ORIGINAL PAPER

MICROPLASTIC DEBRIS IN YOGURT: OCCURRENCE, CHARACTERIZATION, AND IMPLICATIONS FOR HUMAN HEALTH

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Abstract. *The present study aimed to identify and characterize the microplastics (MPs) extracted from conventional and organic yogurt, sold in large hypermarket chains in Romania. In this respect, the morphology and chemical composition of MPs, as well as the health risks generated by their presence in yogurt were important to investigate. In the yogurt samples were identified by optical microscopy a reasonably high number of microparticles (black, blue, red, gray, etc.): ~2236 / kg in conventional yogurt and ~2266 / kg in organic yogurt. The micro-FTIR analysis along with OPUS v.7.5 software's library revealed their composition. The complexity of the study was not generated only by the analytical methods used to characterize the MPs but also by the isolation process required for this. Therefore, the results revealed the presence of microparticles of cotton, cellulose, wool, raffia, and flax, (considered natural microparticles), but also mixtures with polymers (acrylic, nylon, polyester, cellophane, polyurethane, polyethylene, etc.), considered synthetic microparticles. Based on the above results could be established the correlations and the statistical approach, information that may serve or act as an incentive for milk and dairy product processors to try to find the source of contamination, starting with the raw material, continuing with the processing chain, and ending with the final product.*

Keywords: *yogurt; natural fiber; syntetic fiber; micro-FTIR; statistical approach.*

1. INTRODUCTION

The goal of rational nutrition is to ensure sufficient consumption of nutrients important for human health [1, 2]. However, at this time, consumers lack the information on making the right food-purchasing decisions that contribute to a healthy life. In this sense, one affected category by those aforementioned decisions is referring to milk and dairy products which are some of the most healthy and valued foods, however, are insufficiently consumed worldwide [1]. Throughout time, milk and dairy products were considered the most nutrient-dense foods that provide energy, high-quality protein, and micronutrients such as vitamins and minerals [3-9]. An adequate amount of essential minerals (i.e., calcium, potassium, phosphorous, magnesium, and zinc, in easily absorbable forms) often means aiming for a balanced diet [10].

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Particularly, dairy products provide a package of nutrients that are difficult to obtain in low-dairy or dairy-free diets (i.e., dairy-restrictive diet or vegan) [11]. On the other hand, several studies revealed that a dairy-free diet does not allow many people to meet the recommended daily calcium requirement for a healthy life [11-15].

In Codex Alimentarius - Standard for Fermented Milks (CXS 243/2003) yogurt is defined as a "dairy product obtained by fermentation of lactose to lactic acid by the action of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*" [16]. Due to its taste/flavor and nutritional content, yogurt is one of the most widely consumed fermented foods, being used in gastronomy and/or as a culinary component in many cultures around the world [17]. Yogurt is mainly obtained by fermenting cow's milk using lactic acid bacteria (LAB) [18]. To obtain yogurt, a series of technological operations are usually necessary, including milk cleaning, homogenization, heat treatment (pasteurization) and fermentation. Based on how these operations are combined, different types of yogurts with completely different physical qualities can be obtained. Furthermore, by changing manufacturing procedures/recipes, physical and chemical characteristics such as viscosity and firmness can change even within the same type of yogurt [19].

The need to extend the shelf life of milk, rather than throwing it away, led to the initial manufacture of fermented milk products. The first methods of making yogurt were based on empirical knowledge, without standard operating procedures or examination of all the phases involved in the process, then its production became mechanized and standardized at the end of the 20th Century. Yogurt manufacturing has attracted significant attention over the past 20 years, both for scientific and commercial purposes. Research has led to the development of new dairy products with improved sensory properties, especially texture, and human health benefits (probiotic cultures, bioactive chemical fortification), and as a result, there is increasing consumer demand for yogurt and dairy products related ferments [20-22].

The question is whether these milk products which are so appreciated today by all people (from children to seniors) can represent a hidden danger in terms of emerging contaminants as a result of human activities. If so, then what potential unseen contaminants, which cannot be quantified and are not yet regulated in terms of limits, risk, and cause-effect relationship, can represent a risk to consumers' health?

Based on these concerns, the current study aimed to investigate the presence of possible microplastics in fermented milk products, more specifically in yogurt, knowing the appreciation enjoyed by this food among the population regardless of age. Microplastics (MPs) as emerging contaminants are chemicals that are not currently regulated and there exist concerns regarding their impact on human health. To a greater understanding of the nature, extent, and impacts of the presence of microplastics in dairy products, this research used the statistical approach to investigate closer, the sources concerning the MPs occurrence. The release of microplastics from different sources is mainly influenced by weathering conditions corroborated with anthropic pollution, which can change the composition of raw milk collected from the farmers, as well as by other factors such as the transport of raw milk, the production and packaging process of dairy products (i.e., yogurt plain low-fat or fruit low-fat), the packaging type, the protective equipment of factory workers, etc. Zhao et al. [23] revealed that long-term wearing of masks and disinfection for reuse can promote the release of microplastics. On the other hand, mask/capeline-derived microplastics are mainly transparent and small-sized (<1 mm) polypropylene fibers as already mentioned by several authors [23-25]. The discussions can take shape not only around the masks/capeline currently used in milk processing and production factories but also around latex gloves or special protective equipment that can release MPs during yogurt production. As MPs act as vectors, it is possible that they also transport other emerging contaminants (such as heavy metals, PAHs, and other synthetic organic compounds), thus amplifying the harmful effects on human health (due to ingestion or inhalation). All these hypotheses were investigated by statistical analysis, along with performed analytical techniques such as optical microscopy and micro-Fourier transform infrared spectroscopy.

2. MATERIALS AND METHODS

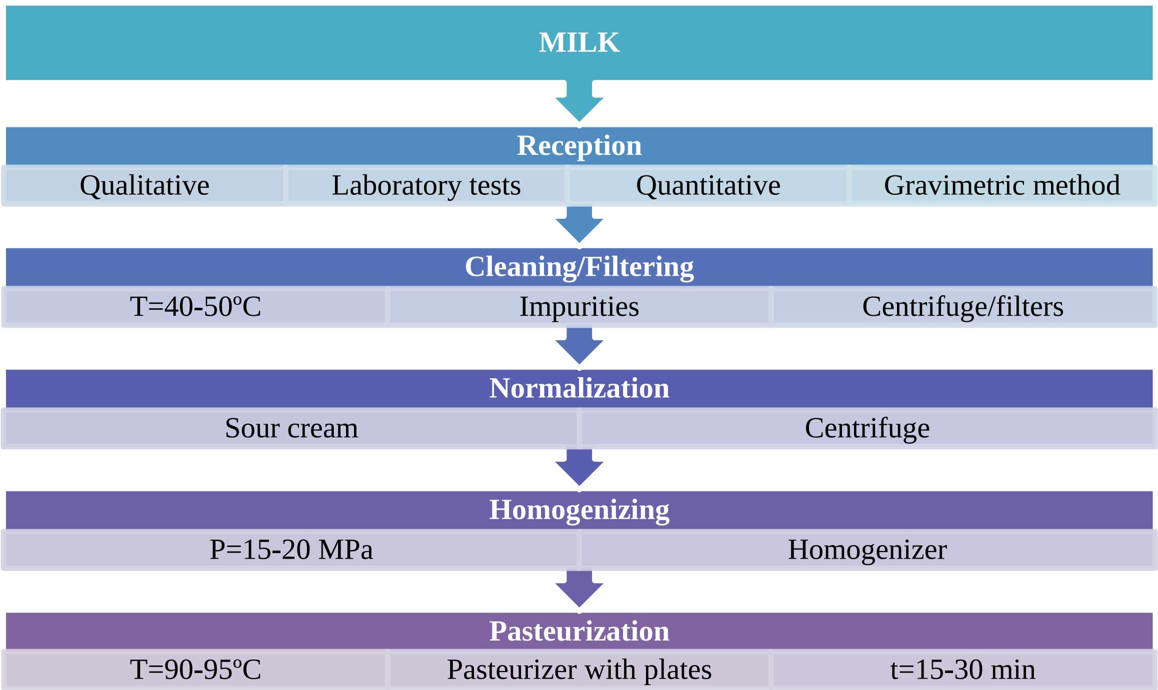
2.1. MATERIALS AND REAGENTS

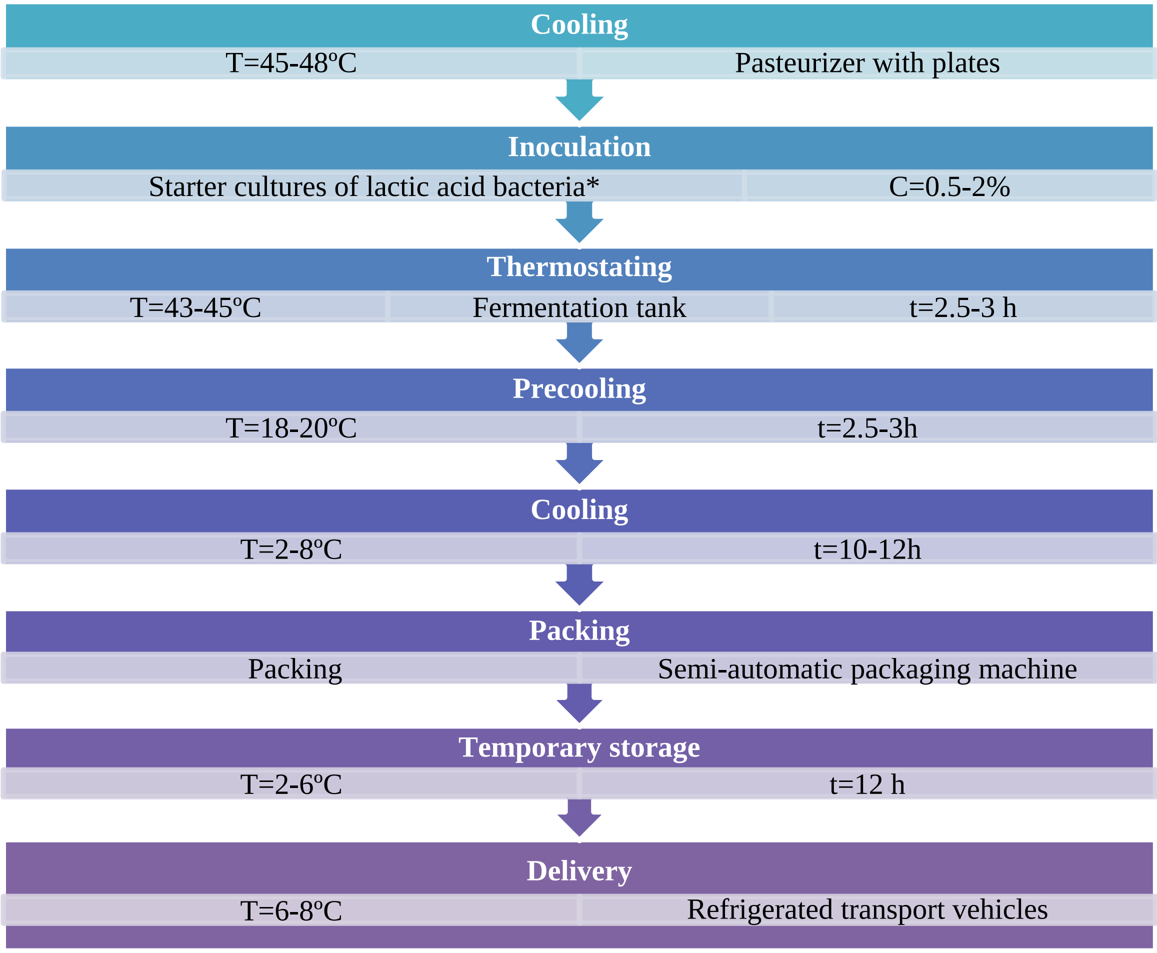
The high purity reagents (analytical grade) used for analysis were purchased from Merck (KGaA, Germany) and Sigma-Aldrich (Saint Louis, USA). In addition, the liquid ones were filtered before use to avoid any accidental contamination of samples (including ultrapure water).

2.2. SAMPLING AND SAMPLE PREPARATION

2.2.1. Samples

Yogurt manufacturing has been in the attention over the past 20 years, both for scientific and commercial purposes. Research has led to the development of dairy products with improved sensory qualities, appreciated especially through their texture but also for human health benefits (probiotic cultures, bioactive chemical fortification). As a result, there is greater consumer demand for yogurt and related fermented milk products [20-22]. The yogurt's production involves a series of milk processing stages, which starts with milking the animal and ends with wrapping it in a product meant for daily consumption (Fig. 1).





* *Live bacterial strains of Streptococcus salivarius subsp. thermophilus and Lactobacillus delbrueckii subsp. Bulgaricus.*

Figure 1. Yogurt production technology [21].

To examine the presence and type of MPs in eleven conventional yogurts with varied fat content (from 0.1 to 3.0%), and in six organic yogurts with varied fat content (from 0.9 to 3.8%), in the spring of 2023 was purchased yogurt from twelve popular brands (all from Romanian markets, random selection). The samples were labeled according to the nutritional qualities mentioned on the packaging (Tables 1 and 2) and were kept under refrigerated conditions (2-6°C) until analysis.

Table 1. General presentation of conventional yogurt samples.

Sample code	Nutritional value / 100 g yogurt						Fat content [%]	Packaging type
	Energy value		Saturated fatty acids [g]	Carbohydrates [g]	Protein [g]	Salt [g]		
	[kJ]	[kCal]						
I_1	121	29	0.1	3.9	3.2	0.12	0.1	Plastic
I_2	143	34	0.1	4.1	3.8	0.09	0.1	Plastic
I_3	334	81	1.3	11.7	3.6	0.10	2.0	Plastic
I_4	55	100	1.8	3.7	3.2	0.06	3.0	Plastic
I_5	139	33	0.1	4.5	3.6	0.1	0.1	Plastic
I_6	256	61	2.0	3.8	7.0	0.04	2.0	Plastic
I_7	128	33	0.1	4.1	3.8	0.1	0.1	Plastic
I_8	117	52	1.7	3.3	3.3	0.1	2.7	Glass
I_9	256	61	1.3	2.7	8.0	0.1	2.0	Plastic
I_{10}	240	57	2.0	4.5	3.5	0.1	2.8	Plastic
I_{11}	223	53	1.7	3.8	3.2	0.1	2.8	Plastic

Table 2. General presentation of organic yogurt samples.

Sample code	Nutritional value / 100 g yogurt						Fat content [%]	Packaging type
	Energy value		Saturated fatty acids [g]	Carbohydrates [g]	Protein [g]	Salt [g]		
	[kJ]	[kCal]						
I_2B	244	58	2.0	3.7	3.0	0.1	3.5	Plastic
I_3B	306	73	0.4	13.9	1.5	0.02	0.9	Plastic
I_4B	256	61	2.3	3.7	3.1	0.06	3.8	Plastic
I_6B	291	70	2.1	4.5	5.0	0.2	3.5	Plastic
I_8B	277	66	2.0	4.8	4.0	0.18	3.5	Glass
$I_{11}B$	260	62	2.2	3.8	3.2	0.1	3.8	Plastic

The selection criteria for the yogurt samples were as follows: relatively low-fat content, brand popularity among consumers, affordable price, purchase share, and safety criteria based on the international food quality classification, i.e., organic or conventional. To get a better picture of the impact on health and the associated risks were added two more yogurts intended/marketed for children (Fig. 2).

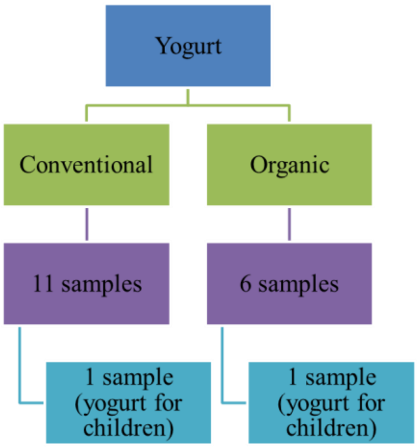


Figure 2. Selection and sampling of yogurt samples.

Given the potential contamination risk of the samples, their preparation (including sampling), was carried out in a clean room (according to ISO 14644-1:2015, class 1000 - ISO6) at the Institute of Multidisciplinary Research for Science and Technology of Valahia University of Targoviste. The non-textile (i.e., cotton) protective equipment (gown) and particle-free nitrile gloves were worn throughout the sample preparation process, and lab coat sleeves were tucked and secured inside the gloves. Laboratory equipment was washed at least three times with anhydrous ethanol and ultrapure water. The samples were handled carefully and covered with aluminum foil until the time of filtration. Experiments were performed in triplicate and the reported data were obtained by averaging the results.

2.2.2. Microparticles isolation protocol

Vessels/materials used for microparticles isolation and subsequent investigations (e.g., Erlenmeyer beakers, graduated cylinders, pipettes, spatulas, and Petri dishes) were cleaned with anhydrous ethanol and ultrapure water, then sterilized at 100°C for 48 h in the Venticell® oven (BMT Medical Technology, Brno, Czech Republic). The protocol for the microparticles isolation from conventional and organic yogurt samples was carried out according to the patent application [26]. According to the patent application, the isolation process is carried out in three steps:

A. Pretreatment: 5 g of the sample mixed with 1 g of NaOH and 500 mL of ultrapure water;
B. Digestion: the obtained mixture (in the pretreatment step) was homogenized at 150 rpm for 10 minutes, using the IKA® RT 5 shaker (IKA, Staufen, Germany) and ultrasonicated for 20 minutes at temperatures of 30ºC using the ultrasonic bath type VWR® Ultrasonic Cleaner USC - TH (VWR International, Radnor, United States of America);
C. Filtration: was performed on a cellulose membrane with a pore size of 12-15 µm (cellulose filters, VWR® Grade 413, VWR International, Radnor, United States of America) using a 3-station stainless-steel filter manifold (Labbox Labware, Barcelona, Spain), a vacuum pump with a flow rate of 18 L/min. The yogurt samples were kept in a water bath at a temperature of 60ºC until complete filtration [26].

2.3. ANALYTICAL TECHNIQUES

2.3.1. Optical microscopy

Optical microscopy was used to identify the microparticles on the surface of the filters. Magnification factors were 20X, 25X, 32X, 40X, and 50X, depending on particle size. For image acquisition, a Stemi 2000c microscope (Carl Zeiss, Oberkochen, Germany), Carl Zeiss Axiocam 105 digital video camera, and Zen software (Carl Zeiss, Oberkochen, Germany) were used. Optical microscopy was performed to identify and characterize the microparticles in terms of shape, color, and texture.

2.3.2. Micro-Fourier-transform infrared spectroscopy

The Fourier Transform Infrared Spectroscopy (FTIR) is a non-destructive analytical method that brings to discussion relevant information about the molecular structure of organic components. Lately, FTIR and Raman spectroscopy have been used more for the chemical characterization of food samples, due to the necessity generated by the human health safety measures linked to an increased consumption of new low or high-processed food products. Using both high-resolution micro-FTIR and micro-Raman imaging on food samples allows observation and mapping of the MPs distributions at a micrometer scale. For the current study, the micro-FTIR investigation was performed using the Vertex 80v FTIR spectrometer (Bruker, Billerica, United States of America) equipped with a Bruker Hyperion 2000 microscope. The system uses an MCT detector (mercury cadmium telluride) cooled with liquid nitrogen. Before proceeding to the analysis of dairy product samples, it was selected the Attenuated Total Reflection (ATR) work mode, which allows the direct measurement of samples and provides particulars regarding the presence or absence of specific functional groups or chemical structures of polymers. The Ge crystal with refraction index 4 is the component of the ATR objective (20X), and the micro-FTIR analysis of the samples was carried out in the transmission mode with the spectral range between 600-4000 cm^{-1} and 32 scans/sample. The IR spectra were compared with the OPUS v.7.5 software's database, and the polymer type was considered acceptable in the sample when its match with standard spectra was more than 70%.

2.4. DATA ANALYSIS

2.4.1. Pollution Load Index (PLI)

The pollution load index (PLI) was calculated using the following equation:

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

where: C_i – the content of microparticles (expressed as n/kg) determined in yogurt samples and C_0 – the minimum reported average concentration of microparticles in processed food [27-29] (C_0 = 1.68 n·kg^{-1}). Lin et al. [28] defined the hazard levels based on PLI values (Table 3).

Table 3. The risk level criteria for pollution load index of MPs [28]

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

2.4.2. Daily Intake of Microparticles (DIM)

Following the study of Lin et al. [28], it was used the daily intake of microparticles (DIM) equation:

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

where: C_i – the content of microparticles (expressed in n/kg) determined in the yogurt samples; I_r – degree of ingestion (expressed in kg/day); E_f – exposure rate (expressed as d/y); *Ed* – exposure period (expressed as y); *Bw* – body weight (expressed in kg); *At* – the average exposure time (expressed as d). The values of the mentioned parameters are presented in Table 4.

Table 4. The values of parameters for DIM calculation

		I_r [kg/d]	E_f [d/y]	E_d [y]	B_w [kg]	A_t [d]
Yogurt	Adults	0.304	365	70	70	25550
	Children	0.407	365	10	14	3650

2.4.3. Statistical analysis

Statistical analysis of study data was performed using IBM SPSS Statistics. The Pearson correlation coefficient, which can take values between -1 and +1 and is used as a measure of linear dependence between two or more variables, was used to examine significant correlations [33]. Relationships between variables were predicted using a regression analysis.

The independent variable is distributed on the x-axis and the dependent variable is distributed on the y-axis as methodology. Cluster analysis was performed to establish the commonalities between the samples and the nature of the fibers.

3. RESULTS AND DISCUSSION

3.1. OPTICAL MICROSCOPY

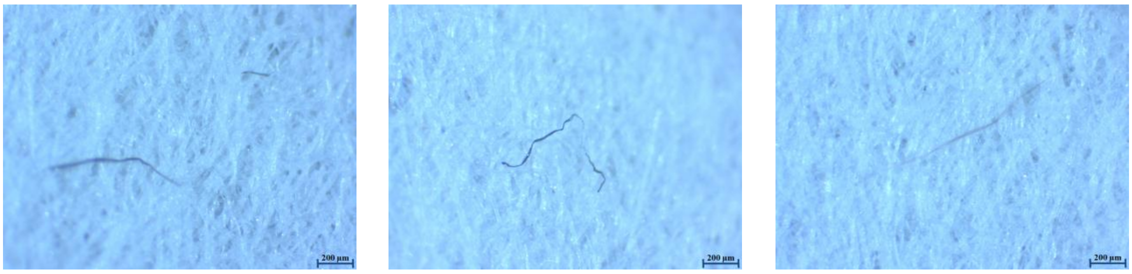
Optical microscopy (OM) is the first analytical method for the determination of contaminants in conventional and organic yogurt. This signifies the initial stage of highlighting the existence of microparticles on the surface of the filter. This method only highlights the presence of particles but cannot identify the nature of the microparticles, therefore a chemical and structural study is required further. For this reason, it was decided that the identified contaminants be generically called microparticles. The microparticles found on the cellulose filters during the examinations were quantified and classified according to certain characteristics (color, shape, etc). The range of shapes (irregular, oval, rhomboidal, film, and fiber) and colors (black, blue, red, brown, grey, yellow, purple, and green) (Figs. 3-4) were highlighted and contributed to the total number of microparticles (expressed as microparticles/kg) (Tables 5 and 6). Not only the filters used in the separation process were the subject of investigation, but also the empty filters, or the filters used to filter the reactants, in which case the result was 0 (no contaminants spotted).

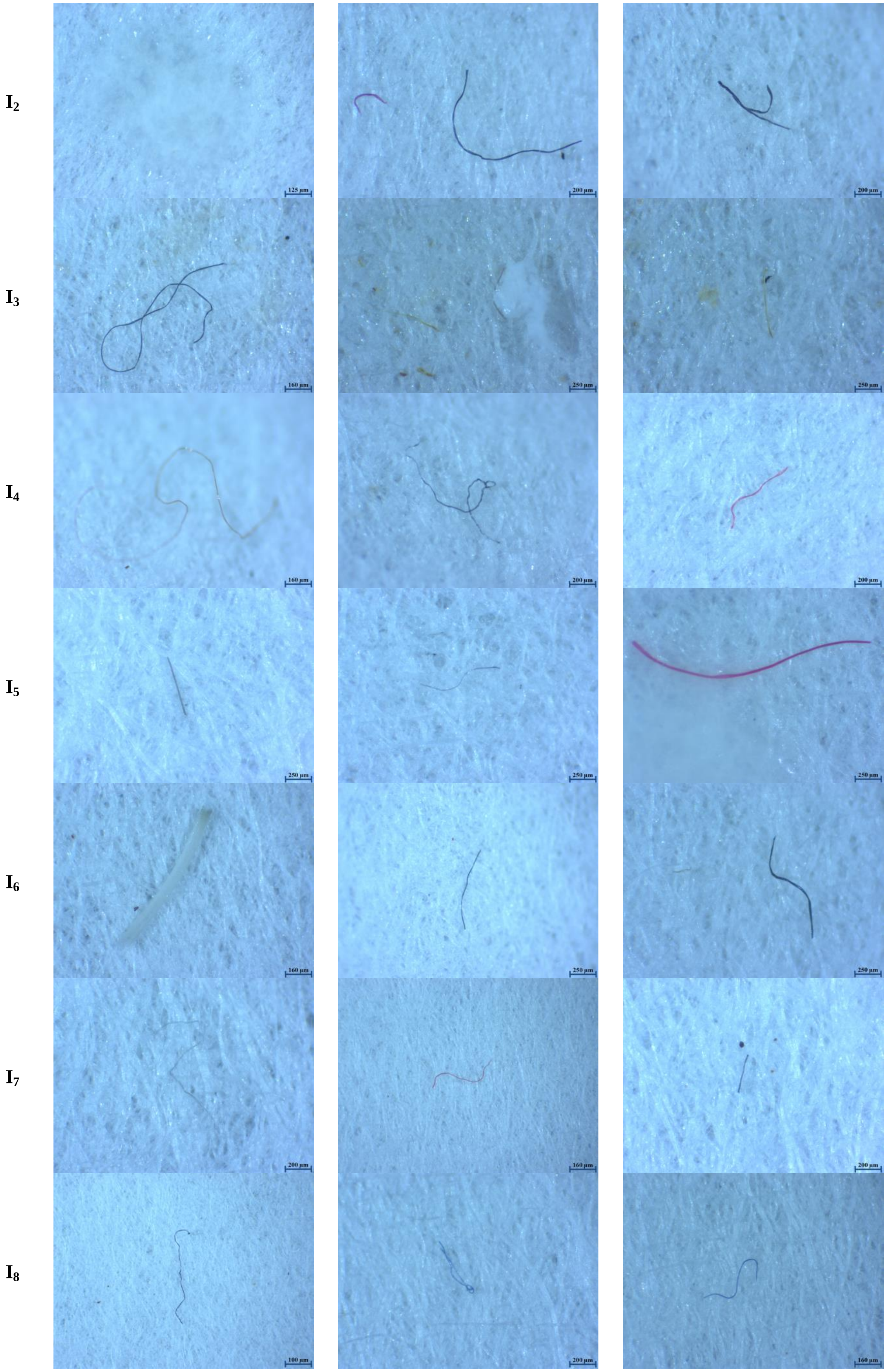
Tabel 5. Optical microscopy on conventional yogurt samples

Sample code	Color and number of microparticles							Total [micro-particles/kg]
	Black	Blue	Red	Brown	Grey	Yellow	Purple	
I_1	1400	*nd**	200	*nd**	1400	200	*nd**	3200
I_2	2200	600	*nd**	*nd**	1000	400	400	4600
I_3	800	*nd**	200	*nd**	200	400	*nd**	1600
I_4	2400	*nd**	1000	800	200	*nd**	*nd**	4400
I_5	600	*nd**	*nd**	*nd**	200	*nd**	200	1000
I_6	800	200	200	*nd**	*nd**	*nd**	*nd**	1200
I_7	200	*nd**	*nd**	*nd**	200	*nd**	*nd**	400
I_8	400	400	200	*nd**	*nd**	*nd**	*nd**	1000
I_9	200	1000	*nd**	*nd**	400	400	*nd**	2000
I_{10}	800	*nd**	400	*nd**	200	*nd**	*nd**	1400
I_{11}	2400	*nd**	*nd**	*nd**	1200	*nd**	200	3800

nd - unidentified*

I_1




I_2
I_3
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I_7
I_8

I_9

I_{10}

I_{11}

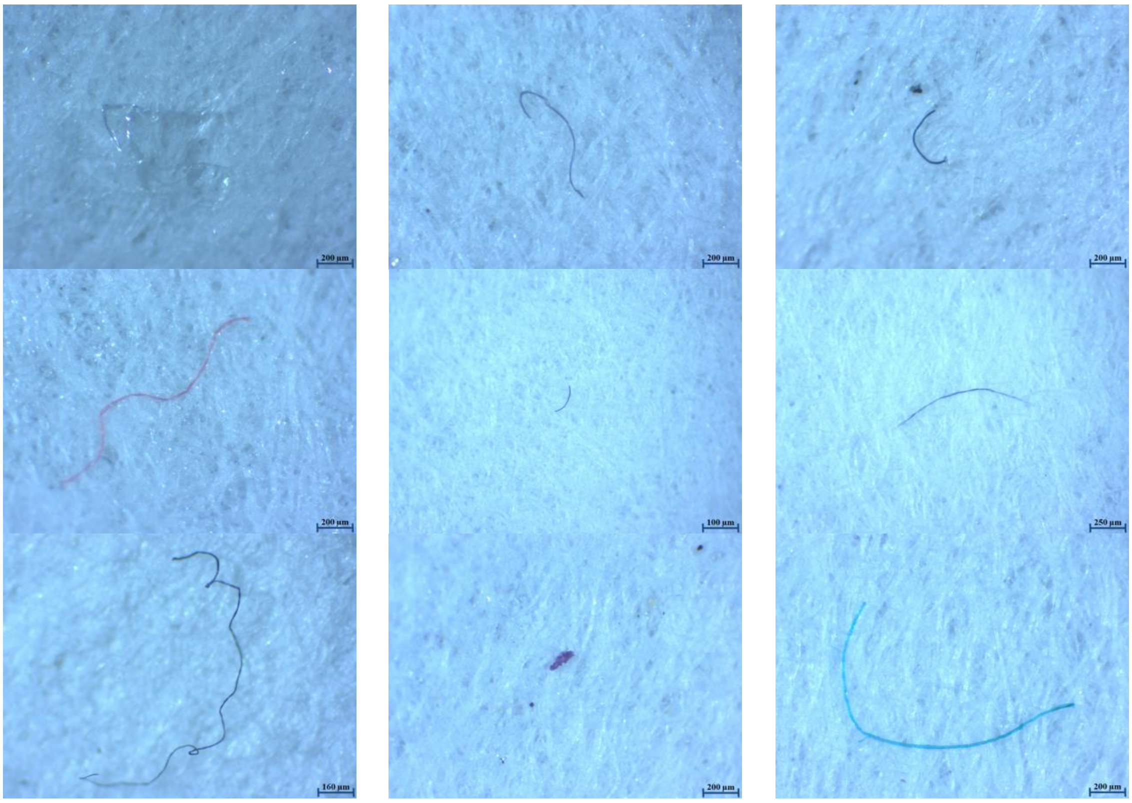


Figure 3. Optical microscopy images of representative microparticles identified in conventional yogurt.

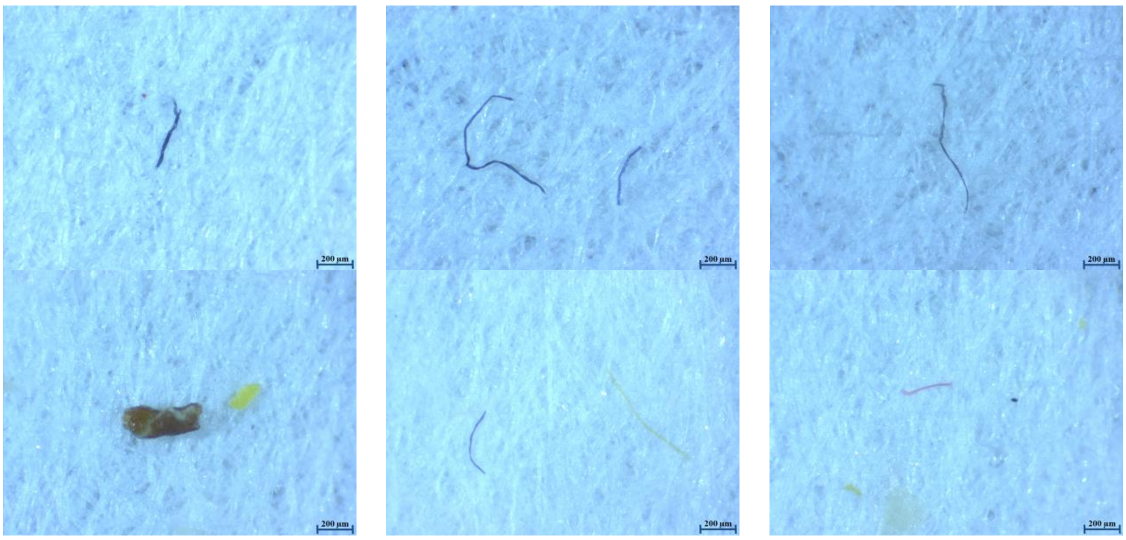
Tabel 6. Optical microscopy on organic yogurt samples

Sample code	Color and number of microparticles							Total [micro-particles/kg]
	Black	Blue	Red	Brown	Grey	Yellow	Green	
I_2B	1800	400	*nd**	*nd**	400	200	*nd**	2800
I_3B	600	*nd**	200	*nd**	*nd**	200	*nd**	1000
I_4B	600	600	*nd**	*nd**	800	400	*nd**	2400
I_6B	400	200	*nd**	*nd**	*nd**	*nd**	200	800
I_8B	400	800	*nd**	200	600	*nd**	*nd**	2200
$I_{11}B$	2600	400	*nd**	*nd**	1000	200	200	4400

nd - unidentified*

I_2B

I_3B



I_4B

I_6B

I_8B

$I_{11}B$

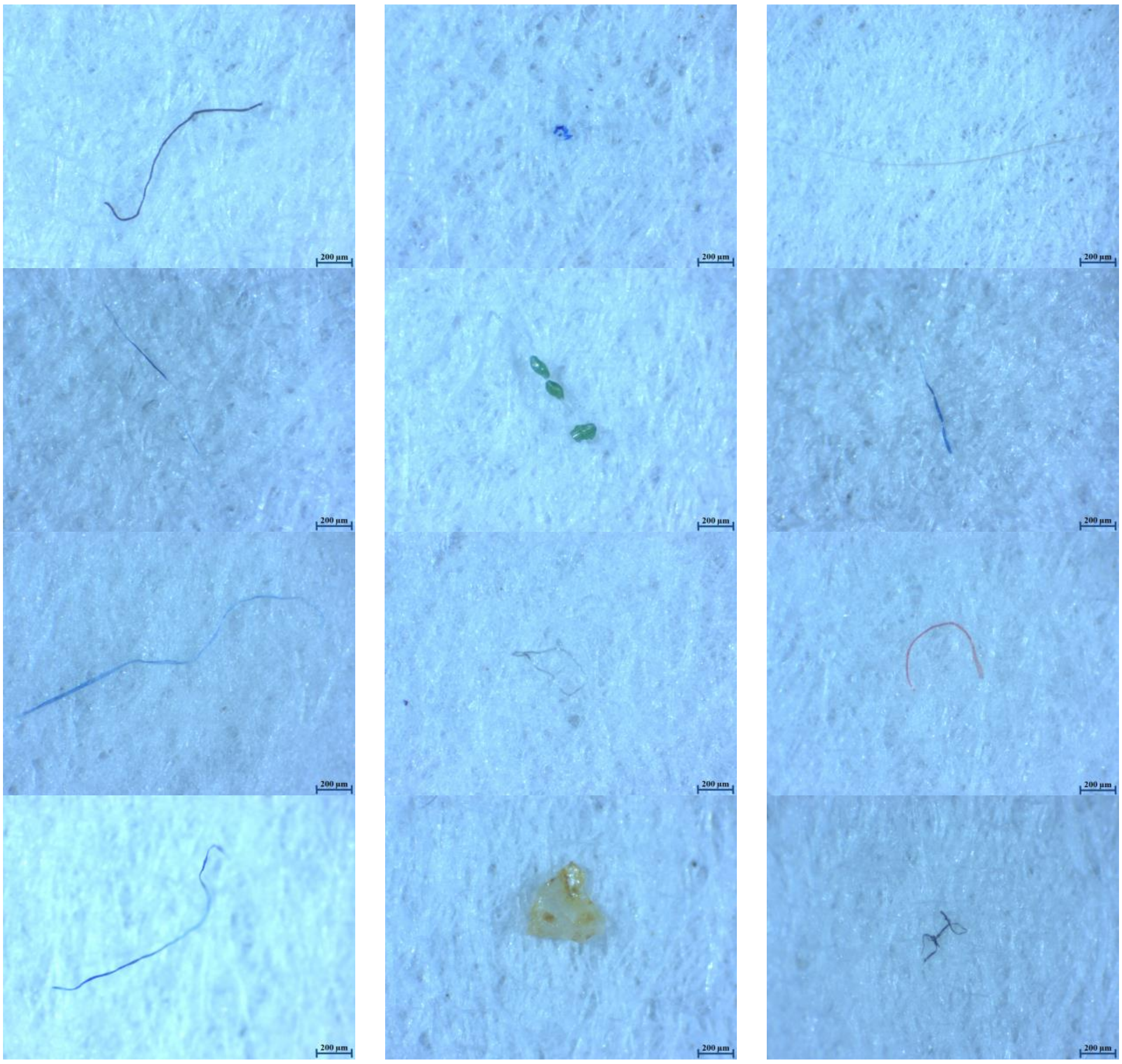


Figure 4. Optical microscopy images of representative microparticles identified in organic yogurt.

The total number of microparticles (Fig. 5) identified in the yogurt samples (conventional and organic) was determined according to Tables 5 and 6. Eleven samples within the category of conventional yogurt were analyzed in which 24,600 microparticles were identified, also, in the category of organic yogurt, six samples were analyzed which led to the identification of 13,600 microparticles. The percentage of colors identified for the conventional yogurt samples were black (50%), gray (20%), red and blue (9%), yellow (6%) and purple and brown (3%). In the case of organic yogurt samples, the percentage was black (48%), gray (21%), blue (18%), yellow (7%), green (3%), red (2%) and brown (1%).

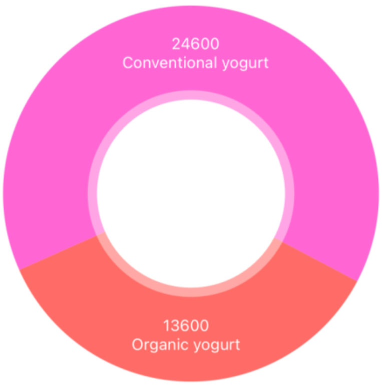


Figure 5. Total number of microparticles identified in conventional and organic yogurt samples

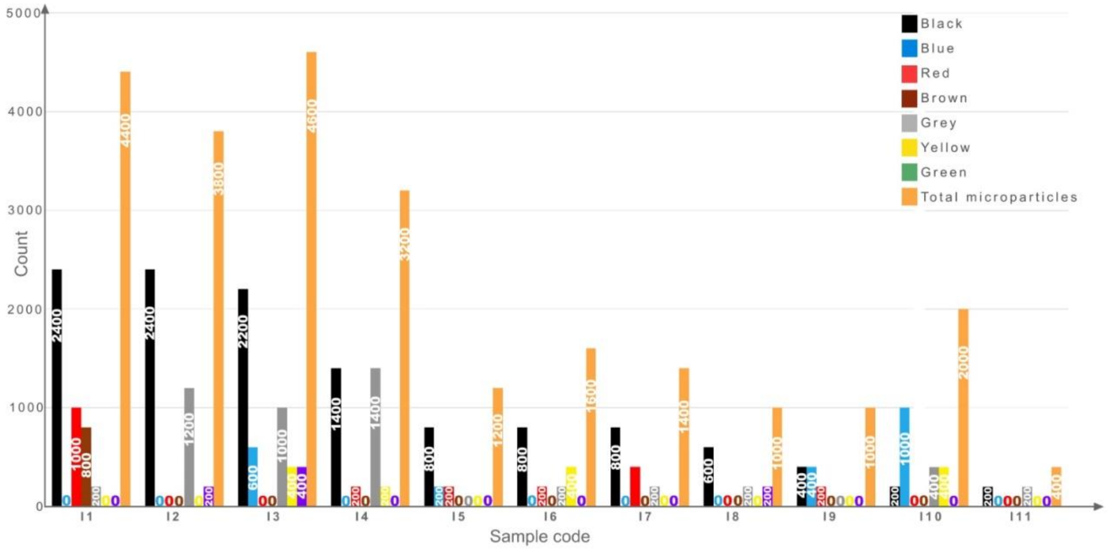


Figure 6. Color graph of microparticles identified in conventional yogurt samples.

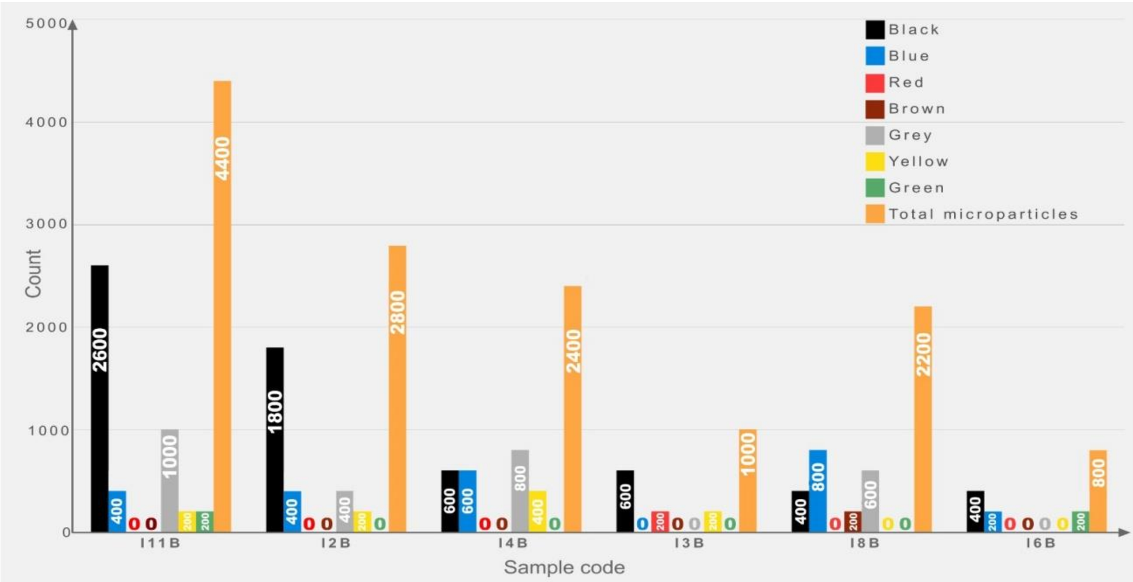


Figure 7. Color graph of microparticles identified in organic yogurt samples.

The predominant color of the microparticles, for both conventional and organic yogurt, was black. The highest number of black microparticles was identified in conventional yogurt, in samples I_4 and I_{11}, while for organic yogurt, the sample with the highest number of black microparticles was I_6B. The highest number of gray microparticles was identified in the case of conventional yogurt (sample I_1), while for organic yogurt, the highest number of microparticles having this color was identified in sample I_6B. In the case of the other colors, we chose to have a discussion only referring us to the samples that had the highest content of microparticles of a certain color. Therefore, the highest amount of blue color microparticles was found in samples I_9 and I_8B, while the yellow ones were attributed to conventional yogurt (samples I_2, $I_{3,}$ and I_9) and organic yogurt (sample I_4B). In addition to the predominant colors, there were also colors such as red, brown, or purple which were mostly identified in organic yogurt (but not exclusively) while the green ones in conventional yogurt.

For the present study, 12 different brands of conventional and organic yogurt were purchased. Five of the brands can be found in both yogurt categories, one brand out of the five is intended for children (samples I_3 and I_3B), and two brands (I8 and I_8B) were selected based on the packaging (glass bottle). Fig. 8 shows the total number of microparticles in accordance with Tables 5 and 6 identified in conventional and organic yogurt samples.

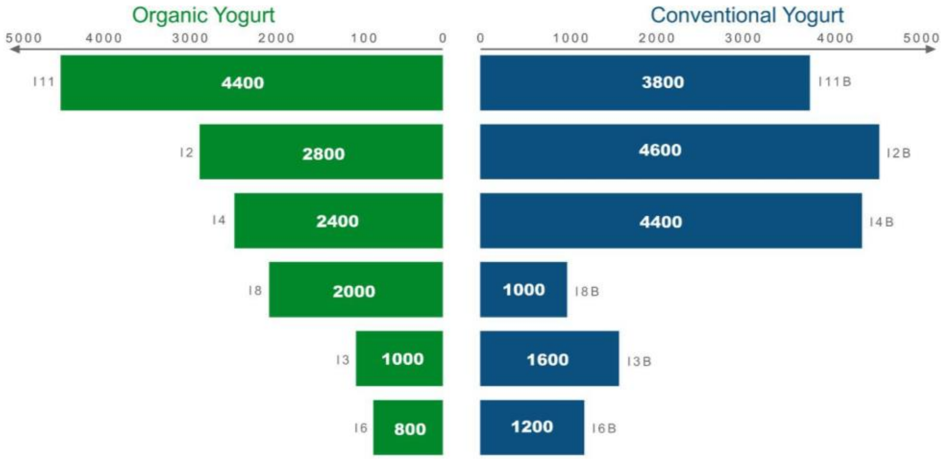


Figure 8. The total number of microparticles for organic and conventional yogurt brands, depending on the brand and the packaging material

The samples with the most microparticles identified in the conventional yogurt category were I_2, I_4, and I_6, while for organic yogurt; the highest number of microparticles was attributed to the I_6B sample. Regarding the samples packaged in glass bottles, the organic yogurt sample (I_8B) had the highest content of microparticles, while in the case of the samples intended for children; the most microparticles were identified in the conventional yogurt (I_3).

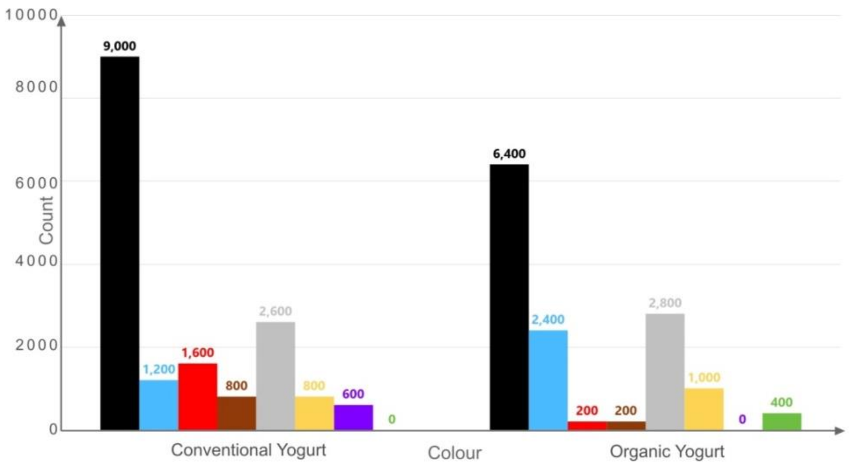


Figure 9. The total number of MPs according to color identified in conventional and organic yogurt

In the figures, the conventional yogurt recorded a total number of 9000 black microparticles while the organic only 6400. Conventional yogurt is leading when it comes to red and brown microparticles as well (1600 vs. 200, respectively 800 vs. 200) while for colors like blue (2400 vs. 1200), grey (2800 vs. 2600), or yellow (1000 vs. 800) put in front the organic yogurt. Other colors were identified as well but were not for both types of yogurt, reason why we cover them only as number and category: 400 green – organic yogurt, 600 purple – conventional yogurt (Fig. 9).

3.2. MICRO-FOURIER-TRANSFORM INFRARED SPECTROSCOPY

Micro-FTIR spectroscopy is one of the best methods to identify sample matrices based on vibrational frequency, even if more extensive/deep research is needed to produce a definitive result. Following the FTIR analysis, each sample was subjected to measurement; Fig. 10 shows some representative measured samples.

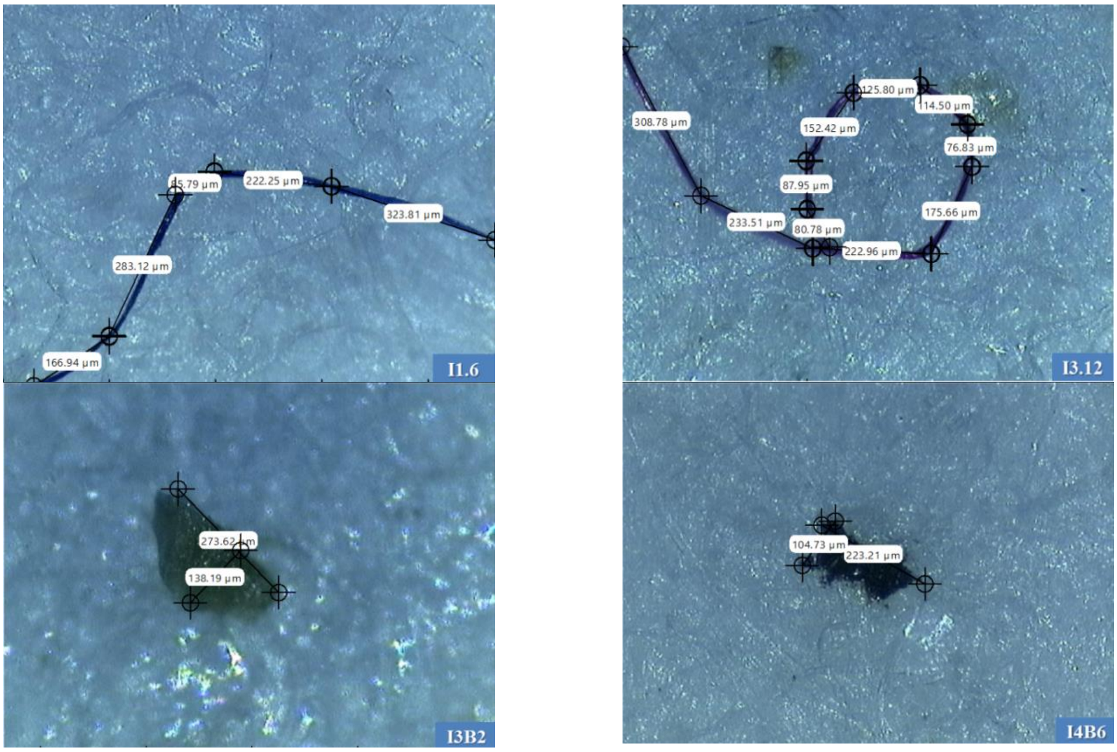


Figure 10. The measurement sequence of the microparticles identified in the conventional (I1.6 and I3.2) and organic (I8B.5 and I4B.6) yogurt samples.

Functional groups of chemical bonds can be identified spectroscopically due to weak, medium, and strong wave numbers and intensity [30]. The identification of the samples is based on the equipment's spectra library, results being shown in Tables 7 and 8.

Tabel 7. Identification of microparticles according to the OPUS v.7.5 library of FTIR from conventional yogurt samples

Sample	Sample Code	Identification according to the OPUS v.7.5 library											Microparticles composition according to the OPUS library	Characterization	
		Cotton	Acrylic	Nylon	Elastane	Cellulose	Cellophane	Polyester	Flax	Wool	Polyethylene	Polyurethane		Shape	Size (LxW) [µm]
I_1	I1.1			✓		✓		✓					mixture 33.33:33.33:33.33	Oval	66.89 (L)
	I1.2	✓											100% natural	Irregular	70.45 (L)
	I1.3	✓	✓										mixture 60:40	Fiber	656.76 (L)
	I1.4	✓											100% natural	Fiber	≈925.85 (L)
	I1.5							✓					100%	Fiber	>789.96 (L)
	I1.6	✓							✓				mixture 60:40	Fiber	>1086.91 (L)
	I1.7			✓		✓		✓					mixture 33.33:33.33:33.33	Fiber	>723.85 (L)
	I1.8					✓							100% natural	Fiber	-
	I1.9					✓							100% natural	Fiber	-
	I1.10					✓							100% natural	Fiber	608.15 (L)
	I1.11	✓											100% natural	Fiber	717.85 (L)
	I1.12	✓				✓							mixture 98:2	Fiber	>1078.26 (L)
	I1.13					✓							100% natural	Fiber	≈265.98 (L)
I_2	I2.1	✓											100% natural	Trapeze	168.38x84.79
	I2.2	✓											100% natural	Irregular	103.17 (L)
	I2.3	✓		✓	✓	✓							mixture 33:16:2:49	Fiber	>966.86 (L)

Sample	Sample Code	Identification according to the OPUS v.7.5 library											Microparticles composition according to the OPUS library	Characterization	
		Cotton	Acrylic	Nylon	Elastane	Cellulose	Cellophane	Polyester	Flax	Wool	Polyethylene	Polyurethane		Shape	Size (LxW) [µm]
	I2.4	✓		✓		✓							mixture 65:25:10	Fiber	>1060.19 (L)
	I2.5	✓											100% natural	Fiber	>789.26 (L)
	I2.6	✓											100% natural	Fiber	-
	I2.7	✓											100% natural	Fiber	>706.07 (L)
I_3	I3.1	✓							✓				mixture 60:40	Irregular	483.84x97.87
	I3.2					✓							100% natural	Trapeze	273.62x 138.19
	I3.3					✓							100% natural	Fiber	162.55 (L)
	I3.4					✓							100% natural	Irregular	476.18x55.30
	I3.5					✓							100% natural	Irregular	118.95 (L)
	I3.6					✓							100% natural	Square	107.61x 104.77
	I3.7	✓	✓	✓		✓							mixture 70:6:11:13	Irregular	74.15 (L)
	I3.8					✓							100% natural	Irregular	101.34 (L)
	I3.9					✓							100% natural	Fiber	-
	I3.10					✓							100% natural	Fiber	326.64 (L)
	I3.11	✓							✓				mixture 60:40	Fiber	>802.44 (L)
	I3.12					✓							100% natural	Fiber	>1579.19 (L)
	I3.13	✓											100% natural	Fiber	320.97 (L)
	I3.14						✓						100%	Fiber	520.52 (L)
	I3.15					✓							100% natural	Fiber	428.32 (L)
	I3.16					✓							100% natural	Fiber	79.73 (L)
	I3.17	✓		✓		✓							Mixture 65:25:10	Fiber	904.72 (L)
	I3.18					✓			✓				mixture 42:58	Fiber	227.73 (L)
	I3.19	✓											100% natural	Fiber	182.33 (L)
	I3.20					✓							100% natural	Fiber	402.68 (L)
	I3.21					✓							100% natural	Fiber	583.63 (L)
	I3.22	✓							✓				mixture 60:40	Fiber	350.95 (L)
	I3.23	✓		✓		✓							Mixture 65:25:10	Fiber	572.54 (L)
	I3.24	✓							✓				mixture 60:40	Fiber	104.95x 763.96
	I3.25	✓		✓									mixture 60:40	Fiber	342.16 (L)
	I3.26	✓		✓		✓							Mixture 65:25:10	Fiber	274.89 (L)
I_4	I4.1	✓							✓				mixture 60:40	Irregular	-
	I4.2	✓											100% natural	Irregular	332.17x 188.77
	I4.3					✓							100% natural	Irregular	239.31x86.77
	I4.4							✓					100%	Irregular	490.71x 116.53
	I4.5	✓											100% natural	Irregular	-
	I4.6	✓											100% natural	Fiber	>890.92 (L)
	I4.7	✓											100% natural	Fiber	252.54 (L)
	I4.8	✓											100% natural	Fiber	>564.45 (L)
	I4.9					✓							100% natural	Fiber	>490.12 (L)
	I4.10					✓							100% natural	Fiber	>710.30 (L)
	I4.11					✓							100% natural	Fiber	-
	I4.12					✓							100% natural	Fiber	202.16 (L)
	I4.13	✓						✓					mixture 68:32	Fiber	-
	I4.14	✓											100% natural	Fiber	>751.22 (L)
	I4.15					✓							100% natural	Fiber	>449.97 (L)
	I4.16	✓											100% natural	Fiber	208.86 (L)
	I4.17	✓		✓		✓							mixture 65:25:10	Fiber	>569.24 (L)
I_5	I5.1					✓							100% natural	Irregular	148.11x 115.61
	I5.2					✓							100% natural	Fiber	-
	I5.3					✓							100% natural	Fiber	-
	I5.4					✓							100% natural	Fiber	>726.78 (L)
	I5.5					✓							100% natural	Fiber	>635.96 (L)

Sample	Sample Code	Identification according to the OPUS v.7.5 library											Microparticles composition according to the OPUS library	Characterization	
		Cotton	Acrylic	Nylon	Elastane	Cellulose	Cellophane	Polyester	Flax	Wool	Polyethylene	Polyurethane		Shape	Size (LxW) [μm]
	I5.6	✓		✓		✓							mixture 65:25:10	Fiber	>984.06 (L)
	I5.7	✓		✓		✓							mixture 65:25:10	Fiber	>1114.83 (L)
	I5.8					✓							100% natural	Fiber	-
	I5.9					✓							100% natural	Fiber	>933.65 (L)
	I5.10	✓											100% natural	Fiber	>947.45 (L)
	I5.11					✓							100% natural	Fiber	>956.03
	I5.12					✓							100% natural	Fiber	-
I_6	I6.1					✓							100% natural	Square	214.13x 177.46
	I6.2					✓							100% natural	Irregular	173.54 (L)
	I6.3	✓	✓	✓		✓							mixture 70:6:11:13	Fiber	-
	I6.4	✓	✓										mixture 79:21	Fiber	>879.63 (L)
	I6.5					✓							100% natural	Fiber	>679.57 (L)
	I6.6	✓	✓	✓		✓							mixture 70:6:11:13	Fiber	-
	I6.7	✓			✓								mixture 98:2	Fiber	>945.30 (L)
I_7	I7.1	✓							✓				mixture 60:40	Rectangle	179.29x 123.73
	I7.2	✓				✓							mixture 82:18	Circle	92.58x98.88
	I7.3	✓	✓										mixture 60:40	Irregular	106.31x80.33
	I7.4	✓	✓	✓		✓							mixture 70:6:11:13	Rectangle	173.64x 124.58
	I7.5	✓											100% natural	Fiber	>553.86 (L)
	I7.6	✓											100% natural	Fiber	>974.85 (L)
	I7.7	✓							✓				mixture 60:40	Fiber	>1313.55 (L)
	I7.8	✓											100% natural	Fiber	>740.63 (L)
	I7.9	✓											100% natural	Fiber	>828.44 (L)
	I7.10	✓	✓										mixture 79:21	Fiber	>583.74 (L)
	I7.11	✓							✓				mixture 60:40	Fiber	>718.76 (L)
	I7.12			✓		✓		✓					mixture 33.33:33.33:33.33	Fiber	>916.49 (L)
	I7.13					✓							100% natural	Fiber	>792.84 (L)
I_8	I8.1	✓	✓	✓		✓							mixture 70:6:11:13	Irregular	103.34 (L)
	I8.2					✓							100% natural	Irregular	64.27x65.14
	I8.3	✓											100% natural	Irregular	82.44x77.73
	I8.4	✓											100% natural	Irregular	88.29 (L)
	I8.5	✓							✓				mixture 60:40	Irregular	81.53 (L)
	I8.6	✓	✓	✓		✓							mixture 70:6:11:13	Fiber	>1064.41 (L)
	I8.7	✓											100% natural	Fiber	>982.34 (L)
	I8.8	✓											100% natural	Fiber	>957.78 (L)
	I8.9	✓											100% natural	Fiber	>697.63 (L)
	I8.10	✓											100% natural	Fiber	>881.21 (L)
	I8.11					✓							100% natural	Fiber	>623.95 (L)
	I8.12								✓				100% natural	Fiber	>862.27 (L)
	I8.13					✓							100% natural	Fiber	>1123.58 (L)
I_9	I9.1					✓							100% natural	Triangle	333.05x216.3 5
	I9.2	✓	✓										mixture 60:40	Irregular	104.37x77.50
	I9.3	✓	✓						✓				mixture 64:15:21	Rectangle	202.73x 135.51
	I9.4	✓							✓				mixture 60:40	Irregular	163.44x78.81
	I9.5	✓											100% natural	Irregular	280.50x 139.51
	I9.6	✓	✓										mixture 60:40	Irregular	69.14 (L)
	I9.7	✓											100% natural	Irregular	104.95x71.73
	I9.8	✓	✓										mixture 60:40	Irregular	300.59x 418.71
	I9.9	✓	✓	✓		✓							mixture 70:6:11:13	Triangle	235.93x 201.44
	I9.10	✓	✓						✓				mixture 64:15:21	Irregular	-

Sample	Sample Code	Identification according to the OPUS v.7.5 library											Microparticles composition according to the OPUS library	Characterization	
		Cotton	Acrylic	Nylon	Elastane	Cellulose	Cellophane	Polyester	Flax	Wool	Polyethylene	Polyurethane		Shape	Size (LxW) [µm]
	I9.11	✓	✓						✓				mixture 64:15:21	Irregular	186.62x98.18
	I9.12	✓	✓						✓				mixture 64:15:21	Irregular	157.19x85.94
	I9.13	✓											100% natural	Irregular	141.66x72.69
	I9.14	✓	✓										mixture 60:40	Irregular	144.18x 160.65
	I9.15	✓	✓						✓				mixture 64:15:21	Square	470.18x 367.93
	I9.16										✓		100%	Irregular	238.43x 236.75
	I9.17					✓							100% natural	Irregular	119.11x89.20
	I9.18	✓	✓						✓				mixture 64:15:21	Irregular	70.45 (L)
	I9.19	✓	✓						✓				mixture 64:15:21	Irregular	277.46 (L)
	I9.20	✓											100% natural	Rectangle	385.09x 551.13
	I9.21					✓							100% natural	Fiber	578.96 (L)
	I9.22	✓	✓										mixture 79:21	Fiber	>812.47 (L)
	I9.23					✓							100% natural	Fiber	-
	I9.24					✓							100% natural	Fiber	>784.85 (L)
	I9.25	✓											100% natural	Fiber	>807.23 (L)
	I9.26			✓		✓		✓					mixture 33.33:33.33:33.33	Fiber	252.62 (L)
	I9.27			✓		✓		✓					mixture 33.33:33.33:33.33	Fiber	121.62 (L)
	I9.28	✓	✓										mixture 79:21	Fiber	265.01 (L)
	I10.1											✓	100%	Square	588.13x 539.99
	I10.2											✓	100%	Irregular	295.07x176.0 3
	I10.3	✓											100% natural	Square	87.05x84.54
	I10.4	✓											100% natural	Irregular	130.34x 115.12
	I10.5					✓							100% natural	Fiber	>540.90 (L)
I_{10}	I10.6	✓	✓										mixture 60:40	Fiber	>788.37 (L)
	I10.7	✓											100% natural	Fiber	≈860.06 (L)
	I10.8	✓											100% natural	Fiber	373.45 (L)
	I10.9	✓											100% natural	Fiber	408.99 (L)
	I10.10					✓							100% natural	Fiber	272.25 (L)
	I10.11	✓								✓			mixture 70:30	Fiber	>704.67 (L)
	I10.12					✓							100% natural	Fiber	>628.81 (L)
	I10.13	✓											100% natural	Fiber	>829.20 (L)
	I10.14					✓							100% natural	Fiber	>646.91 (L)
	I11.1	✓							✓				mixture 60:40	Irregular	150.34x 115.43
	I11.2	✓											100% natural	Irregular	-
	I11.3	✓							✓				mixture 60:40	Irregular	105.22x73.86
	I11.4	✓			✓								mixture 98:2	Irregular	-
	I11.5	✓											100% natural	Irregular	214.16x82.55
	I11.6	✓	✓	✓						✓			mixture 23:25:20:32	Irregular	809.78x 421.67
	I11.7	✓		✓		✓							mixture 65:25:10	Irregular	98.57x69.38
I_{11}	I11.8	✓											100% natural	Irregular	119.38x87.35
	I11.9	✓											100% natural	Irregular	108.76x88.98
	I11.10	✓							✓				mixture 60:40	Irregular	224.94x 187.66
	I11.11	✓											100% natural	Irregular	149.45 (L)
	I11.12					✓							100% natural	Oval	98.57x66.08
	I11.13	✓							✓				mixture 60:40	Rectangle	80.33x69.30
	I11.14	✓	✓	✓		✓							mixture 70:6:11:13	Rectangle	127.38x 103.58
	I11.15	✓	✓	✓		✓							mixture 70:6:11:13	Irregulat	394.29x

Sample	Sample Code	Identification according to the OPUS v.7.5 library											Microparticles composition according to the OPUS library	Characterization	
		Cotton	Acrylic	Nylon	Elastane	Cellulose	Cellophane	Polyester	Flax	Wool	Polyethylene	Polyurethane		Shape	Size (LxW) [µm]
															136.95
	I11.16	✓	✓										mixture 60:40	Irregulat	121.54x93.95
	I11.17	✓							✓				mixture 60:40	Fiber	>793.80 (L)
	I11.18	✓						✓					mixture 85:15	Fiber	-
	I11.19	✓							✓				mixture 60:40	Fiber	>558.24 (L)
	I11.20	✓							✓				mixture 60:40	Fiber	-
	I11.21	✓							✓				mixture 60:40	Fiber	>845.50 (L)
	I11.22	✓							✓				mixture 60:40	Fiber	-
	I11.23	✓		✓		✓							mixture 65:25:10	Fiber	>804.00 (L)
	I11.24	✓											100% natural	Fiber	646.05 (L)
	I11.25	✓											100% natural	Fiber	>857.40 (L)
	I11.26	✓											100% natural	Fiber	778.90 (L)
	I11.27	✓							✓				mixture 60:40	Fiber	616.95 (L)
	I11.28	✓							✓				100% natural	Fiber	306.78 (L)
	I11.29	✓			✓								mixture 98:2	Fiber	>669.41 (L)
	I11.30	✓	✓										mixture 60:40	Fiber	513.53 (L)
	I11.31	✓											100% natural	Fiber	-

Tabel 8. Identification of microparticles according to the OPUS v.7.5 library of FTIR from organic yogurt samples

Sample	Sample Code	Identification according to the OPUS v.7.5 library							Microparticles composition according to the OPUS library	Characterization	
		Cotton	Acrylic	Nylon	Elastane	Cellulose	Polyester	Flax		Shape	Size (LxW) [µm]
I_2B	I2B1	✓		✓		✓			mixture 65:25:10	Irregular	91.84 (L)
	I2B2	✓							100% natural	Fiber	>968.47 (L)
	I2B3	✓						✓	mixture 60:40	Fiber	439.44 (L)
	I2B4	✓	✓						mixture 60:40	Fiber	180.76 (L)
	I2B5					✓			100% natural	Fiber	338.23 (L)
	I2B6					✓			100% natural	Fiber	920.2 (L)
	I2B7						✓		100%	Fiber	>935.60 (L)
	I2B8	✓	✓						mixture 60:40	Fiber	520.22(L)
	I2B9	✓			✓				mixture 98:2	Fiber	559.21 (L)
	I2B10	✓	✓					✓	mixture 64:15:21	Fiber	>796.31 (L)
I_3B	I3B1	✓						✓	mixture 60:40	Irregular	247.35x105.17
	I3B2	✓						✓	mixture 60:40	Fiber	344.38 (L)
	I3B3					✓			100% natural	Fiber	181.69 (L)
	I3B4					✓			100% natural	Fiber	>773.97 (L)
	I3B5	✓						✓	mixture 60:40	Fiber	622.86 (L)
	I3B6	✓						✓	mixture 60:40	Fiber	>706.37 (L)
	I3B7	✓	✓	✓		✓			mixture 70:6:11:13	Fiber	582.21 (L)
	I3B8	✓						✓	mixture 60:40	Fiber	>523.21 (L)
	I3B9	✓	✓	✓		✓			mixture 70:6:11:13	Fiber	210.44 (L)
	I3B10					✓			100% natural	Fiber	>442.02 (L)
I_4B	I4B1	✓							100% natural	Irregular	204.79x117.25
	I4B2	✓	✓						mixture 50:50	Irregular	121.40 (L)
	I4B3							✓	100% natural	Irregular	98.57 (L)
	I4B4	✓			✓				mixture 98:2	Irregular	66.69x55.27
	I4B5	✓							100% natural	Irregular	67.74x69.14
	I4B6	✓							100% natural	Irregular	223.21x104.73
	I4B7						✓		100%	Fiber	138.40 (L)
	I4B8	✓							100% natural	Fiber	>826.31 (l)

Sample	Sample Code	Identification according to the OPUS v.7.5 library							Microparticles composition according to the OPUS library	Characterization	
		Cotton	Acrylic	Nylon	Elastane	Cellulose	Polyester	Flax		Shape	Size (LxW) [µm]
	I4B9	✓		✓		✓			mixture 65:2510	Fiber	560.99 (L)
	I4B10	✓							100% natural	Fiber	>904.48 (L)
	I4B11					✓			100% natural	Fiber	473.71 (L)
	I4B12	✓			✓				mixture 98:2	Fiber	638.70 (L)
	I4B13	✓	✓	✓		✓			mixture 70:6:11:13	Fiber	-
	I4B14	✓		✓		✓			mixture 65:2510	Fiber	470.75 (L)
I_6B	I6B1	✓	✓						mixture 60:40	Fiber	173.90x121.11
	I6B2			✓		✓	✓		mixture 33.33:33.33:33.33	Irregular	-
	I6B3	✓							100% natural	Fiber	>891.35 (L)
	I6B4	✓							100% natural	Fiber	576.08 (L)
I_8B	I8B1					✓			100% natural	Fiber	373.20 (L)
	I8B2	✓		✓		✓			mixture 65:25:10	Fiber	836.71 (L)
	I8B3	✓	✓						mixture 60:40	Fiber	>755.24 (L)
	I8B4	✓							100% natural	Fiber	>834.01 (L)
	I8B5	✓	✓						mixture 79:21	Fiber	>1045.16 (L)
	I8B6	✓	✓						mixture 79:21	Fiber	≈428.27 (L)
	I8B7	✓	✓						mixture 79:21	Fiber	210.56 (L)
	I8B8	✓	✓						mixture 79:21	Fiber	>780,43 (L)
	I8B9	✓	✓						mixture 79:21	Fiber	≈116.34 (L)
$I_{11}B$	I11B1	✓							100% natural	Square	267.95x248.20
	I11B2	✓		✓		✓			mixture 65:2510	Square	264.07x251.84
	I11B3	✓						✓	mixture 60:40	Fiber	>721.36 (L)
	I11B4	✓						✓	mixture 60:40	Fiber	>739.67 (L)
	I11B5	✓							100% natural	Fiber	-
	I11B6			✓		✓	✓		mixture 33.33:33.33:33.33	Fiber	>1017.53 (L)
	I11B7			✓		✓	✓		mixture 33.33:33.33:33.33	Fiber	>502.26
	I11B8			✓		✓	✓		mixture 33.33:33.33:33.33	Fiber	>845.54

A total of 236 (181 from conventional yogurt and 55 from organic yogurt) microparticles were analyzed by micro-FTIR technique. Following the analysis, they were divided into natural and synthetic. Natural microparticles are those that have a 100% natural composition or a mixture of natural fibers (for example, cotton, linen, cellulose, and wool) according to Tables 7 and 8. Synthetic microparticles are those that have in their composition a mixture of natural and polymeric compounds or are 100% polymers (acrylic, nylon, elastane, polyester, polyethylene, or polyurethane).

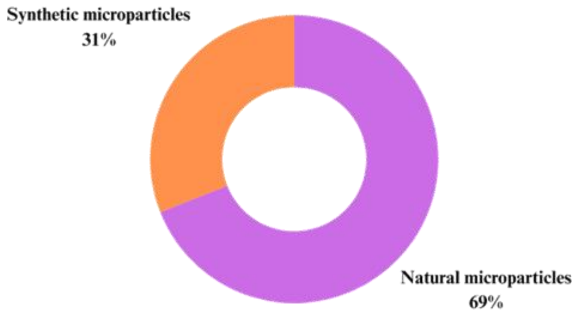


Figure 11. The nature of microparticles identified by the micro-FTIR technique in conventional yogurt samples.

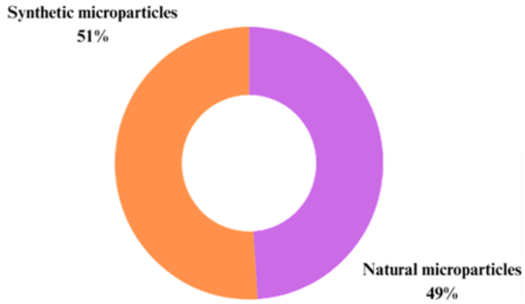


Figure 12. The nature of microparticles identified by the micro-FTIR technique in organic yogurt samples.

According to Figs. 11 and 12, organic yogurt had the highest distribution of synthetic microparticles (51% in organic yogurt and 31% in conventional yogurt). In terms of natural microparticles, conventional yogurt had the highest distribution (69%), while organic yogurt showed 49% natural microparticles.

Each microparticle was placed in a size class according to its dimension. In Table 9, based strictly on the microparticles dimension it was considered justified the use of a color code class to define those microparticles which could add a higher risk for human health. In this respect, the green color refers to the lowest health risk, while the red is attributed to microparticles at the highest risk.

Table 9. Size classes

Category	Size class C_x [μm]				
	[C_A] <50	[C_B] 50-100	[C_C] 100-500	[C_D] 500-1000	[C_E] >1000
Organic Yogurt	3	4	23	24	1
Conventional Yogurt	22	16	70	65	7

Complementary, the discussion may include the amount and the chemical structure of microparticles, which definitely could modify the health risk scale. Therefore, for the C_A class, 22 microparticles having a length under 50 μm came from conventional yogurt and 3 from organic yogurt; 16 microparticles from conventional yogurt and 4 from organic yogurt were attributed to class C_B; 70 microparticles detected in conventional yogurt along with 23 microparticles detected in organic yogurt are the subject of C_C class. The last two classes (C_D and C_E) registered 65 microparticles which were having as source the conventional yogurt and 7 microparticles the organic one, respectively 24 originated from conventional yogurt, and 1 from organic one.

All 236 microparticles analyzed by the FTIR technique were also characterized in terms of shape (Fig. 13). Seven different shapes were assigned to them (oval, irregular, trapeze, square, rectangle, circle, and triangle), as well as the term, "fiber".

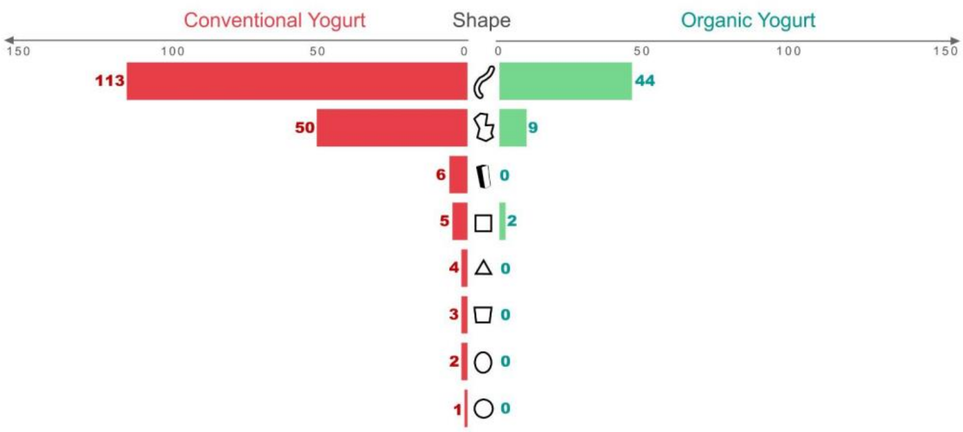


Figure 13. The shape distribution (attributed to microplastics identified in conventional and organic yogurt)

In both varieties of yogurt (conventional and organic), the identified fibers were numerous (113 fibers in the conventional yogurt and 44 in the organic one). Regarding their shape, 50 irregular fragments were identified in the conventional yogurt and 44 in the organic yogurt. The fragments identified in the conventional yogurt samples had the following shapes: oval (2), trapezoid (2), square (5), rectangular (6), circle (1) and triangular (1). No oval, rectangular, circular, or triangular fragments were identified in the organic yogurt. The content of microparticles in the yogurt samples (conventional and organic) is very variable so in the conventional yogurt samples, there was a variation from 400 to 4600 microparticles/kg (Table 5), as for the organic yogurt (from 800 to 4400 microparticles /kg) (Table 6). The pollution loading index (PLI) was calculated for each sample for the two yogurt categories using equation (1).

Figure 14. Pollution load index (PLI) for analyzed conventional yogurt samples

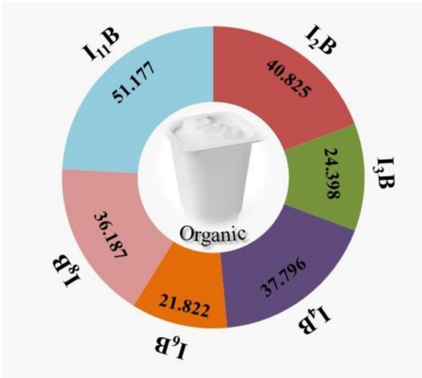


Figure 15. Pollution load index (PLI) for analyzed organic yogurt samples

The pollution loading index (PLI) varied in the conventional yogurt samples from 15,430 (sample I_7) to 52,327 (sample I_2), while for the organic yogurt samples, they were from 21,822 (sample I_6B) to 51,177 (sample $I_{11}B$). The colors used in Figs. 14 and 15 indicate the level of pollution (Table 3). In conventional yogurt, four samples (I_1, I_2, I_4, and I_{11}) show a very high risk, samples I_3 (the sample intended for children) and I_9 have a high risk, while samples (I_5, I_6, I_8, and I_{10}) and sample I_7 presents a medium and low risk.

In the case of organic yogurt, samples I_2B and $I_{11}B$ have a very high risk, samples I_4B and I_8B have a high risk, while samples I_3B (the sample intended for children) and I_6B present a medium risk.

Based on equation (2), was calculated the daily intake of microparticles; the results are presented in Fig. 16 for adults and Fig. 17 for children.

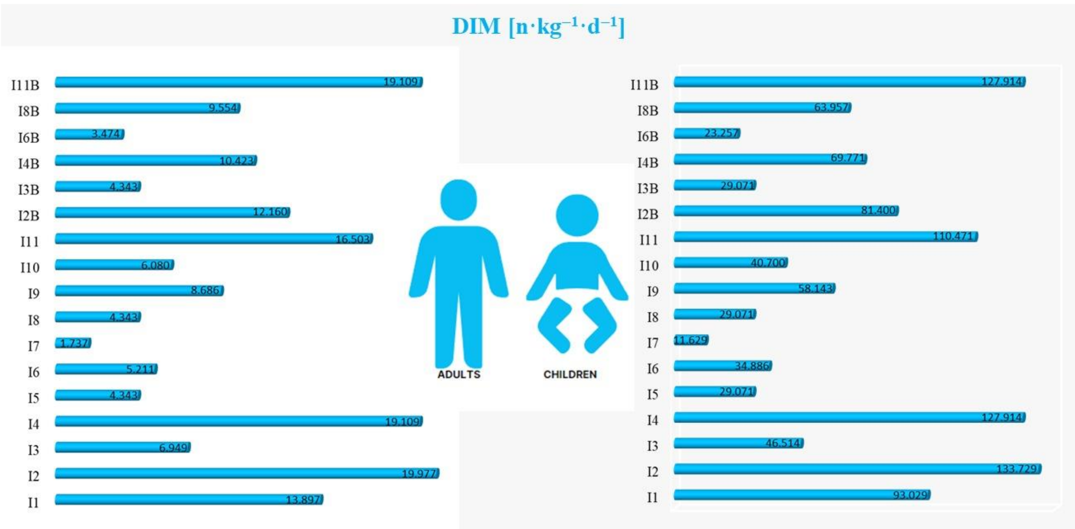


Figure 16. DIM values for adults from yogurt samples.

Figure 17. DIM values for children from yogurt samples.

Conventional yogurt recorded a DIM value for children between 12.443 (n/(kg·d)) and 143.090 (n/(kg·d)), and as for adults between 1.859 (n/(kg·d)) and 21.376 (n/(kg·d)). For organic yogurt, the DIM value recorded for children ranged between 24.885 (n/(kg·d)) and

136.868 (n/(kg·d)), also for adults the recorded value was between 3.717 (n/(kg·d)) and 20.446 (n/(kg·d)).

Performing the Pearson correlation between the variables "type of fiber" and "source of occurrence" highlights an average relationship of dependence (R=0.540), in this case, it can be stated that the type of fiber is influenced by the source of occurrence (Table 10).

Table 10. Correlations

		Type of Fiber	Occurrence source
Pearson Correlation	Type of Fiber	1.000	.540
	Occurrence source	.540	1.000
Sig. (1-tailed)	Type of Fiber	.	.000
	Occurrence source	.000	.
N	Type of Fiber	86	86
	Occurrence source	86	86

With the help of the regression equation, the coefficient of determination can be observed, namely $R^2 = 0.292$, which proves that 29.20% of the variation of the dependent variable "type of fiber" can be explained as function of "source of occurrence" (independent variable) of microparticles (Table 11).

Table 11. 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	.540[a]	.292	.283	29.695	.292	34.590	1	84	.000	1.775

a. Predictors: (Constant), Occurrence source

b. Dependent Variable: Type of Fiber

To identify relatively homogeneous groupings based on predefined characteristics (Microparticle composition according to the OPUS library), hierarchical cluster analysis was performed for 103 microparticles identified in conventional and organic yogurt samples, according to Tables 7 and 8. Figs. 18 and 19 present the cluster analysis for the analyzed samples, based on the variables: type and nature of microparticles. The synthetic fibers that had in their composition acrylic, nylon, elastane, cellophane, polyester, polyurethane, and polyethylene were selected. The dendogram was made from 57 microparticles analyzed by the micro-FTIR technique from the category of conventional yogurt, and to create the dendogram for the microparticles identified in the organic yogurt samples, 28 microparticles were taken into account.

The cluster analysis made it possible to group the microparticles identified in the conventional yogurt samples according to the type of fibers considered synthetic and group them according to the composition of the mixtures. Cluster analysis grouped the 57 samples out of 181 into fourteen clusters. For blends "Cotton & Elastane", "Cotton, Acrylic, Nylon & Cellulose", "Cotton, Nylon & Cellulose", "Cotton & Nylon", "Cotton, Acrylic & Flax", "Cotton & Acrylic", "Cotton, Acrylic, Nylon & Wool", "Cotton, Nylon, Elastane & Cellulose", and "Cotton & Polyester", the potential sources of the appearance of microparticles in conventional yogurt are: the textile materials used in the sanitization and cleaning of the udders of the animals, the clothing of the workers who operate and handles the raw material (milk) from the farms where the milk comes from, but also from dumping waste in inappropriate spaces (ponds, rivers, fields). For the previously mentioned clusters, 51 samples were grouped out of a total of 57, and the difference of 6 samples was also grouped into four clusters: "Polyester", "Polyurethane", "Polyethylene", and "Cellophane". The potential sources of the origin of the polymers are the plastic materials used to make different types of packaging. Storing the packaging in places not in areas close to animals may accidentally become animal feed, as it is well-known that ruminants feed on almost anything (including plastic packaging).

For the mixture "Nylon, Cellulose & Polyester" and "Polyester", respectively, four and two microparticles identified in the organic yogurt samples were grouped. For the two grouped mixtures, the potential sources of origin are textile materials used in the hygiene and udder cleaning process of animals, as well as the clothing of the workers who operate or handle the raw material (milk) in the milk origin farms; the plastic packaging used in the packaging of the finished products, and so on. For the blends "Cotton & Acrylic", "Cotton, Acrylic & Flax", "Cotton & Elastan", and "Cotton, Nylon & Cellulose", the samples were grouped as follows: (1) 10 microparticles, (2) 1 micropatch, (3) 3 microparticles, and (4) 8 microparticles. Clothing, textiles, and packaging are potential sources of microparticles.

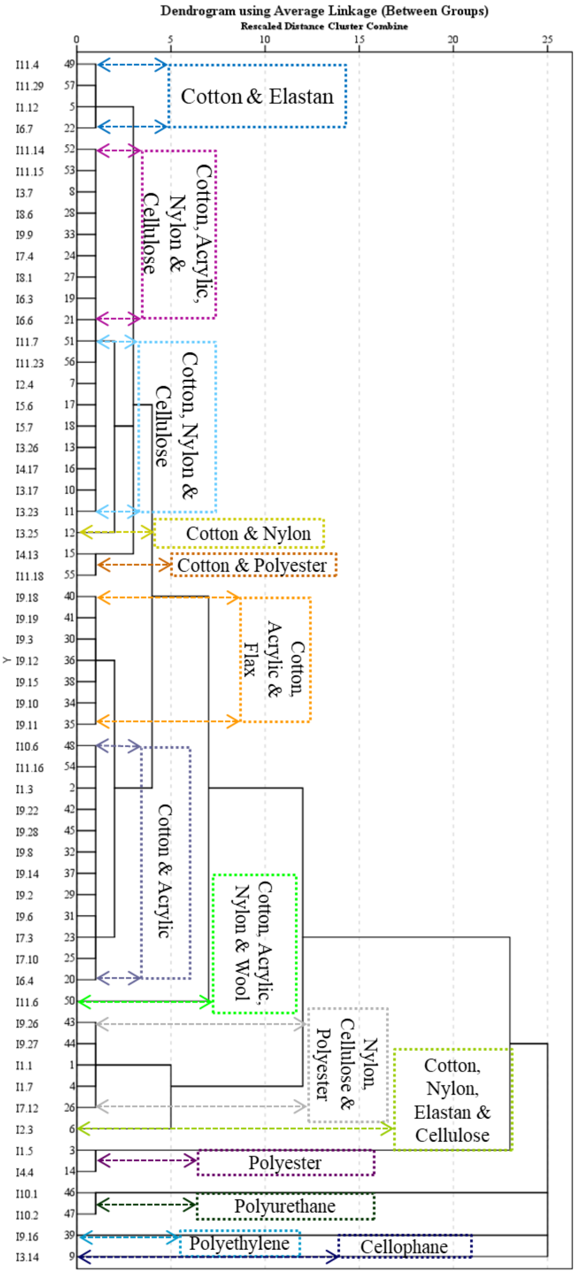


Figure 18. Dendrogram plot tab - synthetic microparticles identified in the conventional yogurt.

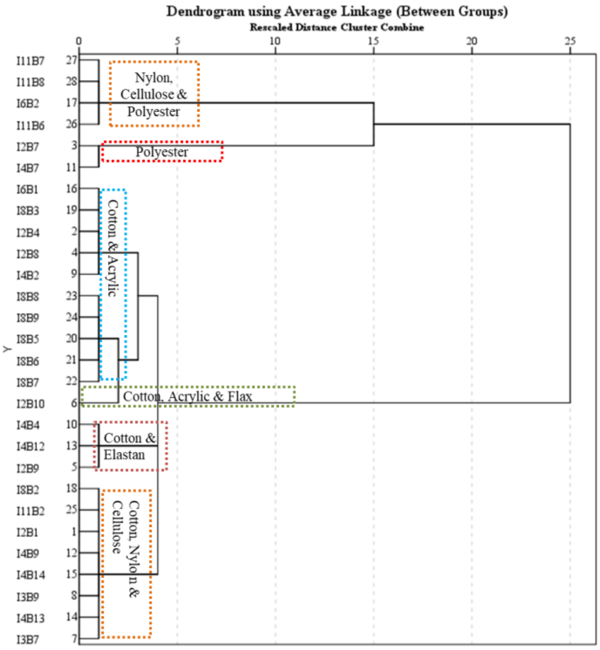


Figure 19. Dendrogram plot tab - synthetic microparticles identified in the organic yogurt.

The milk processing factories, like many other industries, are in constant development thus, the big producers but not only them, are investing in process automation, making the involvement of human resources to be as minimal as possible. Inside an automated factory, the contamination with microplastics due to human error decreases considerably, so the only weakness is still given by the raw milk quality as well as by the effects generated by the animal's feeding environment and the food.

4. CONCLUSIONS

The present study confirms the presence of natural and synthetic microparticles in fermented dairy products (conventional and organic yogurt) isolated according to the method described in the patent application [26]. They were also characterized from a physical and chemical point of view. All analyzed samples (17 samples) showed variations in number, color, shape, size, and type of fiber (natural or synthetic). The composition of the microparticles was established according to the OPUS v.7.5 library. It was admitted natural fibers as those microparticles that had in their composition only organic materials (cotton, cellulose, flax, and wool), while synthetic ones (microplastics) those microparticles of nylon, polyester, acrylic, elastane, etc., but also the ones which show mixtures with natural fibers.

Following this study, the authors showed a high potential risk for human health based on the number of microparticles detected/kg in 17 samples of yogurt. For conventional yogurt, the smallest amount of microparticles was detected for sample I_7 (400 microparticles/kg) while the highest was recorded for sample I_2 (4600 microparticles/kg). In the case of organic yogurt, the microparticle content was similar and ranged between 800 and 4400 microparticles/kg (samples I_6B and $I_{11}B$, respectively).

After establishing the type of microplastics detected, it was continued by referring to the potential sources of their origin, starting in this process with the module for collecting and processing the milk used and until obtaining the finished product.

The overall number of microparticles was equal to 38200 (distributed in 17 kg of yogurt), from which 14562 were natural fibers, and 23638 were synthetic fibers (microplastics). Concerning the pollution load index (PLI), all 17 samples were classified according to their hazard level as follows: 6 samples in the very high level, 4 samples in the high level, 6 samples in the medium level, and 1 sample in the low level.

The absence of knowledge and procedures/standards regarding microplastic analysis is a challenge for the future. In this sense, this study was intended as a starting point for future research referring to the presence of MPs in milk-based foods and for achieving a better assessment of potential human exposures and risks to human health.

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