MICROPLASTICS CONTAMINATION OF DAIRY PRODUCTS WITH HIGH-FAT CONTENT - OCCURRENCE AND ASSOCIATED RISKS

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This study aims to investigate the presence of microplastics in dairy products in terms of morphology and chemical composition. Through optical microscopy, microparticles of different colors were identified: ~942 per kg of conventional butter, ~833 per kg of organic butter, and ~800 per kg of sour cream. Micro-FTIR technique through OPUS v.7.5 library has identified in the microparticles' composition as having more natural than synthetic compounds. The correlations obtained by the statistical approach could serve or act as an incentive for milk processors to try finding the source of contamination.

Keywords: microplastic, microparticles, dairy products, optical microscopy, micro-FTIR, health risk, statistical approach.

1. Introduction

The evidence regarding the presence of microplastics (MPs) in humans was recently revealed, these being included in the category of emerging contaminants (ECs) [1-4]. Due to the chemical composition and small sizes (i.e., less than 5 mm), MPs are slowly degraded and easily inhaled or/and ingested by humans [5, 6]. The scientific concerns show that the first pathway of human exposure to MPs is ingestion, mainly through the consumption of contaminated food and water [4, 7]. From a chemical composition point of view, it was revealed that their complex composition acts as a vector for different chemical contaminants adsorbed on their surfaces such as pyrene [8], phthalates and bisphenol A [9, 10], heavy metals [11], polycyclic aromatic hydrocarbons (PAHs) [12], dioxins and dioxin-like compounds (DLCs) [4], pharmaceuticals [13], and serve as carriers of pathogenic microbes, therefore threats to humans and aquatic ecosystems. Usually, the most commonly known morphological shapes of microplastics are fiber, fragment, pellet, film, and foam of different colors [14]. The most predominant types of MPs are fibers

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(synthetic or mixture), and several studies showed that they are easily released from clothes/protection or work equipment during different processes [15-17]. It was previously mentioned that risk awareness is important but can this be enough to prevent the MPs from reaching into our organism? Well, a leading actor in the quality of assured life is given by the food industry, the raw material as well as manufacturing processes or the packaging known of being the final step before distribution it may increase or decrease the contamination by inhalation/ingestion. The storage environment and temperature may also affect food quality; therefore, the fragmentation of MPs and passing them into food depends on the type of polymer structure as well as climatic/microclimatic conditions. Apart from chemical composition, the occurrence and abundance of microplastics represent valuable indicators, but unfortunately, less investigated when assessing contamination levels with MPs of foods, particularly dairy products. On the other hand, taking into consideration the chemical risk of MPs from animal feeds or pollution levels with nano/microplastics can be difficult to quantify the potential human health risk of them. Packaging made of plastics is an important source in terms of the occurrence of MPs [18, 19]. Briefly, the polymeric materials known as plastics are achieved by a complex polymerization process of one kind or different monomers, and various additives are incorporated during production. The release of plastic additives from the packaging by leaching represents a huge issue worldwide [20]. Li et al. [21] revealed that processed foods are more likely to be contaminated with microplastics than non-processed foods. The expected answer for the aforementioned is linked to the manufacturing and packaging processes [21].

Currently, to characterize MPs as too small to be chemically analyzed by conventional techniques, a series of analytical methods were developed [22-25]. In this regard, for a precise investigation of micro/nanoparticles in terms of chemical composition can be used micro-Fourier transform infrared spectroscopy (micro-FTIR), micro-Raman spectroscopy, thermal analysis (differential scanning calorimetry (DSC), and thermal desorption gas chromatography-mass spectrometry (TGC-MS)) [26-31]. Since the development of mercury-cadmium telluride (MCT) detector technology, the micro-FTIR technique has become preferential in microplastic characterization [31]. This technique takes over IR signals at high spatial resolution (beam size < 5 μm) allowing the characterization of compositionally complex MPs samples. In addition, what is very important and represents an advantage compared to the conventional FTIR technique, or compared to other techniques, is the fact that micro-FTIR allows in situ, non-destructive, and non-invasive analysis without the demand of other stages in sample preparation. On the other hand, the microscopy technique provides information regarding the microparticles' morphology mainly about shape and size. Imaging software and microscopy allow the determination of the average particle size as well as the microparticle number. Currently, several microscopy techniques, such as atomic force microscopy (AFM), fluorescence microscopy, scanning electron microscopy

(SEM), phase contrast microscopy, and stereo microscopy, have provided the first data in terms of the morphology of microparticles/microplastics from different samples, including dairy products [22, 23, 32]. One of the main problems in detecting microplastics in dairy products may be, on the one hand, the complex composition of the organic matrix of milk and dairy products, particularly the high-fat contents, and on the other hand, the fact that isolation of MPs must be achieved by a method that involves the using of safe, efficient, and eco-friendly reagents [3, 22, 23].

The current study highlighted that MPs from dairy products were investigated for the first time, in terms of morphology and chemical composition, to give the scientific world the possibility to take cause-effect research to another level of knowledge related to the safety of human life. The study of MPs morphology, in conjunction with analytical investigations regarding the chemical composition of MPs, as well as with the risk effect of compositionally dairy products with high-fat content (i.e., butter and sour cream) by identifying microplastic particles of small/different sizes on human health, is an assumed objective of the present research. The findings from this study regarding the occurrence, isolation, and characterization of MPs, based on the correlations established by the statistical approach, surely will be helpful to producers of milk and dairy products.

2. Materials and Methods

2.1. Materials and reagents

All used reagents (Merck, KGaA, Germany, and Sigma-Aldrich, Saint Louis, USA) were of high purity (analytical grade). In addition, the liquid ones were filtered before use to avoid accidental contamination of the samples (including ultrapure water) or possible interferences in chemical investigations.

2.2. Sampling and sample preparation

To investigate the occurrence and type of MPs in eighteen dairy products with high-fat content, i.e., eleven popular butter brands (conventional/organic, Tables 1 and 2) and seven Romanian sour cream brands (Table 3), were sampled in the spring of 2023. As a complement, for this study, all the above-mentioned products were purchased exclusively from Romanian stores. The fat content of butter varied between 80-82% and of the sour cream between 20-25%. The samples taken were coded, characterized in terms of nutritional properties as stated on the package label (Tables 1-3), and stored in the refrigerator (2-6°C) until analysis. The selection criteria for the two categories of dairy products were: (1) high-fat content, brand popularity among consumers, packaging type, affordable price among consumers, the share of sales, and for butter the safety criteria regarding the international classification of food quality, i.e. organic or conventional, were also included. Considering the contamination risk that may occur it was decided that all sample preparation (including the sampling stage) to be performed under strict regulation of the laboratory environment where

no kind of airborne particles may interfere. Therefore, these activities were carried out in a cleanroom (according to ISO 14644-1:2015, Class 1000 - ISO6) at the Institute of Multidisciplinary Research for Science and Technology of Valahia University of Targoviste.

Table 1

General presentation of conventional butter samples

Sample code	Nutritional value / 100 g butter						Fat content [%]	Packaging type
	Energy value		Saturated fatty acids [g]	Carbohydrates [g]	Protein [g]	Salt [g]		
	[kJ]	[kcal]						
U_1	3060	744	55	1.0	0.5	0.02	82	Waxed paper
U_2	3092	738	50	0.4	0.2	0.10	82	Waxed paper
U_3	2980	725	49	0.7	0.5	0.20	80	Glass bottle
U_4	3054	743	49	0.4	0.8	0.05	82	Waxed paper
U_5	3051	742	53	0.1	0.9	0.01	82	Waxed paper
U_6	3073	739	49	0.1	0.1	0.06	82	Waxed paper
U_7	2985	726	56	0.5	0.7	0.02	80	Waxed paper
U_8	2977	724	48	0.3	0.7	0.05	80	Waxed paper

Table 2

General presentation of organic butter samples

Sample code	Nutritional value / 100 g butter						Fat content [%]	Packaging type
	Energy value		Saturated fatty acids [g]	Carbohydrates [g]	Protein [g]	Salt [g]		
	[kJ]	[kcal]						
U_6B	3073	739	49	0.1	0.1	0.06	82	Waxed paper
U_7B	3056	743	55	0.6	0.7	0.04	82	Waxed paper
U_8B	2977	724	48	0.3	0.7	0.05	80	Waxed paper

Table 3

General presentation of sour cream samples

Sample code	Nutritional value / 100 g sour cream						Fat content [%]	Packaging type
	Energy value		Saturated fatty acids [g]	Carbohydrates [g]	Protein [g]	Salt [g]		
	[kJ]	[kcal]						
S_1	849	203	13	2.9	2.8	0.10	20	Plastic bottle
S_2	1020	247	15	3.3	2.3	0.06	25	Plastic bottle
S_3	828	200	13	2.9	2.8	0.10	20	Plastic bottle

Sample code	Nutritional value / 100 g sour cream						Fat content [%]	Packaging type
	Energy value		Saturated fatty acids [g]	Carbohydrates [g]	Protein [g]	Salt [g]		
	[kJ]	[kcal]						
S_4	832	202	12	2.5	2.9	0.20	20	Plastic bottle
S_5	842	204	14	3.2	2.8	0.10	20	Plastic bottle
S_6	996	242	16	2.0	2.2	0.06	25	Plastic bottle
S_7	822	199	12	2.2	2.6	0.10	20	Plastic bottle

Furthermore, the non-textile lab coats (i.e., cotton) and particle-free nitrile gloves were worn all this time while the sleeves of the lab coats were in advance inserted and secured inside the gloves. The glass vessels were sequentially rinsed at least three times with anhydrous ethanol and ultrapure water. Samples were carefully handled and were covered with aluminum foil, till the isolation process. The protocol of MPs isolation for each sample was repeated three times and the used data was obtained by averaging the three repetitions.

2.2.1. Microplastics isolation protocol

The protocol for the isolation of MPs from dairy product samples with high-fat content (i.e., butter and sour cream) was achieved according to the patent application [23]. Firstly, the vessels/materials used for MPs isolation and further investigations (e.g., Erlenmeyer beakers, graduated cylinders, pipettes, spatulas, and Petri dishes) were properly cleaned with anhydrous ethanol and ultrapure water, then sterilized at a temperature of 100°C for 48 hours in Venticell® oven (BMT Medical Technology, Brno, Czech Republic). The MPs isolation protocol requires the following steps: A. pretreatment of samples with reagents for fat, protein, and carbohydrate digestion; B. digestion; C. mixtures filtering and MPs isolation. For the MPs isolation from the butter samples regardless of the fat content, 8 g of sample were mixed with 4 g of sodium dodecyl sulfate, 1 g of sodium hydroxide, and 500 mL of distilled water. The resulting mixture was homogenized for 10 minutes at 150 rpm using IKA® shaker RT 5 (IKA, Staufen, Germany); thus, the obtained sample was ultrasonicated for 20 minutes at 30°C by using ultrasonic bath type VWR® Ultrasonic Cleaner USC – TH (VWR International, Radnor, USA). Filtration was carried out on a cellulose membrane with pore size 12-15 µm using a three-position stainless steel filtration system (Labbox Labware, Barcelona, Spain) connected to a vacuum pump at 18 L/min flow rate of on cellulose filters with 12-15 µm porosity, VWR® Grade 413 (VWR International, Radnor, United States of America). The mixture was kept in a water bath at a temperature of 60°C until complete filtration [23]. Similar to the previously described protocol (i.e.,

butter), for the MPs isolation from the sour cream samples, 5 g were mixed with 0.5 g of sodium dodecyl sulfate, 0.6 g of sodium hydroxide, and 500 mL of distilled water. The mixture was homogenized for 10 minutes at 150 rpm, then was ultrasonicated for 20 minutes at 30°C, and finally, was filtrated [23].

2.3. Analytical techniques

2.3.1. Optical microscopy

Microparticles found on the surface of the filters were identified using optical microscopy. The magnification factors used were 25X, 32X, 40X, and 50X, depending on the particle size and to highlight certain aspects of interest. In this sense, the Stemi 2000c microscope coupled with the Axiocam 105 digital video camera (Carl Zeiss) was used as well as Zen software for the image acquisitions. Optical microscopy was important for the identification and physical characterization of microparticles (shape, color, texture).

2.3.2. Micro-Fourier-Transform Infrared Spectroscopy

Fourier Transform Infrared Spectroscopy (FTIR) is a non-destructive and non-invasive analytical technique that provides important information in terms of the molecular structure of organic components. In the past few decades, this method has been used extensively for the chemical characterization of food samples, together with Raman spectroscopy, under the umbrella of vibrational spectroscopies. Currently, it was demonstrated that the employment of both high-resolution micro-FTIR and micro-Raman imaging enables visualization and mapping of the MPs distributions on a micrometer scale in food samples, leading to an advanced understanding in terms of chemical composition. These features, barely accessible with other analytical techniques, may provide fundamental information in terms of MPs occurrence in food samples, as well. In this research, the micro-FTIR technique was performed by Vertex 80 FTIR spectrometer (Bruker, United States) equipped with a Bruker Hyperion 2000 microscope. The Hyperion 2000 FTIR system uses an MCT detector (mercury cadmium telluride) cooled with liquid nitrogen. The chosen method for the analysis of dairy product samples was attenuated total reflection (ATR), which allows the direct measurement of samples. The ATR objective (20x), is equipped with a Ge crystal (refraction index 4). Micro-FTIR analysis was performed in transmittance mode, 600–4000 cm^{-1} spectral range, with 32 scans per sample. The obtained IR spectra were compared with the database on the OPUS v.7.5 software, and a polymer type was considered acceptable in the sample when the match with standard spectra was greater than 70% according to several studies [33-36].

2.4. Data analysis

2.4.1. Pollution Load Index (PLI)

The pollution load index (PLI) was described by Tomlinson et al. [37] as:

$$PLI = \sqrt{\frac{C_i}{C_0}} \quad (1)$$

where: C_i represents the content of MPs (expressed as n/kg) determined in butter and sour cream samples and C_0 represents the minimum reported average concentration of microparticles in processed food [3, 38] (C_0 = 1.68 n·kg^{-1}). Lin et al. [3] established hazard levels according to PLI values (Table 4).

Table 4

The risk level criteria for pollution load index of MPs [3]

PLI	Hazard level	Color assigned to the Hazard level
< 10	Very low hazard	
10–20	Low hazard	
20–30	Medium hazard	
> 30	High hazard	
-	Very high hazard	

2.4.2. Daily Microparticles Intake

Daily microparticles intake (DIM) can be calculated using the following equation as described by Lin et al. [3]:

$$DIM = \frac{C_i \cdot I_r \cdot E_f \cdot E_d}{B_w \cdot A_t} \ [\mathrm{n/(kg \cdot d)}] \quad (2)$$

where: C_i represents the microparticles content (expressed as n/kg) determined in the butter and sour cream samples; I_r represents the degree of ingestion (expressed in kg/d); E_f represents the exposure rate (expressed as d/y); E_d represents the exposure period (expressed as y); B_w represents body weight (expressed in kg); A_t represents the average exposure time (expressed as d). The values of the mentioned parameters are presented in Table 5.

Table 5

The values of parameters for DIM calculation

		I_r [kg/d]	E_f [d/y]	E_d [y]	B_w [kg]	A_t [d]
Butter	Adults	0.013	365	70	70	25550
	Children	0.010	365	10	14	3650
Sour cream	Adults	0.026	365	70	70	25550
	Children	0.020	365	10	14	3650

2.5. Statistical analysis

IBM SPSS Statistics for Windows software (Version 21.0, IBM Corp., United States of America) was used for the statistical processing of the study data. Significant correlations were analyzed using the Pearson coefficient, which is used as an indicator of linear dependence between two or more variables, determining the dependence relationship between them, which can take values between −1 and +1 [39]. A regression analysis was performed to predict the links between the variables. As a methodology, the dependent variable is distributed on the *y*-axis, while the independent variable is distributed on the *x*-axis. To determine the similarities between samples and/or fibers, a cluster analysis was performed.

3. Results and Discussion

The prevalence of microparticles in food chains and their long-term impact pose a significant threat to human health. Given their ubiquitous nature, rapid and reliable characterization is critical [40]. The large number of particles that must be analyzed in samples creates significant challenges in this research field.

3.1. Optical microscopy

The first analytical technique for identifying the impurities from dairy products is optical microscopy (OM). It represents the preliminary step for highlighting the presence of microparticles on the filter surface. It is important to note that this technique aims to highlight the presence of particles but does not reveal the existence of MPs, for which the chemical and structural analysis will be necessary, therefore it was decided to address at this point to those impurities as microparticles. During the investigations, some microparticles were detected on the cellulose filters and were characterized and classified based on some aspects (i.e. color, and shape). The range of colors (black, blue, red, brown, yellow, gray, etc.) and shapes (irregular, oval, rhomboidal, film, and fiber) (Figures 1-3) were highlighted and helped to determine the total number of microparticles (represented as microparticles/kg) (Tables 6-8).

Table 6

Optical microscopy on conventional butter samples

Sample code	Color and number of microparticles								Total [micro-particles/kg]
	Black	Blue	Red	Green	Brown	Grey	Yellow	Purple	
U_1	750	250	125	*nd**	250	*nd**	125	*nd**	1500
U_2	125	125	*nd**	125	*nd**	*nd**	*nd**	*nd**	375
U_3	1125	*nd**	125	*nd**	*nd**	*nd**	*nd**	*nd**	1250
U_4	*nd**	125	125	*nd**	125	*nd**	*nd**	*nd**	375
U_5	625	250	*nd**	*nd**	*nd**	*nd**	125	*nd**	1000
U_6	250	125	125	*nd**	*nd**	375	*nd**	*nd**	875
U_7	375	500	*nd**	250	*nd**	*nd**	250	125	1500
U_8	375	250	125	*nd**	*nd**	125	125	*nd**	1000

nd - unidentified*

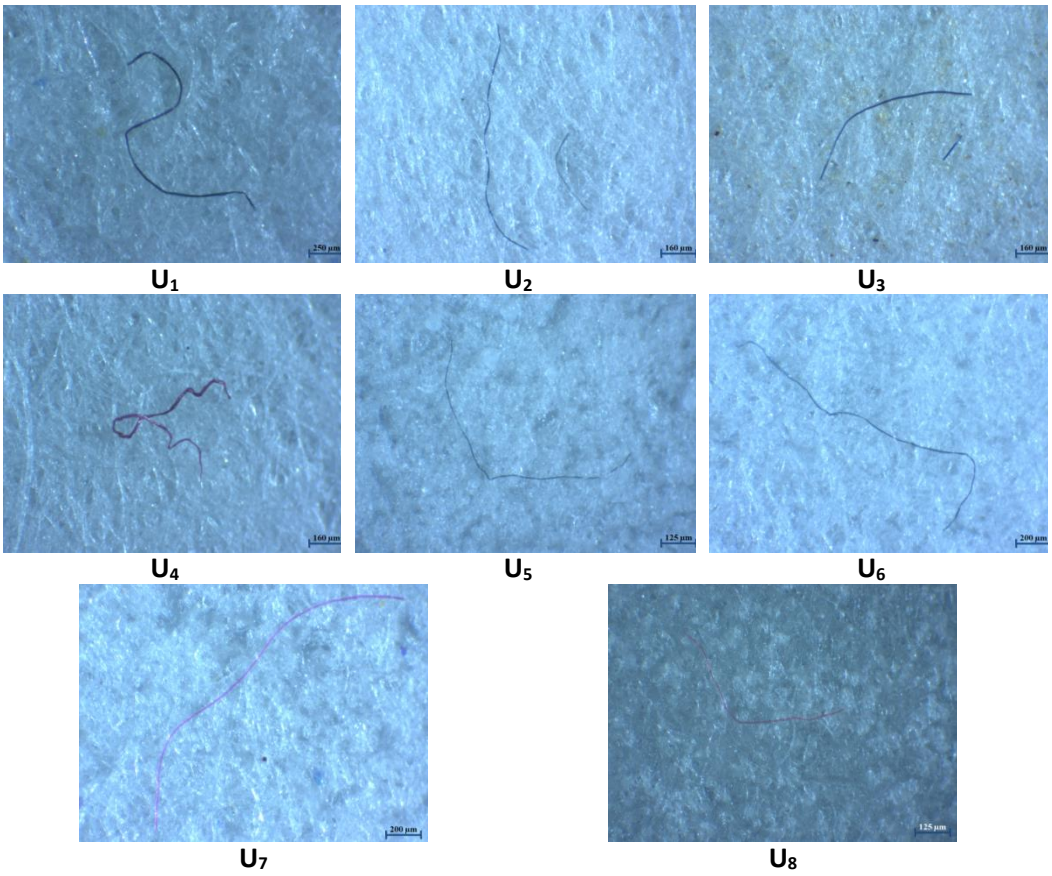

Figure 1. Representative microparticles in conventional butter samples.

Table 7

Optical microscopy on organic butter samples

Sample code	Color and number of microparticles							Total [micro-particles/kg]
	Black	Blue	Red	Brown	Grey	Yellow	Purple	
U6B	750	*nd**	125	*nd**	*nd**	125	*nd**	1000
U7B	125	375	*nd**	*nd**	125	*nd**	*nd**	625
U8B	375	125	*nd**	125	*nd**	125	125	875

nd - unidentified*

Figure 2. Representative microparticles in organic butter samples.

Table 8

Optical microscopy on sour cream samples

Sample code	Color and number of microparticles						Total [micro-particles/kg]
	Black	Blue	Green	Grey	Yellow	Purple	
S_1	400	*nd**	*nd**	*nd**	*nd**	*nd**	400
S_2	600	*nd**	*nd**	200	*nd**	*nd**	800
S_3	600	200	*nd**	*nd**	200	*nd**	1000
S_4	1000	*nd**	200	*nd**	*nd**	*nd**	1200
S_5	600	200	*nd**	200	200	*nd**	1200
S_6	600	*nd**	*nd**	*nd**	*nd**	*nd**	600
S_7	200	*nd**	*nd**	*nd**	*nd**	200	400

*nd** - *unidentified*

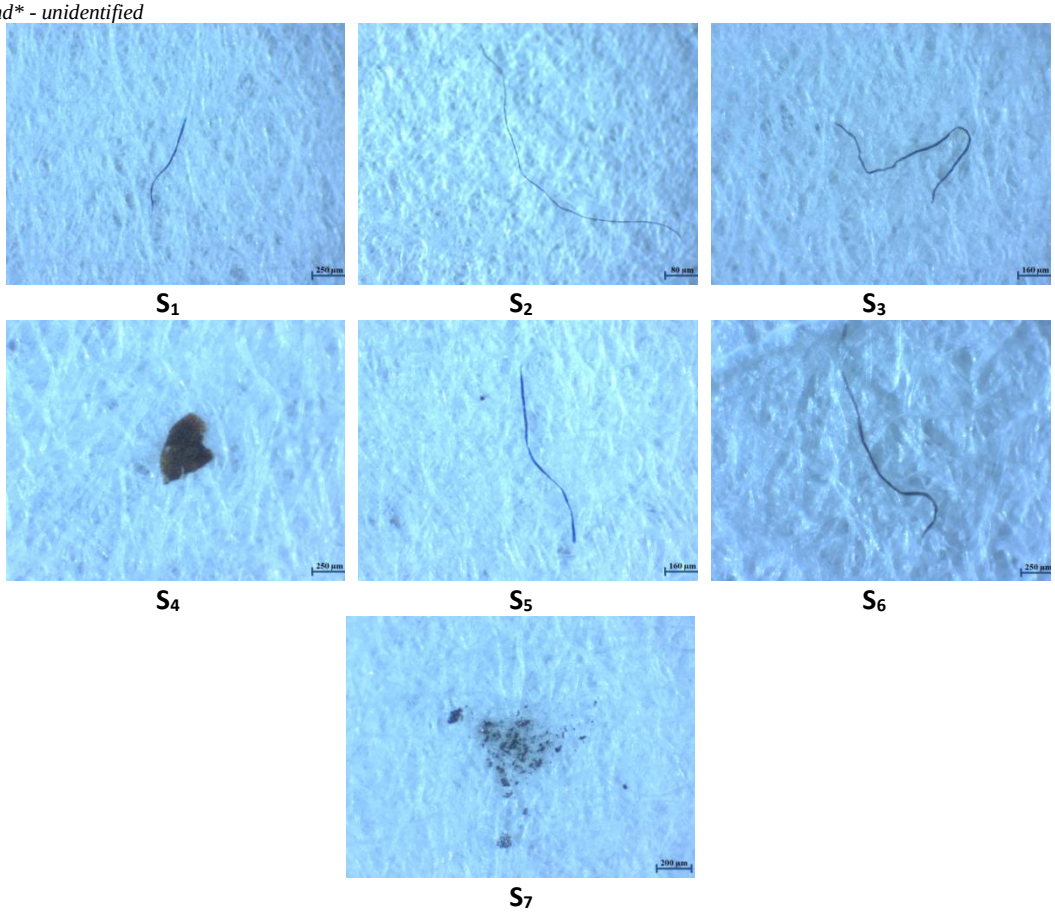

Figure 3. Representative microparticles in sour cream samples.

It is important to note that the investigations were carried out not only on the filters used in the separation process, but also on empty filters, or those used to filter the reagents, in the case of the latter the result was zero. The total number of microparticles identified in analyzed samples (conventional or organic butter and conventional sour cream) is presented in Figure 4. The highest number of microparticles was determined in the conventional butter where eight samples were

analyzed, followed by the conventional sour cream and organic butter. The number of microparticles (split by color) identified in the butter (conventional or organic) and sour cream samples are presented in Figures 5, 7, and 9.

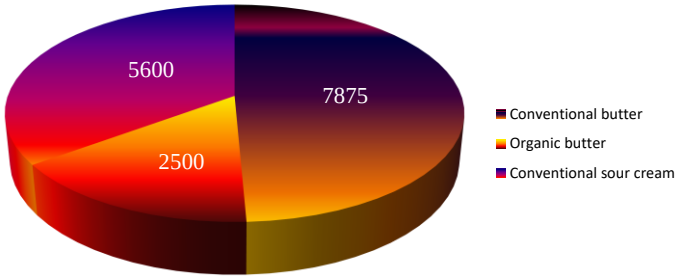

Figure 4. Total microparticles identified in butter and sour cream samples

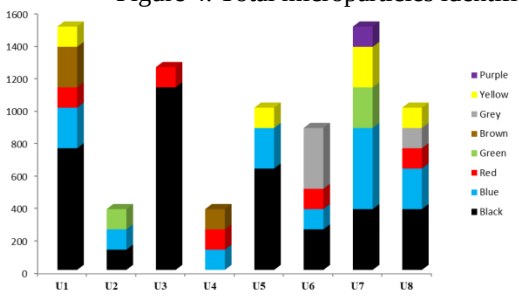

Figure 5. Plot of microparticles colors identified in conventional butter samples.

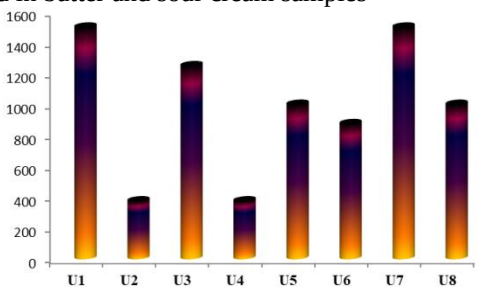

Figure 6. The total number of microparticles identified in conventional butter samples.

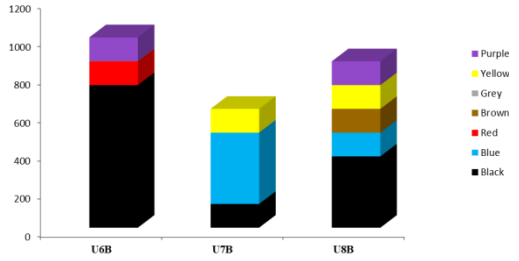

Figure 7. Plot of microparticles colors identified in organic butter samples.

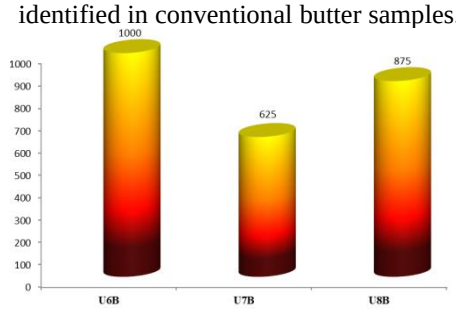

Figure 8. The total number of microparticles identified in organic butter samples.

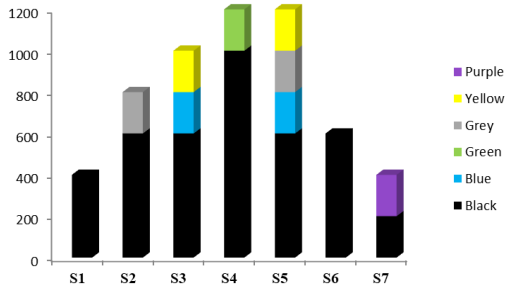

Figure 9. Plot of microparticles colors identified in conventional sour cream samples.

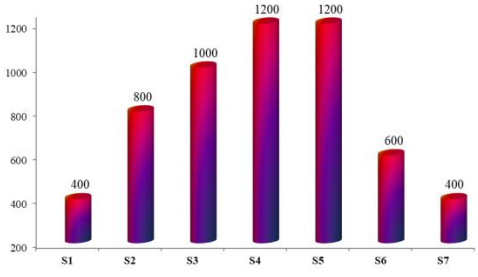

Figure 10. The total number of microparticles identified in conventional sour cream samples.

In the conventional butter samples eight colors were identified (black, blue, red, green, brown, grey, yellow, and purple), seven colors in the organic butter samples (black, blue, red, brown, grey, yellow, and purple) and six colors in the conventional sour cream samples (black, blue, green, grey, yellow, and violet). Figures 6, 8 and 10 show the total number of microparticles identified in the analyzed butter and sour cream samples.

The predominant color of microparticles/filters is black, with a proportion of 46% for conventional butter, 50% for organic butter, and 71% for sour cream. Furthermore, it may be added that in the conventional U_3 butter sample, 1125 microparticles/kg were identified, 750 black microparticles/kg in the U_6B organic butter sample, and the sour cream, the blackest particles (1000 microparticles/kg) were found in the S_4 sample. The second predominant color was blue, thus, in the conventional butter samples the percentage concentration was 21%, in the organic butter sample 20%, and in the cream 7%. The maximum value of blue microparticles for each diary product category was as follows: U_7 with 500 microparticles/kg, U_7B with 375 microparticles/kg, S_3 and S_5 samples with 200 microparticles/kg. Other colors identified in the analyzed samples are: red – 8% in conventional butter samples and 5% in organic butter samples, green – 5% in conventional butter samples, and 4% in conventional sour cream samples. Brown fibers were identified in conventional and organic butter (5%) and by a very small percentage of other colors like grey, yellow, and purple in all three categories.

3.2. Micro-Fourier-Transform Infrared Spectroscopy

An in-depth chemical and morphological characterization of microparticles can be achieved by micro-FTIR imaging. Although micro-FTIR spectroscopy requires many more on-point investigations to obtain a concluding result, it is still one of the most effective ways to identify the sample matrices based on vibrational frequency. After the spectrum background correction was made, all analyzed spectra showed different weak, medium, and strong vibrational frequencies. In spectroscopic terms, the weak, medium, and strong peaks (intensity), together with the wave numbers, allowed the assignment of the functional groups in the organic compounds [30]. Tables 9, 10, and 11 show the identifications of the samples analyzed according to the spectral library. The measurement sequence was carried out for each microparticle after the FTIR analysis (Figure 11).

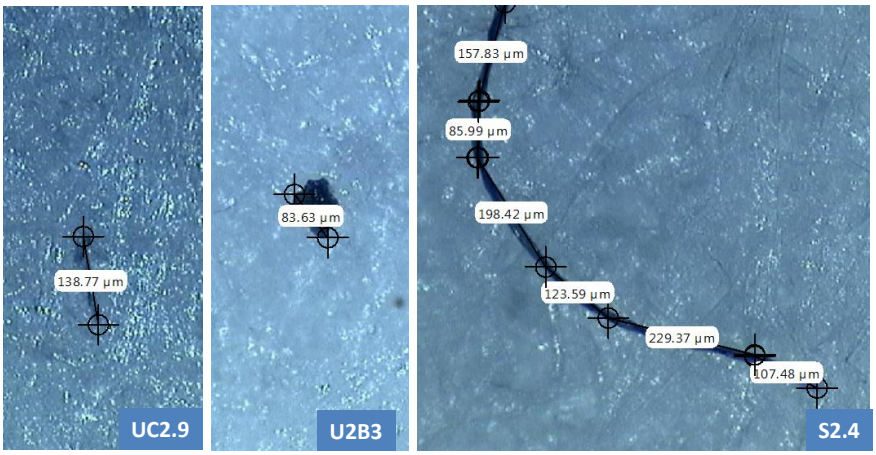

Figure 11. Microparticle measurements sequence.

Table 9

Identification of microparticles according to the OPUS v.7.5 library of FTIR from conventional butter samples

Sample	Sample Code	Identification according to the OPUS v.7.5 library						Microparticles composition	Characterization	
		Cotton	Acrylic	Nylon	Elastane	Cellulose	Wool		Shape	Size (LxW) [µm]
U_1	U1.1	✓		✓		✓		mixture 65:25:10	Irregular	43.69 (L)
	U1.2	✓	✓					mixture 73:21	Irregular	170.62 x 69.14
	U1.3	✓					✓	mixture 70:30	Fiber	344.83 (L)
	U1.4	✓					✓	mixture 70:30	Fiber	>1443.57 (L)
	U1.5	✓					✓	mixture 70:30	Film	256.88 (L)
	U1.6	✓					✓	mixture 70:30	Fiber	≈788.25 (L)
	U1.7	✓						100% natural	Fiber	>704.82 (L)
	U1.8	✓		✓		✓		mixture 65:25:10	Fiber	>1022.95 (L)
	U1.9	✓	✓					mixture 79:21	Fiber	303.12 (L)
U_2	U2.1					✓		100% natural	Irregular	167.23 x 76
	U2.2	✓		✓		✓		mixture 65:25:10%	Oval	163.06 x 132.44
	U2.3	✓	✓					mixture 79:21	Irregular	85.30 x 52.79
	U2.4	✓	✓	✓		✓		mixture 70:6:11:13	Square	41.03 x 41.03
	U2.5	✓	✓					mixture 79:21	Film	457.49 x 168.45
	U2.6	✓						100% natural	Irregular	90.92 x 73.55
U_3	U3.1	✓	✓			✓		mixture 60:20:20	Film	137.97 (L)
	U3.2					✓		100% natural	Film	361.4 (L)
	U3.3	✓	✓					mixture 79:21	Fiber	439.17 (L)
	U3.4	✓	✓					mixture 60:40	Fiber	261.52 (L)
	U3.5	✓	✓					mixture 79:21	Fiber	>929.14 (L)
	U3.6	✓						100% natural	Fiber	>1434.98 (L)
	U3.7	✓	✓	✓		✓		mixture 70:6:11:13	Fiber	870.87 (L)
	U3.8	✓	✓					mixture 79:21	Fiber	≈726.02 (L)
	U3.9	✓	✓	✓		✓		mixture 70:6:11:13	Fiber	388.89 (L)
	U3.10	✓					✓	mixture 70:30	Fiber	639.08 (L)
	U3.11	✓	✓					mixture 79:21	Fiber	221.6 (L)
	U3.12					✓		100% natural	Fiber	341.03 (L)
U_4	U4.1					✓		100% natural	Irregular	immeasurable
	U4.2	✓			✓			mixture 92:8	Irregular	122.74 x 58.91
	U4.3					✓		100% natural	Irregular	125.74 x 52.79
	U4.4	✓	✓	✓		✓		mixture 70:6:11:13	Irregular	53.65 (L)
	U4.5					✓		100% natural	Irregular	93.22 x 70.80
	U4.6					✓		100% natural	Fiber	225.01 (L)
	U4.7	✓	✓					mixture 60:40	Fiber	184.18 (L)
U_5	U5.1					✓		100% natural	Irregular	225.51 x 262.42
	U5.2					✓		100% natural	Irregular	673.90 x 341.51
	U5.3	✓			✓			mixture 92:8	Triangle	641.23 x 577.04

Sample	Sample Code	Identification according to the OPUS v.7.5 library						Microparticles composition	Characterization	
		Cotton	Acrylic	Nylon	Elastane	Cellulose	Wool		Shape	Size (LxW) [µm]
	U5.4	✓	✓					mixture 79:21	Irregular	64.51 (L)
	U5.5	✓	✓					mixture 79:21	Fiber	308.98 (L)
	U5.6					✓		100% natural	Fiber	337.46 (L)
U_6	U6.1	✓	✓	✓		✓		mixture 70:6:11:13	Irregular	123.04 x 63.01
	U6.2					✓		100% natural	Fiber	>682.83 (L)
	U6.3					✓		100% natural	Fiber	>736.19 (L)
	U6.4					✓		100% natural	Fiber	>696.68 (L)
	U6.5	✓	✓					mixture 60:40	Fiber	>635.21 (L)
	U6.6	✓	✓	✓		✓		mixture 70:6:11:13	Fiber	>762.7 (L)
	U6.7	✓	✓	✓		✓		mixture 70:6:11:13	Fiber	>758.04 (L)
U_7	U7.1					✓		100% natural	Square	165.89 x 150.66
	U7.2	✓	✓					mixture 60:40	Irregular	129.09 x 93.99
	U7.3	✓	✓					mixture 60:40	Irregular	207.12 x 81.27
	U7.4	✓	✓					mixture 60:40	Irregular	116.09 x 87.82
	U7.5	✓	✓	✓		✓		mixture 70:6:11:13	Irregular	283.84 x 70.74
	U7.6	✓	✓					mixture 60:40	Irregular	151.28 x 138.36
	U7.7	✓	✓					mixture 60:40	Fiber	304.16 (L)
	U7.8	✓	✓					mixture 60:40	Fiber	>980.13 (L)
	U7.9	✓		✓	✓	✓		mixture 33:16:2:49	Fiber	>783.71(L)
	U7.10					✓		100% natural	Fiber	>814.03(L)
	U7.11					✓		100% natural	Fiber	>827.92(L)
	U7.12					✓		100% natural	Fiber	260.52 (L)
U_8	U8.1	✓	✓	✓		✓		mixture 70:6:11:13	Trapezoid	206.42 x 102.58
	U8.2	✓	✓					mixture 79:21	Irregular	132.75 x 74.79
	U8.3	✓	✓					mixture 79:21	Diamond	166.50 x 137.57
	U8.4					✓		100% natural	Irregular	205.16 x 83.31
	U8.5	✓						100% natural	Film	immeasurable
	U8.6	✓	✓	✓		✓		mixture 70:6:11:13	Fiber	>556.14 (L)
	U8.7	✓	✓	✓		✓		mixture 70:6:11:13	Fiber	147.70 (L)
	U8.8	✓	✓					mixture 60:40	Fiber	198.85 (L)
	U8.9	✓	✓					mixture 50:50	Film	138.77 (L)

A total of 133 samples (microparticles) were analyzed by the micro-FTIR technique for the three categories of dairy products (conventional and organic butter and sour cream) and were characterized from the point of view of the microparticles' nature: 65 natural and 68 synthetic microparticles (Figures 12, 13, and 14). It is considered as natural microparticles those microparticles that present a 100% natural composition or a mixture of natural fibers (e.g., cotton, wool, raffia, cellulose, etc.) according to Garside and Wyeth [36] (Tables 9, 10, and 11). Synthetic microparticles are those microparticles that have in their composition a mixture of natural and polymeric compounds (acrylic, nylon, or elastane).

Table 10

Identification of microparticles according to the OPUS v.7.5 library of FTIR from organic butter samples

Sample	Sample Code	Identification according to the OPUS v.7.5 library				Microparticle composition	Characterization	
		Cotton	Acrylic	Cellulose	Flax		Shape	Size (LxW) [µm]
U_6B	U6B1	✓	✓			mixture 79:21	Oval	170.62 x 71.12
	U6B2	✓	✓		✓	mixture 79:21	Film	143.12 (L)
	U6B3	✓	✓			mixture 79:21	Irregular	≈117.82 x 64.95
	U6B4	✓	✓			mixture 79:21	Rectangular	103.71 x 56.80
	U6B5	✓	✓			mixture 79:21	Irregular	144.35 x 113.60
	U6B6	✓	✓		✓	mixture 60:40	Irregular	99.52 x 108.52
	U6B7	✓	✓			mixture 79:21	Fiber	282.51 (L)
	U6B8	✓				100% natural	Fiber	159.61 (L)

Sample	Sample Code	Identification according to the OPUS v.7.5 library				Microparticle composition	Characterization	
		Cotton	Acrylic	Cellulose	Flax		Shape	Size (LxW) [µm]
	U6B9	✓	✓			mixture 79:21	Fiber	527.48 (L)
	U6B10	✓	✓			mixture 50:50	Fiber	>945.68 (L)
U_7B	U7B1	✓	✓			mixture 79:21	Sphere	72.29 (D)
	U7B2	✓	✓			mixture 79:21	Oval	105.77 x 78.16
	U7B3	✓	✓			mixture 50:50	Square	83.63 x 81.81
	U7B4			✓	✓	mixture 42:58	Irregular	86.77 (L)
	U7B5	✓	✓			mixture 79:21	Fiber	>605.76 (L)
	U7B6			✓		100% natural	Fiber	≈771.02 (L)
	U7B7	✓				100% natural	Fiber	>635.28 (L)
	U7B8	✓	✓		✓	mixture 64:15:21	Fiber	716.45 (L)
	U7B9			✓		100% natural	Fiber	209.30 (L)
	U7B10	✓	✓			mixture 79:21	Fiber	812.72 (L)
U_8B	U8B1	✓				100% natural	Irregular	141.04 (L)
	U8B2	✓	✓			mixture 60:40	Irregular	107.80 x 101.34
	U8B3	✓	✓			mixture 79:21	Fiber	911.03 (L)
	U8B42	✓	✓			mixture 60:40	Film	immeasurable

Table 11

Identification of microparticles according to the OPUS v.7.5 library of FTIR from conventional sour cream samples

Sample	Sample Code	Identification according to the OPUS v.7.5 library				Microparticle composition	Characterization	
		Cotton	Acrylic	Cellulose	Flax		Shape	Size (LxW) [µm]
S_1	S1.1	✓			✓	mixture 60:40	Irregular	47.22 (L)
	S1.2	✓				100% natural	Film	291.10 x 55.88
	S1.3	✓				100% natural	Fiber	>810.96 x 73.73
	S1.4	✓				100% natural	Fiber	173.10 (L)
	S1.5	✓				100% natural	Fiber	≈838.78 (L)
	S1.6	✓			✓	mixture 60:40	Fiber	>1028.68 (L)
S_2	S2.1	✓			✓	mixture 60:40	Oval	104.95 (L)
	S2.2	✓	✓			mixture 60:40	Irregular	immeasurable
	S2.3			✓		100% natural	Fiber	>949.68 (L)
	S2.4	✓				100% natural	Fiber	>902.68 (L)
	S2.5			✓		100% natural	Film	immeasurable
S_3	S3.1			✓		100% natural	Irregular	86.37 (L)
	S3.2			✓		100% natural	Fiber	>1747.91 (L)
	S3.3	✓				100% natural	Fiber	>709.22 (L)
S_4	S4.1			✓		100% natural	Irregular	332.98 x 293.15
	S4.2			✓		100% natural	Fiber	>1143.87 (L)
	S4.3	✓				100% natural	Fiber	>213.66 (L)
	S4.4			✓		100% natural	Fiber	>975.68 (L)
	S4.5	✓				mixture 60:40	Fiber	>760.51 (L)
	S4.6			✓		100% natural	Fiber	≈305.23 (L)
	S4.7	✓			✓	mixture 60:40	Fiber	494.75 (L)
S_5	S5.1	✓				100% natural	Triangle	49.00 x 79.05
	S5.2			✓		100% natural	Irregular	immeasurable
	S5.3			✓		100% natural	Fiber	>868.49 (L)
	S5.4	✓				100% natural	Fiber	>259.24 (L)
	S5.5			✓		100% natural	Fiber	≈975.07 (L)
	S5.6			✓		100% natural	Fiber	576.7 (L)
S_6	S6.1	✓				100% natural	Irregular	150.76 x 107.80
	S6.2	✓	✓		✓	mixture 64:15:21	Fiber	>777.62 (L)
	S6.3			✓		100% natural	Fiber	644.95 (L)
	S6.4			✓		100% natural	Fiber	747.94 (L)
	S6.5				✓	100% natural	Fiber	237.27 (L)
S_7	S7.1	✓			✓	mixture 60:40	Irregular	55.30 (L)
	S7.2				✓	100% natural	Oval	66.83 (L)
	S7.3			✓		100% natural	Irregular	>1026.40 x >349.43
	S7.4	✓				100% natural	Fiber	739.64(L)

According to Figures 12-14, organic and conventional butter had the highest distribution of synthetic microparticles (80% in organic butter and 56% in conventional

butter), while conventional sour cream had the lowest distribution (22%). In terms of natural microparticles, sour cream had the highest distribution (78%), while conventional and organic butter accounted for 44% and 20%, respectively.

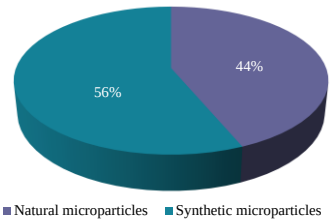

Figure 12. The nature of microparticles identified by the micro-FTIR technique in conventional butter samples.

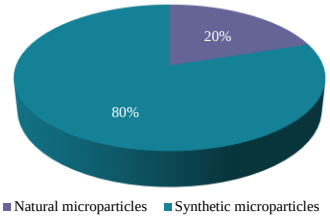

Figure 13. The nature of microparticles identified by the micro-FTIR technique in organic butter samples.

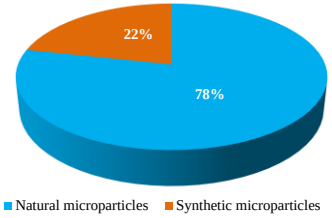

Figure 14. The nature of microparticles identified by the micro-FTIR technique in sour cream samples.

The microparticles content in butter (conventional and organic) and sour cream samples is highly variable. In the conventional butter samples, was recorded a variation from 375 to 1500 microparticles/kg (Table 6), and in organic butter from 625 to 1000 microparticles/kg (Table 7). In the case of sour cream, the variation of microparticles content ranged between 400 and 1200 microparticles/kg (Table 8).

Based on the pollution load index (PLI) equation, for conventional and organic butter samples, the recorded variations were from 14.940 (U_2 and U_4 samples) to 29.881 (U_1 and U_7 samples). For the conventional butter samples and organic butter samples, values ranged from 19.288 (sample U_7B) to 24.398 (sample U6B). According to Table 4, the samples of conventional and organic butter fall either into a low-danger level (green color) or a high-danger one (red color) (Figure 15) of pollution/contamination.

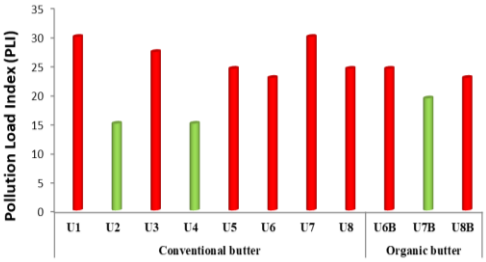

Figure 15. The pollution load index (PLI) of analyzed butter samples

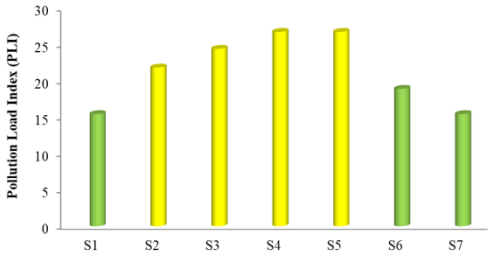

Figure 16. The pollution load index (PLI) of analyzed sour cream samples

The pollution loading index (PLI) of the conventional sour cream pods varied from 15.430 (S_1 and S7 samples) to 26.726 (S_4 and S_5 samples). The sour cream samples are included in low and medium levels of risk (hazard) (Figure 16) according to Table 4.

Using the second equation, the daily intake of microparticles for children and adults was calculated. Figures 17 and 18 show the results for butter (conventional and organic) and sour cream (conventional).

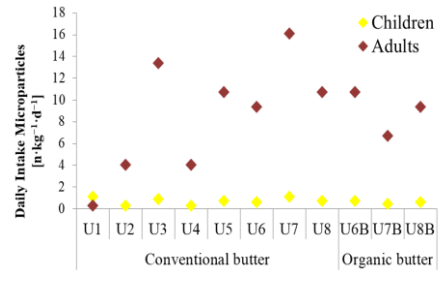

Figure 17. The daily intake of micro-fibers (DIM) in the analyzed butter samples.

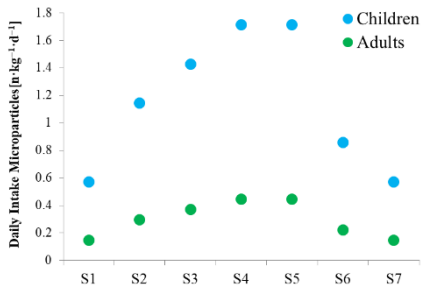

Figure 18. The daily intake of micro-fibers (DIM) in the analyzed sour cream samples.

Conventional butter samples recorded a DIM value for children between 0.268 and 1.071 n/(kg·d) and between 0.279 and 16.071 n/(kg·d) for adults, while organic butter reached values between 0.446 and 0.714 n/(kg·d) for children and from 6.696 to 10.714 n/(kg·d) for adults. Conventional sour cream samples recorded a DIM value for children between 0.571 and 1.714 n/(kg·d) and for adults, DIM values were between 0.149 and 0.446 n n/(kg·d).

The Pearson correlation coefficient is used to determine the relationship between "microparticle type" and potential sources of occurrence. Table 12 highlights the presence of a significant correlation between the two variables (R = 0.595), which proves that the type of microparticle defines the source of occurrence.

Table 12

Correlations between the type of microparticle and the sources of origin in conventional and organic butter samples

		Microparticle type	Occurrence source
Microparticle type	Pearson Correlation	1	0.595**
	Sig. (2-tailed)		0.000
	N	96	96
Occurrence source	Pearson Correlation	0.595**	1
	Sig. (2-tailed)	0.000	
	N	96	96

***. Correlation is significant at the 0.01 level (2-tailed).*

The forecast of privileged values at the source of origin of microparticles from the analyzed samples was made based on the equation that describes the link between the microparticle type and the source of occurrence by the linear regression method. The effect size indicators in the case of simple linear regression are R and adjusted R^2, in this case, it is R=0.595, so there is a large effect of the source of occurrence on microparticle type. The coefficient of determination R^2=0.354, which means that 35.4% of the variance of the dependent variable "Microparticle type" can be explained by the variance of the independent variable (source of occurrence).

Table 13

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics					Durbin-Watson
					R Square Change	F Change	df1	df2	Sig. F Change	
1	0.595[a]	0.354	0.345	2.159	0.354	37.280	1	95	0.000	1.290

[a] Predictors: (Constant), Occurrence source; [b] Dependent Variable: Microparticle type

In Table 13, the unstandardized (B) and standardized (beta) regression coefficients are presented, as well as the t-test results for each of these coefficients. For simple linear regression, beta (standardized coefficient β = 0.595) is the correlation coefficient between the independent variable and the dependent variable. The unstandardized coefficient (Table 14) for the variable ethical attitudes at the political level is b=3.183 and represents the slope of the regression line. The unstandardized coefficient for the constant is a= -73.033 and represents the intercept. It can be write the regression equation Y=a+b·X=-73.033+3.183X to predict the growth of microparticles based on the source of occurrence.

Table 14

Coefficients[a]

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
(Constant)	-73.033	12.813		-5.700	0.000
Occurrence source	3.183	0.521	0.595	6.106	0.000

[a] Dependent Variable: Microparticle type

To analyze whether the predictor variable and the outcome variable are linearly related, visual verification was carried out using a scatter plot. In assessing

the relationship between ethical attitudes at the political level and ethical attitudes at the social level, the scatter plot in Figure 19 indicates that there is a moderate linear positive relationship between the two variables, which is supported by the correlation coefficient (r = 0.595).

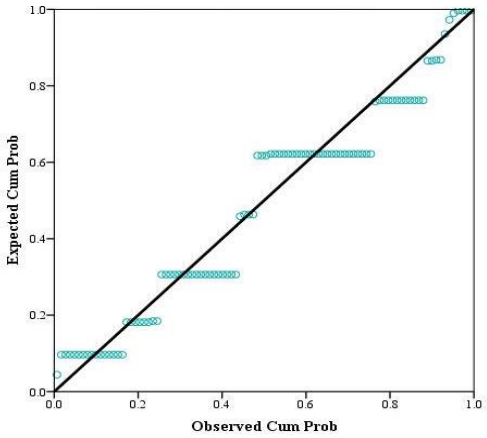

Figure. 19. Moderate positive linear relationship between the two variables. Normal P-P plot of regression standardized residual dependent variable.

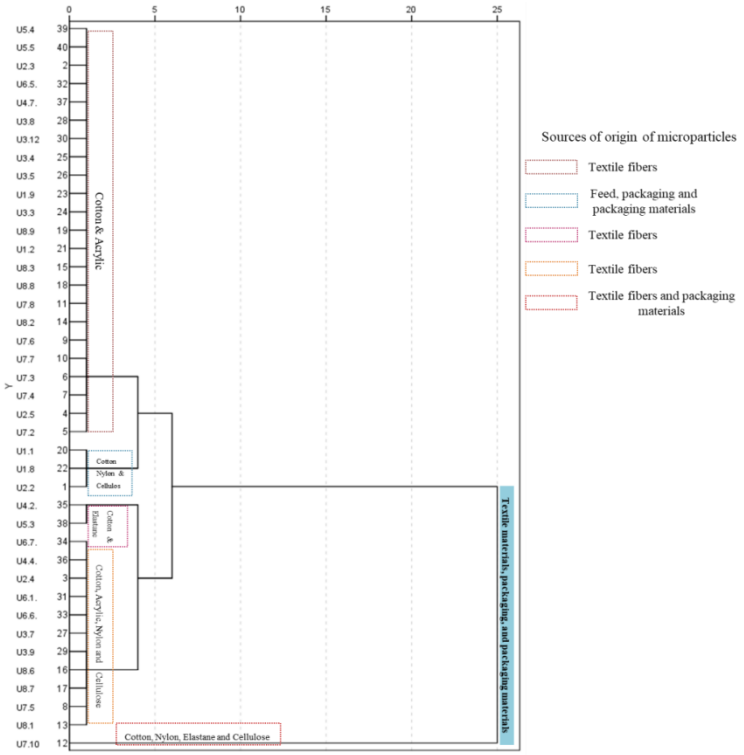

Figure 20. Dendrogram plot tab - distribution of textile fibers, feed, packaging materials for conventional butter samples

The hierarchical cluster analysis is a procedure for identifying relatively homogeneous groups in terms of cases or variables based on predetermined characteristics. Figures 20 and 21 present an analysis of the hierarchical clusters made for conventional and organic butter, and the default variables were the type of microparticle and the nature of the microparticle. For both categories of butter, synthetic fibers were selected that had nylon, acrylic, and/or elastane in their composition.

The dendrogram (hierarchical cluster) of conventional butter was obtained on data collected from 40 synthetic microparticles (Figure 20), while the organic butter was obtained on data collected from 20 synthetic microparticles (Figure 21).

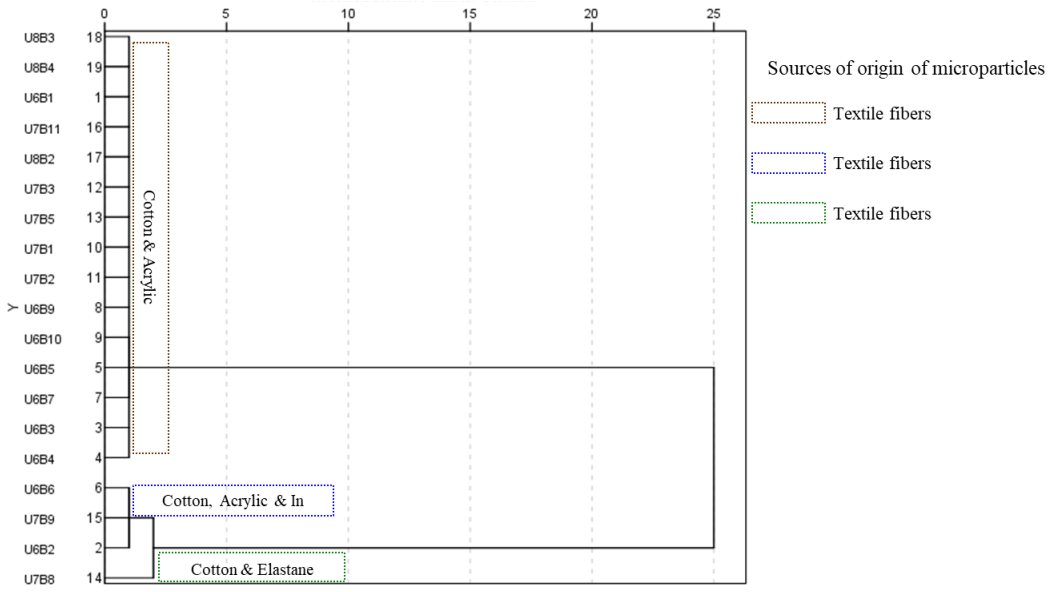

Figure 21. Dendrogram plot tab - distribution of textile fibers for organic butter samples

Based on the cluster analysis, it was possible to group the conventional butter samples according to microparticle (e.g. cotton, cellulose, linen, wool, nylon, acrylic, etc.) and group them according to the composition of the blends. Cluster analysis grouped the 40 samples into five categories and assigned potential sources of occurrence. For the cotton and acrylic blend, 23 conventional butter samples were identified. In the mixture, cotton and acrylic come from the textile industry, and the potential sources of provenance could be clothes, materials used in the milk industry for straining, and the preparation of raw and auxiliary materials to be used in the technological field. The mixture of cotton, nylon, and cellulose, which could come from animal feed and food packaging but also from ingesting packaging found on pastures and meadows, was identified in three samples. Cotton in combination with elastane and the mixture of cotton, acrylic, nylon, and cellulose was identified in two respectively ten samples, which may come from the textile

industry during the work equipment use (blouses, trousers, vests etc.), protection (disposable caps and masks) and cloths. The mixture of cotton, nylon, elastane, and cellulose was identified in a sample that may come from the textile industry and the blisters used in the packaging of medicines.

Cluster analysis made it possible to group the organic butter samples according to fiber type (e.g., cotton, cellulose, linen, wool, nylon, acrylic, etc.) and group them according to the composition of the blends. Cluster analysis grouped the 25 samples into three categories and assigned potential sources of origin. For the cotton-acrylic blend, cotton, acrylic, and linen, 14 and 3 organic butter samples were identified, respectively. In a mixture, cotton and acrylic, but also a mixture consisting of cotton, acrylic, and linen can come from the textile industry, and the potential sources of appearance are clothes, cloths, materials used in the milk industry for straining, as well as the preparation of raw and auxiliary materials. Cotton, in combination with elastane, was identified in one sample. The mixture can come from the textile industry during the use of work equipment (blouses, trousers, and vests), protective equipment (caps and disposable masks) and cloths.

4. Conclusions

Microplastics-bound hazard pollutants are ingested through foods, including milk and dairy products. An absence of knowledge and procedures/standards in terms of microplastic analysis is a challenge for the future. Following the analyses, it can attribute the term microplastics to all synthetic microparticles (identified in a mixture with natural fibers). Therefore, from all 133 microparticles identified in the three categories of dairy products, 51.13% of them represent microplastics (synthetic microparticles), while the differences are attributed to natural microparticles. So far, according to our knowledge, no studies exist regarding the investigations on microplastics (type of polymer, morphological characterization, occurrence, abundance, source, risk, etc.), from a complex matrix of milk, such as dairy products with a high-fat content (i.e., sour cream, butter). In this respect, this study wants to be a point of start for future original research related to the presence of MPs in milk-based foods to a better assessment of potential human exposure and human health risks.

Funding:

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