the same feed to produce butter with different textural characteristics and a healthier fatty acid composition. This was supported by a study by Bobe et al. [30], who found that butter samples from milk from cows with a more unsaturated milk fatty acid composition were more spreadable, softer, and less sticky. Thus, the phenotypic variation in milk fatty acid composition among cows fed the same diet is sufficient to produce butter with different textural properties. Meanwhile, Lapčíková et al. [46] found no overall relationship between the composition of milk fat in the samples of butter, spreads, and shortenings available on the Czech market and the values of their textural parameters (i.e., springiness, cohesiveness, and stringiness).

4. Conclusions

The selected structural (spreadability, hardness, adhesive force, and adhesiveness) and physicochemical (water content, water distribution, plasma pH, color, acid value, peroxide number, saponification number, and fatty acid profile) parameters of the butter and butter substitutes tested in this study were correlated with factors such as the type of sample, measuring temperature and physicochemical composition.

The highest spreadability value (i.e., poorer spreadability of the product on the bread) value was obtained for butter samples at 4 °C, and they were significantly inferior to butter substitutes at the same temperature. Butter samples at 4 °C were also characterized by high hardness, which was significantly higher than butter substitutes at the same temperature.

Statistical analysis of the spreadability, hardness, adhesive strength, and adhesiveness results obtained for the butter and butter substitute samples in this study revealed correlations between the textural parameters studied. These were different for the butter and butter substitute samples tested at 4 °C, as well as between hardness and spreadability for samples tested 30 min after removal from the refrigerator. In the case of the butter samples, only very few strong correlations between the spreadability of the products and their other analyzed characteristics were found. In contrast, such correlations abounded for the butter substitute samples.

The butter substitutes had significantly higher water content values than the butter samples. No clear relationship was found between the composition of the butter and butter substitute samples and the values of the textural parameters, including spreadability. The a^*, b^*, and L^* butter color components of the butter substitutes compared with the standard gave surprisingly similar results for each component, despite the differences in chemical composition and textural parameters, and the differences due to different technological processes.

Analysis of a number of variable measurements of the spreadability and acid number, saponification number or percentage of fatty acids in the butter samples, or even the percentage of each fatty acid identified in the butter samples, did not reveal significant strong relationships between these parameters and the spreadability of the butter samples measured at different temperatures.

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