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Microplastics in Honey, Beer, Milk and Refreshments in Ecuador as Emerging Contaminants

Milene F. Diaz-Basantes [1], Juan A. Conesa [2,*] and Andres Fullana [2]

[1] Department of Food Technology, Central University of Ecuador, Quito EC1701, Ecuador; mfdiaz@uce.edu.ec
[2] Department of Chemical Engineering, University of Alicante, 03690 Alicante, Spain; andres.fullana@ua.es
* Correspondence: ja.conesa@ua.es

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Abstract: According to the latest research, marine products have the greatest potential for microplastic (MPs) contamination. Therefore, their presence in terrestrial food has not managed to attract much attention—despite the fact that in the future they may represent a serious environmental risk. Research conducted in Europe and the US has indicated the presence of MPs in tap water, bottled water, table salt, honey, beer and snails for human consumption. The presence of MPs in food has not yet been evaluated in Latin America. This work focused on evaluating two widely consumed beverages: milk and soft drinks. Furthermore, honey and beer samples were analyzed and compared to findings in the literature. All products were sourced in Ecuador. In order to determine correlations with the intensity of anthropogenic activity, samples of both industrially processed and craft products were studied. For the analysis, an improvement of previous techniques used to determine MPs in honey was applied. This technique uses microfiltration followed by degradation of organic matter with hydrogen peroxide—and finally, continuous rinsing with deionized water. Size ranges were established between 0.8–200 mm. The number of microplastics found was between 10 and 100 MPs/L, with an average of around 40 MPs/L. The sizes of the particles found in the study are in the range of 13.45 and 6742.48 μm for the fibers, and between 2.48 and 247.54 μm for the fragments. From the composition analysis carried out with FTIR, we were able to confirm the presence of 12% of microplastic. The results generally showed a greater presence of MPs compared to those registered in Europe, probably due to processing methods rather than environmental pollution. Regarding composition, the main microplastics found were polyethylene, polypropylene and polyacrylamide.

Keywords: liquid food; drinks; craft process; industrial process; food safety; beverages

1. Introduction

For a long time, plastics have made our lives easier. They are used in almost all daily activities. Their presence in different forms, such as different synthetized materials, has also caused harm to the environment [1]. Every day, articles for human use—from simple ornaments to complicated high-scale structures—are generated from synthetic polymers [2]. The shelf life of these items is varied: some elements have an extended use and others are of single and ephemeral use, such as packaging and disposable utensils. The latter has a higher impact on the environment, their short life requires controlled disposal, but their actual final discarding is the result of insufficient policy measures on the management of solids. Recycling processes are unattractive in many countries, which is why plastic waste ultimately ends up in regional and continental aquatic sinks [3].

Due to its versatility and availability for human use, plastic is regarded as one of the best food-conservation materials, based on economic and sustainable considerations. The presence of plastic in the environment, however, has made it an enemy of humanity [4,5]. The way plastics are processed present considerable potential. Today, plastics are even considered a potential innovation in the construction sector, in industry generally and in the food conservation industry, even under consumers' critical gaze [6].

According to PlasticsEurope, as of October 2019, gross plastic production amounted to 359 million tons per year, with at least 32.5% of demand being covered using recycled plastic [2]. Most of the plastic wastes and their debris are released into the marine environment, which acts as a final sink. However, on their way to the oceans and seas, the particles can remain suspended in the air and continental areas. They can also be deposited into countless foods, processed or not. Added to this are the millions of fibers and synthetic particles released from the wear of clothing which is subject to the erosive activity of washers and dryers [7].

The presence of microplastics in wastewaters has two main sources: a primary source that comes from the granular raw material called "mermaid tears" or "nurdles" used to mold the new plastic items, as well as microparticles of polymers added in cosmetic products as exfoliants and abrasives; and the secondary source corresponds to the erosion and fragmentation of larger plastics [6]. Those fragments, fibers, granules, flakes and pellets that measure between one nanometer and five millimeters long are called microplastics [8]. Particles this size present the specific surface allowing to convert them into vectors of contamination of a broad spectrum of toxics and microorganisms.

Some scientists have downplayed their importance by comparing the possible incidence of microplastics on human health with that of other persistent microparticles in the environment. However, microplastics have attracted increasing attention at the end of this decade because of their constant growth and their toxic effects [9–12].

Currently, due to the difficulty in identifying the progressive consequences of their presence, microplastics have been classified as emerging pollutants. Their generalized presence and their capacity to be a vector of pollution are considered reasons for them to be listed in health alerts issued by regulatory entities [8,13,14].

The effects suggested as high incidence reported from micro and nanoplastics, range from the persistence within digestive systems of the beings that ingest it, to the derivation of toxic effects of different chemical compounds that accompany them and that against degradation they are exposed and of those toxic solutes and pathogenic bacteria present in the sea and that adhere to their high specific surface [15–18].

Among the toxicological effects of microplastic adjuncts, such as PBA, retardants such as bisphenol A and pigments, hormonal disrupting action, inhibition of appetite, impaired neurological and metabolic development, cancer stimulators, as well as generation of the immune system disorders have been reported [19–21].

Jiang [22] in his research has shown that the entry of MPs into biotic organisms can cause physical and oxidative stress, necrosis, apoptosis, inflammation and other immune responses.

A study by Toussant [23] records information from at least 200 food research in general, with an emphasis on edible marine animals, such as fish, bivalves, crustaceans, marine mammals, turtles and shorebirds, which constitute a representative contribution to the contamination of food chains in general. The evaluation of terrestrial food is still poor as mentioned by several regulatory entities.

Furthermore, atmospheric currents carry debris to the terrestrial environment, which also feeds the marine sink, though they first leave their mark on the continents. To assess the incidence of microplastics, different researchers studied honey, beer and table salt in Europe and China. Bottled and tap water was assessed on all continents by an American research group, who demonstrated that the presence of plastic can begin to compete with greenhouse gases in terms of ubiquity and impact on environmental deterioration [24].

Evidence of the significance of microplastics in the continental environment has not yet been determined. The main reason lies in the difficulties of identification and quantification as identification methods are costly and time-consuming. The most accessible method is optical and electronic microscopy, a methodology that can be error-sensitive and requires confirmation via instrumental methods that validate the microparticle's composition. Moreover, the toxic composition analysis is cumbersome in all cases [17–20].

Plastics' high resistance to degradation allows them to be notably resilient in nature. The marine food chain is not the only food chain to be contaminated due to the microplastics intake by the lower levels of zooplankton [21]. Pollution of terrestrial food has also been recorded, although only in a limited number of studies. Liebezeit et al. already considered the presence of microplastics in honey and beer, terrestrial foods that have no relation to the marine chain [25–27].

Many MPs have also been observed in the air, including synthetic polymers from various anthropogenic sources. The friction of components during the different stages of food technology, the microparticles from the degradation of clothes in washing and drying processes that spread to the environment are the simplest source of contribution of microplastics to unprocessed and industrialized foods [10,28–33].

The diversity and variable magnitude of air currents contribute to distributing all the microparticles [31]. They can settle into aquatic sinks and terrestrial cultivation areas or industrial production. Industrial processes, despite their constant quality controls, may unintentionally allow for the presence of microplastics. Microparticle control regulations applying to industries do not consider the dimensions of these microparticles in industrial environments [34]. Regulations exist for particulates measuring less than 10 and 2.5 micrometers in diameter; the control methods, however, are limited. The control of microplastics in craft processes is even more critical, as the investment required makes it unprofitable. Factors such as plant location in relation to anthropogenic activities and climatic conditions are little considered when assessing the presence of MPs within industrial foods.

Currently there are few studies on microplastic contamination in the terrestrial food chain, updated information refers to the presence of microplastics in tap water [10], bottled water [35], table salt [36], honey [27], beer [26] and snails for human consumption [37]. Due to insufficient data and food studied, regulatory agencies request more information [38]. This situation has led us to focus this research on determining the presence of microplastics in industrially and handcrafted packaged and processed liquid foods.

2. Materials and Methods

2.1. Sample Collection

Samples analyzed in the present work were natural source fluids (milk and honey) and liquid foods available on the Ecuadorian market that require water as raw material (beer and soft drinks). A total of 14 soft drink trademarks, 15 beer samples (8 industrial and 7 craft), 10 skim milk samples and 14 honey samples packaged by hand and packaged industrially were collected. Table 1 records the characteristics of the samples analyzed. The production processes of each food analyzed allowed identifying the possible sources of contamination as well as the interaction of the sources of pollution with each raw material and technological service.

Table 1. Characteristics of the analyzed samples.

Food	Analyzed Sample Volume (mL)	Specific Characteristics
Skim milk	1000	Fat < 1% wt, packed in polyethylene covers or Tetra Pak
Honey	700	Honey packaged by hand or industrially in glass containers
Refreshments	500	Refreshing drink with citrus flavor to orange or lemon packaged in PET bottle or Tetra Pak
Beer	750	Lage industrial or artisanal beer bottled in glass

In this investigation, the different samples were taken considering the highest possible homogeneity and reducing the contribution of microplastics through the container as much as possible. In the case of honey and beer, samples packed in glass were preferred. To offer more information on the characteristics of the analyzed foods, Table 1 is included.

In the case of beverages and milk, the available presentations were packaged in polyethylene, so the presence of another type of polymer in the microplastics would indicate that the origin on the contamination is not only the packaging but other points in the production process.

The influence of air pollution is evaluated by looking for the relationship with atmospheric conditions. By analyzing the location of processing plants, considering industrial or craft categories and the characteristics of anthropogenic activity close to the production plants, we assessed the influence of the geographical region on the pollution of processing areas, adjusted to regional weather conditions. Figure 1 shows the regional distribution of the different processing and/or packaging plants of the food under study.

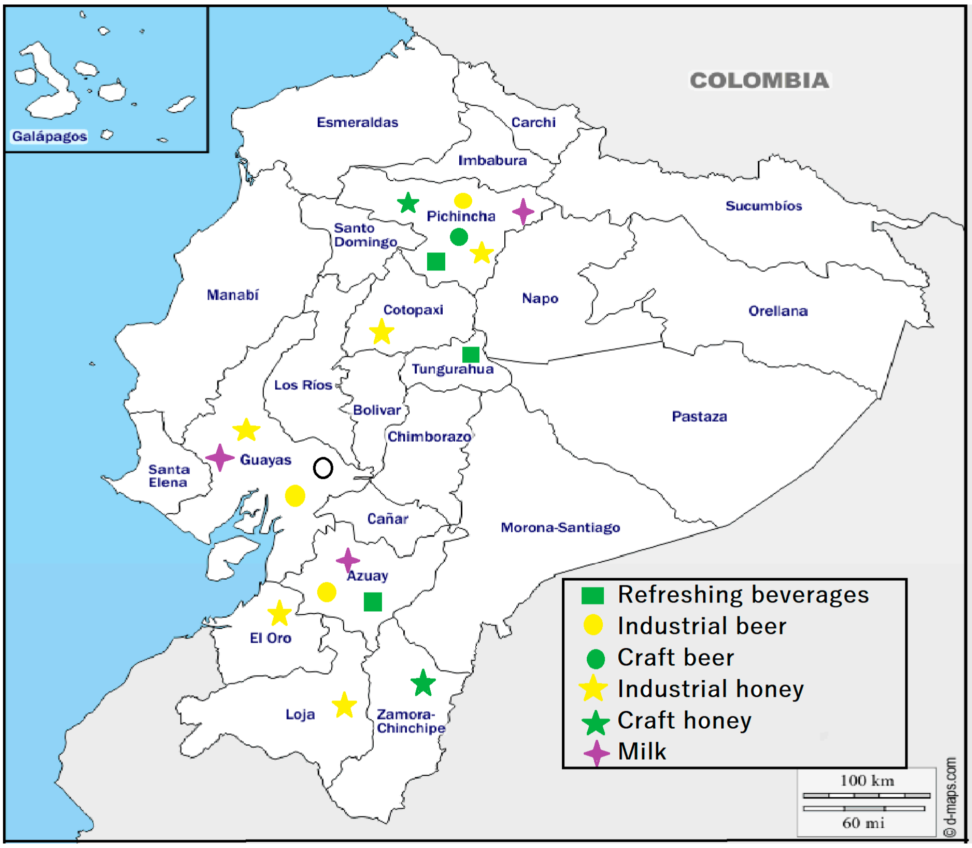
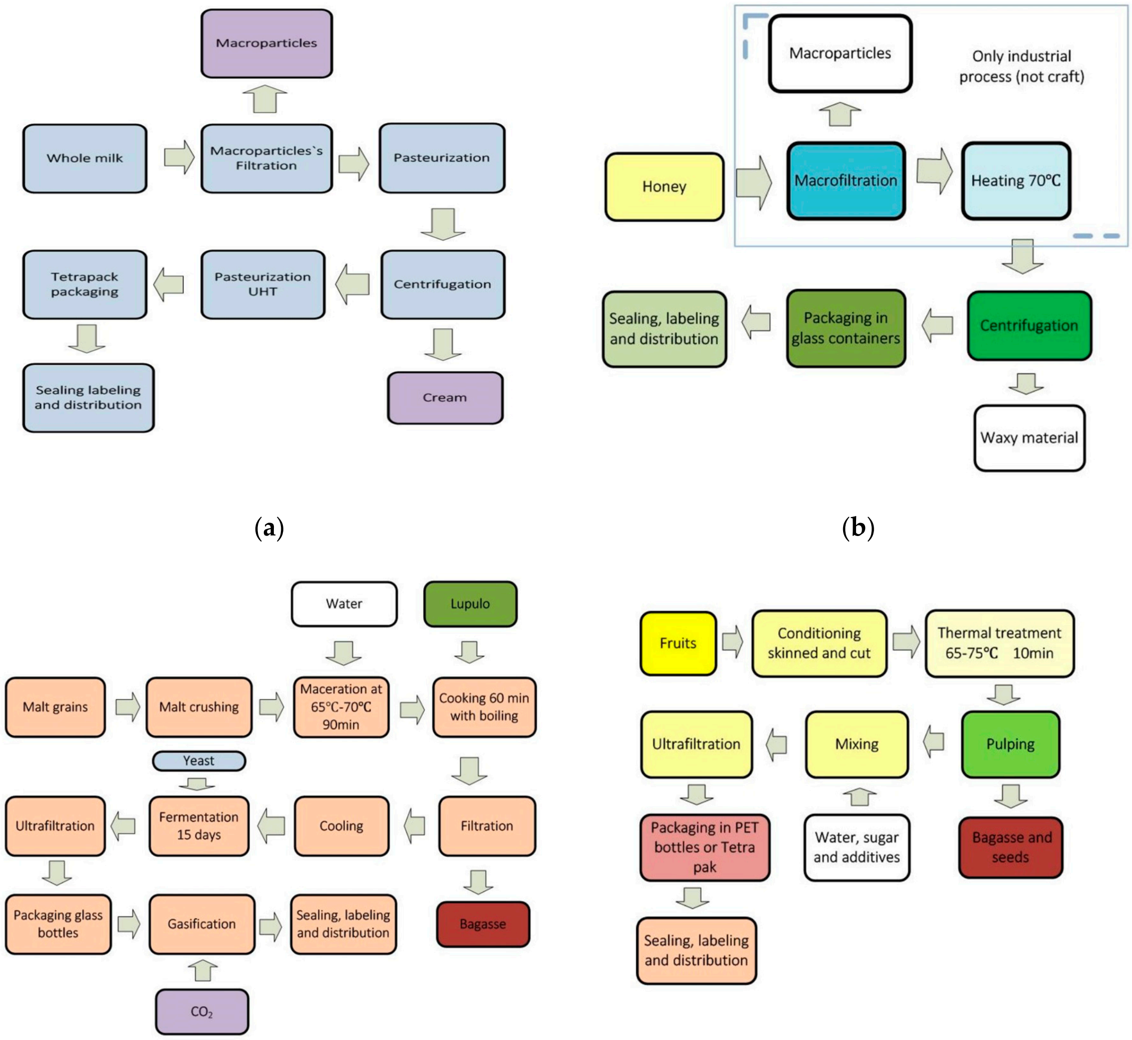


Figure 1. Geographical location of the production sites of the samples analyzed in Republic of Ecuador. Resource: Free maps by https://d-maps.com/carte.php?num_car=3402&lang=es. Beer and honey were evaluated considering the industrial and craft processes (batch processes developed by small producers with minimum control conditions). For soft drinks and milk are exclusively industrial. The samples of beer obtained are those available in the Ecuadorian market.

Figure 2 shows the production processes of skim milk, honey, beer and soft drinks with their respective streams, possible vectors of contamination by MPs in the process. Beer and soft drinks use water, which can be considered their main source of microplastics.



(**a**) (**b**)

(**c**) (**d**)

Figure 2. Production processes of samples tested: (**a**) Industrial process of skimmed milk packed; (**b**) process of honey packed, industrial and craft; (**c**) industrial process for beer production; (**d**) industrial process for citric refreshing beverages.

2.2. Experimental Procedure

A 500-mL portion taken of each sample of milk, beer or beverages. A dilution 1:1 with 500 mL type I distilled water (HPLC grade) was prepared. These solutions were also passed through a 250 µm brass/stainless sieve (Gilson Company Inc., Lewis Center, OH, USA), to ensure the removal of thick material that prevents filtration. A copious wash was performed on the sieve with 1 L of type I distilled water. The washing liquid and the filtered liquid were retrieved and 200 mL of H_2O_2 (30 vol%) were added to allow degradation by organic matter oxidation in 72 h. The degraded solution was filtered again through the 250 µm brass/stainless sieve, adapting the technique proposed by several researchers [31–33]. This procedure ensures that the residual organic matter has a very small diameter and does not obstruct the filter media. Subsequently, vacuum filtration was carried out at 55 °C through a 47-mm nylon membrane and a 10-µm pore in order to define a microparticle size range. Each filter used was submerged in 25 mL of absolute ethanol and subjected to ultrasound for 1 h; this procedure allowed evaporating the alcohol. The filter is then submerged in 25 mL of distilled water and sonicated for 15 min at 55 °C to release the particles from the filter. The liquid obtained was filtered through a PTFE (polytetrafluoroethylene) membrane of 1 µm and 50 mm, and the process ended with a copious rinse. The filter used was stored in a Petri box, washed with alcohol and dried in a stove at 50 °C or allowed to dry at room temperature. The honey sample was prepared following the same methodology but at 70 °C [27].

2.3. Determination of Number, Size and Composition of Microparticles

The presence, amount and measurement of the microparticles, whether fibers or fragments, were determined by visual examination with a 10x lens in an inverted microscope of the AmScope brand, with the filter between two glass plates. Fibers are defined as particles of one-dimensional structures whose length far exceeds their diameter and the fragments corresponding to a particle without a defined format whose relationship between its dimensions is minimal [39].

The chemical composition of the particles was defined using the sensitivity of an FT-IR system connected to a microscope, an analytical instrument recommended by several researchers [19,36,40]. Given the countless number of microparticles, a random sample of 10 particles was taken from each filter and its chemical composition was identified. This information was used to extrapolate to the particles' universe.

2.4. Pollution Prevention

To ensure minimal environmental influence on the samples analyzed, the laboratory air was previously cleaned with a Rainbow brand air purifier and the presence of microparticles in the environment and reagents used in the analysis were evaluated. In addition, all processes were performed within a horizontal laminar flow. Blank runs where performed by following the same procedure but with only filter papers. Amounts between 4–9 fibers were found in these blank runs.

3. Results

The results obtained show that the previous tests collaborated with the adequate extraction of the microparticles. Different types of food were tested to support the choice of the methodology used. These tests allowed us to select food samples that were less difficult to analyze, some complications were: in milk, the lipids present slow down the filtration process due to its coalescence capacity. Similarly, the presence of fiber in soft drinks limits the filtration and the degradation was very complex using reagents. In the case of honey, the presence of waxy honey material blocked the pores of the filters, which was compensated by increasing the temperature, it was not necessary to apply lipid reducers, such as lipases or other methods applied to digestion. In the analysis of the beer sample, the industrial or craft origin of the beer was a parameter that influenced the resistance to the filtration process. Insufficient filtration applied to craft beer reduces the ease of filtration. This drink needs a yeast residue to maintain its turbidity and quality characteristics, which requires some care in the production process [40]. These interferences behave in the same way as organic matter that makes the separation of microparticles difficult, as mentioned in the literature [41]. Given these disadvantages, we decided to consider only low-fiber and low-fat milk samples; in the case of honey, no segregation could be performed.

Table 2 reports for each type of food evaluated, the results obtained in quantity and composition related to the microparticles extracted, referring to the volume analyzed. Table 2 also includes data related to the geographical and demographic situation of each place of processing and/or packaging [41–44].

The observed microparticles were classified as fragments and fibers (Table 2), of which a significant and random sample of particles was taken to perform an infrared analysis using FTIR. From these analyses, only 12% of the particles confirmed some plastic composition. The microplastic fragments have dimensions between 2.48–247.54 micrometers and the fibers are in a range of 13.45–6742.48 micrometers.

Table 2. Results of the amount and nature of microparticles found in the different food samples and characteristics of the production site.

Food Sample	Sample No.	Fibers/Liter	* Fiber Particle Size µm	Fragments/Liter	** Fragment Particle Size µm	Total Microparticles	MPs	City of Producer	Population	Type of Process	Type of Sample	Weather Characteristics		Height (m ASL)
												Geographic Weather	Winds (km/h)	
Skim milk	1	116	19.94–1447.34	212	4.48–183.37	328	39	Ibarra	139,721	Industrial	Urban	Tempered	8.3	2220
	2	122	28.45–2329.41	222	3.77–149.11	344	41	Quito	2,690,150	Industrial	Urban	Subtropical	7.4	2800
	3	74	250.53–1175.25	206	3.10–149.20	280	34	Machachi	27,623	Industrial	Urban	Cold	7.8	2945
	4	142	58.31–2127.49	284	5.98–169.77	426	51	Sangolquí	81,140	Industrial	Urban	Subtropical	8.3	2500
	5	144	46.33–2410.05	194	5.52–130.28	338	41	Sangolquí	81,140	Industrial	Urban	Subtropical	8.3	2500
	6	152	119.44–1789.13	230	4.96–81.49	382	46	Tanicuchi	12,831	Industrial	Urban	Cold	9.1	2750
	7	196	79.97–6742.48	236	2.48–86.16	432	52	Cayambe	50,829	Industrial	Urban	Tempered	15	2830
	8	254	83.38–2137.91	190	5.81–101.62	444	53	Cuenca	331,888	Industrial	Urban	Tempered	9.7	2550
	9	86	32.37–1845.67	168	6.28–99.19	254	30	Guayaquil	2,291,158	Industrial	Urban	Hot	8.3	4
	10	34	154.72–3359.87	100	4.19–87.75	134	16	Machachi	27,623	Industrial	Urban	Cold	7.8	2945
Industrial Honey	11	100	166.05–966.42	530	5.63–173.58	630	76	Guayaquil	2,291,158	Industrial	Urban	Hot	10	4
	12	56	181.65–1388.24	190	12.88–139	246	30	Loja	180,617	Industrial	Urban	Hot tempered	13	2065
	13	56	208.79–1063.04	126	14.88–182.96	182	22	Tabacundo	16,403	Industrial	Urban	Cold	6	2877
	14	20	132.12–548.76	552	7.53–83.99	572	69	Machala	241,606	Industrial	Urban	Tropical hot	8	6
	15	76	140.92–3302.68	494	13.38–161.0	570	68	Quito	2,690,150	Industrial	Urban	Subtropical	2	2800
	16	152	264.72–2518.02	278	11.54–88.55	430	52	Quito	2,690,150	Industrial	Urban	Subtropical	2	2800
	17	166	67.18–250.583	350	9.22–129.02	516	62	Tabacundo	16,403	Industrial	Urban	Cold	6	2877
Craft Honey	18	116	106.56–1705.28	342	5.15–175.39	458	55	Tambillo	8319	Craft	Rural	Humid tropical	3	2800
	19	178	84.95–5174.01	678	11.81–139.44	856	103	Guayllabamba	16,213	Craft	Rural	Dry	7	2171
	20	104	394.1–2398.4	798	12.09–226.01	902	108	Tumbaco	49,944	Craft	Urban	Hot	0	2355
	21	134	169.97–2709.71	200	26.79–199.16	334	40	El Chaupi	1456	Craft	Rural	Cold	7.5	3163
	22	82	186.09–1936.77	246	17.54–69.61	328	39	Lasso	1635	Craft	Rural	Cold	18	3038
	23	98	96.44–2566.84	202	19.44–146.46	300	36	Tanicuchi	12,831	Craft	Rural	Cold	18	3849
	24	126	229.95–1630.12	828	12.9–213.7	954	114	Salcedo	53,216	Craft	Urban	Hot tempered	7	2683
	25	106	240.4–2248.01	254	14.86–159.39	360	43	Los Encuentros	3658	Craft	Rural	Desertic	8	822
Refreshing beverage	26	144	63.85–2224.25	350	5.94–145.81	494	59	Guayaquil	2,291,158	Industrial	Urban	Hot	11	4
	27	68	89.64–1015.9	290	8.44–154.69	358	43	Guayaquil	2,291,158	Industrial	Urban	Hot	11	4
	28	36	169.5–1049.18	272	7.57–228.02	308	37	Pelileo	56,573	Industrial	Urban	Tempered	14	2600
	29	54	56.22–2096.24	242	9.05–127.90	296	36	Guayaquil	2,291,158	Industrial	Urban	Hot	11	4
	30	56	67.89–848.98	190	5.62–165.95	246	30	Machachi	27,623	Industrial	Urban	Cold	8	2945
	31	88	15.64–1181.37	178	5.47–247.54	266	32	Quito	2,690,150	Industrial	Urban	Subtropical	3	2800
	32	94	30.95–1166.96	188	8.24–145.25	282	34	Quito	2,690,150	Industrial	Urban	Subtropical	3	2800
	33	32	47.2–819.49	118	7.60–67.56	150	18	Cuenca	331,888	Industrial	Urban	Tempered	16	2550
	34	62	18.16–931.35	180	6.69–65.88	242	29	Guayaquil	2,291,158	Industrial	Urban	Hot	11	4
	35	38	59.29–1130.91	198	8.47–73.25	236	28	Cayambe	50,829	Industrial	Urban	Tempered	15	2830
	36	10	104.6–1446.02	58	7.35–81.59	68	8	Cayambe	50,829	Industrial	Urban	Tempered	15	2830
	37	48	48.63–717.11	140	6.84–69.47	188	23	Guayaquil	2,291,158	Industrial	Urban	Hot	11	4
	38	96	21.92–1174.66	152	8.60–204.05	248	30	Machachi	27,623	Industrial	Urban	Cold	8	2945
	39	66	105.06–2101.67	288	7.01–93.47	354	42	Guayaquil	2,291,158	Industrial	Urban	Hot	11	4

Table 2. *Cont.*

Food Sample	Sample No.	Fibers/Liter	* Fiber Particle Size µm	Fragments/Liter	** Fragment Particle Size µm	Total Microparticles	MPs	City of Producer	Population	Type of Process	Type of Sample	Weather Characteristics		Height (m ASL)
												Geographic Weather	Winds (km/h)	
Industrial Beer	40	60	59.52–1740.24	182	3.505–186.4	242	29	Cumbayá	31,463	Industrial	Suburban	Subtropical	15	2200
	41	98	13.45–1075.55	304	4.97–202.29	402	48	Guayaquil	2,291,158	Industrial	Urban	Hot	11	4
	42	40	36.34–1076.31	396	9.54–131.8	436	52	Guayaquil	2,291,158	Industrial	Urban	Hot	11	4
	43	58	26.32–1388.43	354	8.55–140.995	412	49	Guayaquil	2,291,158	Industrial	Urban	Hot	11	4
	44	18	35.69–588.34	310	8.445–177.48	328	39	Cumbayá	31,463	Industrial	Suburban	Subtropical	15	2200
	45	18	59.12–427.82	496	6.75–200.23	514	62	Guayaquil	2,291,158	Industrial	Urban	Hot	11	4
Craft Beer	46	12	155.01–769.8	110	6.155–155.8	122	15	Puembo	18,000	Craft	Rural	Subtropical	15	2400
	47	56	40.68–500.4	920	6.5–160.345	976	117	Cuenca	331,888	Craft	Urban	Tempered	16	2550
	48	22	40.28–473.68	384	11.35–86.705	406	49	Chongón	36,726	Craft	Rural	Hot	11	10
	49	20	37.05–595.52	126	10.415–72.715	146	18	Quito	2,690,150	Craft	Urban	Tempered	3	2800
	50	20	103.81–1426.3	50	24.73–128.105	70	8	Quito	2,690,150	Craft	Urban	Tempered	3	2800
	51	20	177.07–737.55	96	9.37–104.355	116	14	Quito	2,690,150	Craft	Urban	Tempered	3	2800
	52	52	60.71–548.57	146	10.46–87.655	198	24	Quito	2,690,150	Craft	Urban	Tempered	3	2800
	53	26	95.49–614.89	90	14.16–73.325	116	14	Quito	2,690,150	Craft	Rural	Tempered	3	2800

* fibers are defined as particles of one-dimensional structures whose length far exceeds their diameter. ** fragments corresponding to a particle without a defined format whose relationship between its dimensions is minimal.

Various synthetic plastic materials were observed on a regular basis, identified by their characteristic shape and color. Figure 3 shows some photographs of the plastics detected. Particles between fragments and fibers of green, yellow, red, violet and blue color could be observed. The composition analysis determined the presence of polypropylene, low and high density polyethylene and polyacrylamide, results that confirmed that plastic microparticles are present in the foods studied, the rest of the fiber particles or fragments correspond to cellulose and various inorganic particles, which It could be identified by FTIR or by fluorescent microscopy, it is considered that they correspond to silica and carbon microparticles. The number of MPs found confirm those reported by Liebezeit [25–27] in honey and beer and also supports the evaluations of tap water carried out by Schymanski [35]. As an example, Figure 4 shows some FTIR spectrum of particles found in different food samples. The plastic material identified in each spectrum is indicated. Figure 5 shows the weighting of the presence of MPs in the analyzed foods.

Forty-seven percent of the samples analyzed are in cities with high population density, the three most important cities in the country, Quito, Guayaquil and Cuenca with a number of inhabitants greater than 600,000. Honey and craft beer processed in cities with a high population density present a greater quantity of microplastic particles greater than 100 MPs. Thirty percent of studied foods present 50 or more MPs.

Despite the fact that only 16% of foods with high MPs content are found in urban areas, no trend or relationship was found between the two variables.

The statistical analysis based on non-normal behavior, applying the Kruskal–Wallis test indicates that, with a significance of 95% (K calculated = 333.57814), if there are differences between the averages of MPs present in the different foods. It is notable that the upper averages correspond to bee honey and beer, this behavior may be a result of the conditions of exposure to greater atmospheric pollution and inefficient mechanical separation processes at the time of purification, as well as in the case of honey, to the pollen collection process that bees run and cause a simultaneous collection of microparticles, which could include microplastics.

Infrared analysis with FTIR indicated that 12% of these particles were microplastics, values like those reported by Liebezeit for honey and beer; they are also compatible with evaluations of tap water carried out by Schymanki. This latter author reports values between 28 and 39 particles per L of tap water or packaged drink [35].

Figure 6 shows the average values of microplastics found across the range of foods in the 1-L samples collected. Based on these data, it was possible to establish a value of 32 MPs/L in soft drinks and also in craft beer, 47 MPs/L in industrial beer, 40 MPs/L in skim milk, 54 MPs/L in industrial honey and 67 MPs/L in craft honey.

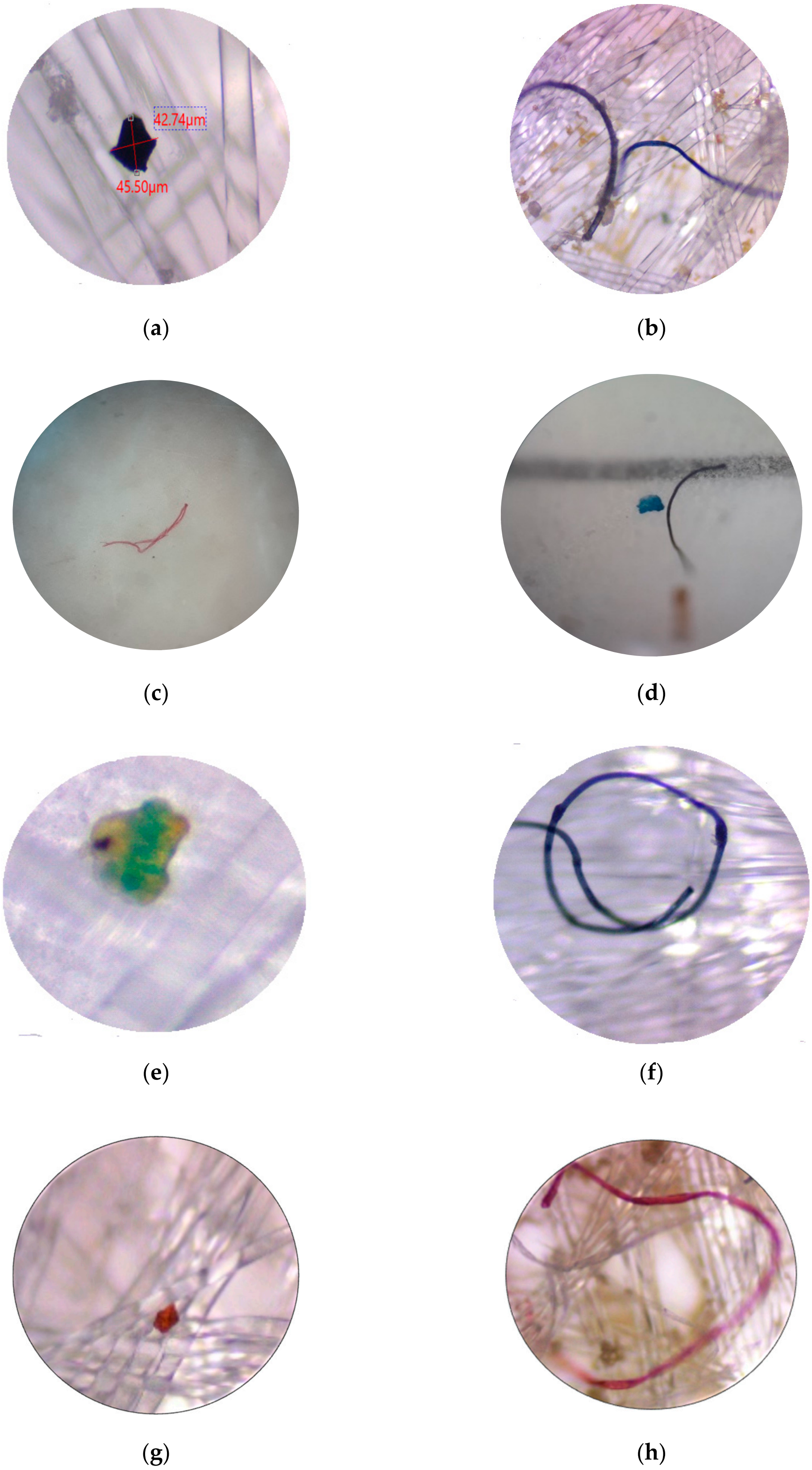
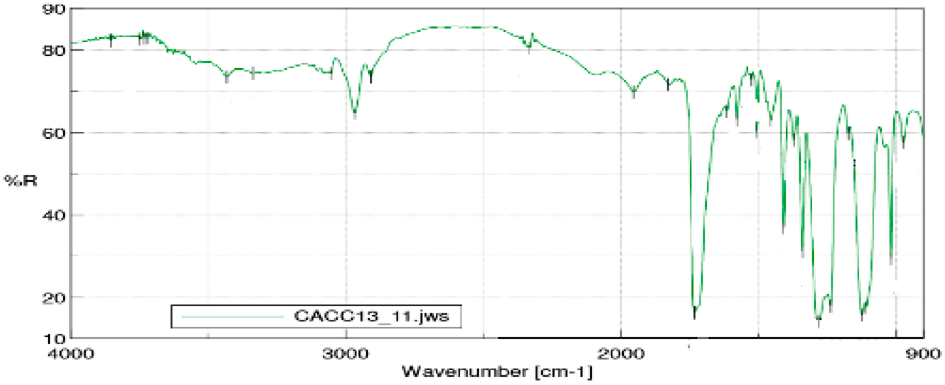


Figure 3. Photographs of microplastics identified by light microscopy in processed or packaged foods in different provinces of Ecuador: (**a**,**b**) refreshing; (**c**,**d**) craft and industrial beer; (**e**,**f**) skim milk; (**g**,**h**) artisanal and industrial honey.



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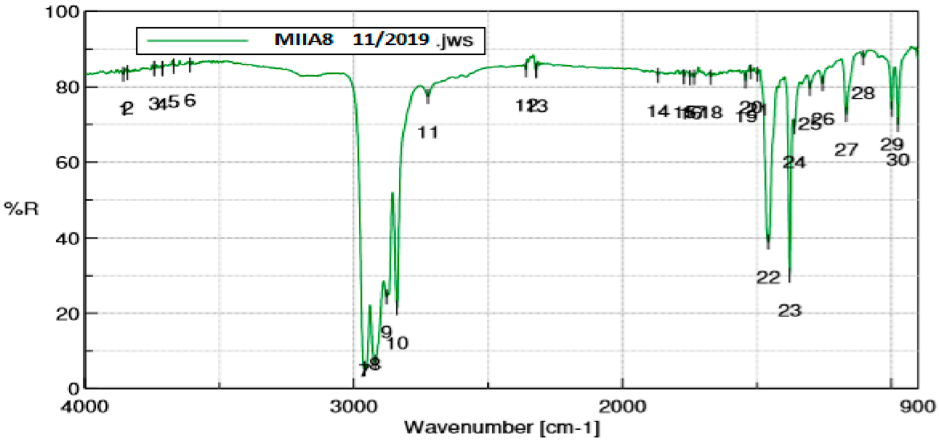

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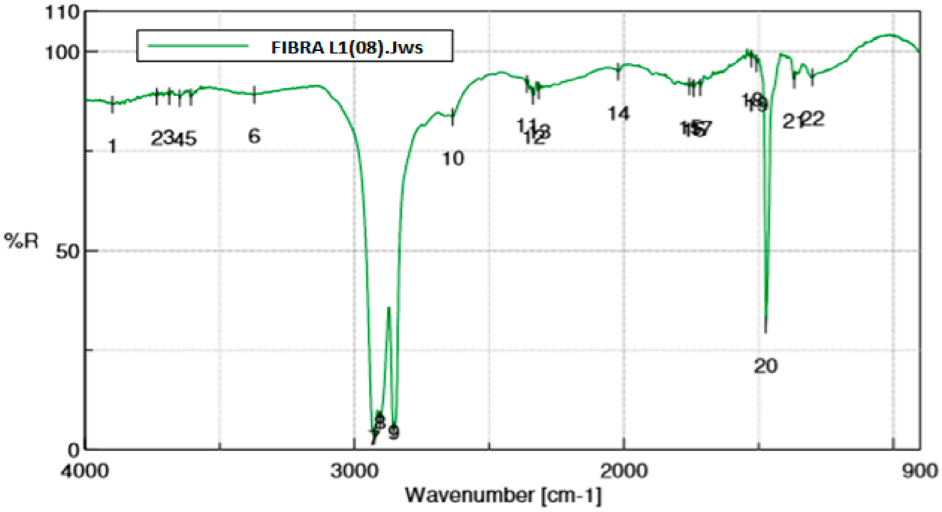
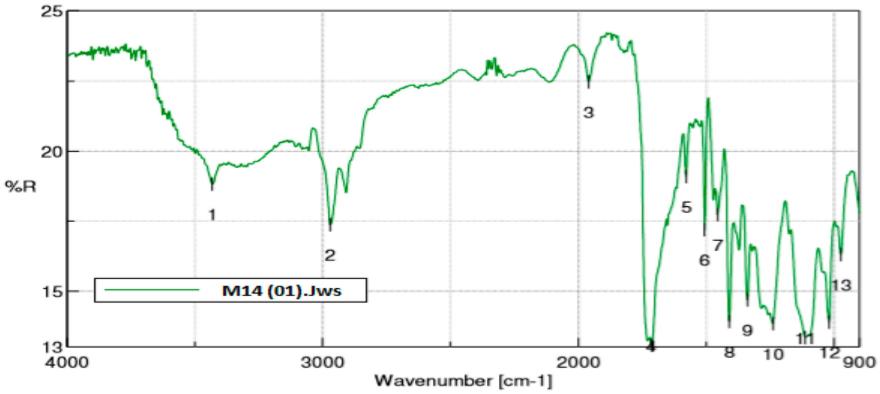


LDPE

Figure 4. *Cont.*



PAA

Figure 4. FTIR spectra synthetic micropolymers in food.

	Microparticles (number)	Microparticles (%)
PP	**17**	**3,21**
HDPE/LDPE	**28**	**5,28**
PAAm	**19**	**3,58**
Other particles	**466**	**87,92**
Total Particles analyzed	**530**	
Microplastic particles	***64***	***12,10***
Microplastic identified as PP or HDPE/LDPE	***45***	***70,30***

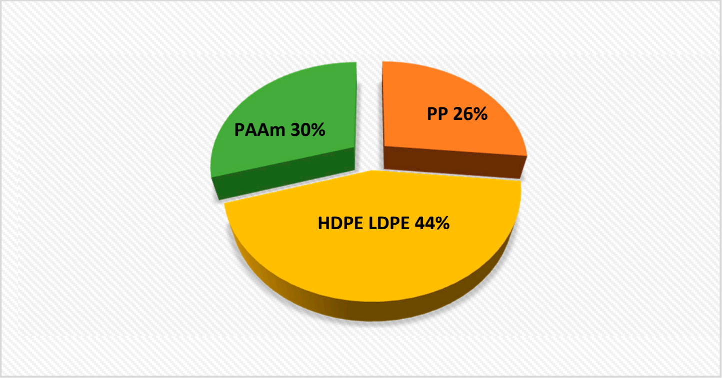


Figure 5. Microparticles analyzed with their relative proportion and weighted distribution of contaminating synthetic polymers found in food. PP—polypropylene; HDPE—high-density polyethylene; LDPE—high-density polyethylene; PAAm—polyacrylamide.

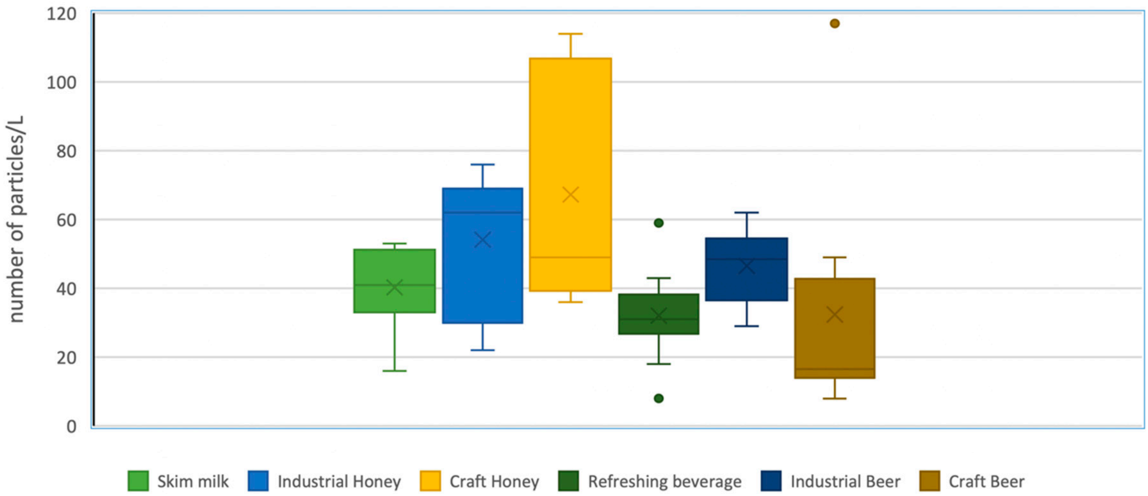


Figure 6. Average values of the number of microplastics found in 1 L of liquid foods analyzed. Microparticles considered in this evaluation comprise fibers and fragments found in beverages, FTIR analysis confirmed the presence of more fibers of polypropylene, low and high-density polyethylene and the fragments correspond to polyacrylamide.

4. Discussion

The results obtained show that the application of the modifications in the methodology proposed by Liebezeit [26] is valid for the extraction of MPs in liquid foods. Furthermore, the specifications established in the selection of food samples, defined in previous tests, facilitated the adequate extraction of microparticles, without affecting their physical structure.

There are several vectors of contamination with microparticles in food products. As an example, Liebezeit mention the materials used in the production process, especially during the filtration step of the beer [26]. Figure 2 shows the main steps of the production processes, each of them can be a source of MPs to the food product. Furthermore, several researchers emphasize atmospheric currents and supply water bodies as contributors to regional microplastic pollution [42].

The present study evaluated the population density of the production areas, as well as the rural or urban condition and the related anthropogenic activities. It is worth mentioning that, although the country's industrial growth is relative, Ecuador is an oil producer subject to a high rate of contamination by waste from crude oil exploitation, the refining process and its automotive fleet, all contributors of microparticulate contamination

The geographical location, whether urban or rural, as well as the anthropogenic activities of the area were generally expected to have a significant impact on the contribution of microparticles. An increase in the number of inhabitants and industrial, commercial, high-intensity agricultural and transport activity is considered to generate greater pollution.

An amplification of the influence of human activity on the presence of microparticles in honey could even be considered, due to the dispersion area of bees, factor of greatest influence on the presence of microparticles in the environment of the honey processing zone by bees and in man packaging. Existing vehicle traffic, proximity to other industries or residential areas, where greater pollution can be generated, can be considered to cause a greater presence of particles, including microplastics.

The great part of microplastic contamination responds to the atmospheric contribution in the productive processes [24,42,45]. Air currents transport large amounts of microparticles from the places of high contamination that end up in food, even more so when atmospheric contamination is not a controlling factor in industrial production. In artisan packaging processes, insufficient resources do not allow for environmental control.

In the case of food composed of water, the MPs enter through this route and given their size, the filtration processes are not a controlling border. In parallel and very often the wear and tear of the processing equipment also contaminates the food. It is essential to carry out corrections on the sources of contamination to reduce their presence in everyday food.

Despite expecting an apparent proportional relationship with air pollution conditions, no defined trend could be observed based on our results. A higher population density and its anthropogenic activities did not correlate with an increase or decrease in the amount of microplastics found. Thus, it would be necessary to increase the information in relation to the specific location of the processing plants which according to new regulations, are often located in industrial parks. The data in Table 2 does not reflect any impact either of weather conditions, such as the presence and speed of winds. The reduction in the number of microparticles is observed only in certain areas subject to high-speed currents, which leads to a greater dispersion of MPs over larger surfaces, reducing the number of microparticles in the region, despite a bigger population or high-pollutant activities.

Furthermore, as commented by Schymanski, beverages packaged in containers whose composition includes polymers may contain microplastics [35]. Another key point regarding the presence of MPs is the origin of the water used for making the different foods; if water is their basic ingredient or they have contact with industrial service water from each country's municipal supply, the treatment applied to water does not usually allow removing microparticles. To date, the level of filtration applied to the foods analyzed is insufficient to eliminate microplastics. In addition, applying a harder filtration process would lead to reducing the nutritional factors that are implicit in milk, honey or soft drinks, as mentioned by Kosuth and Pivokonsky [10,46].

Likewise, when performing a cumulative analysis of consumption, the percentage of plastics recorded in these foods would imply an incremental intake of synthetic microparticles reaching the values projected by Cox [32]. The latter estimated the intake of microplastics to range between 81,000 to 121,000 per year. The author included in the calculation the presence of microplastics in the air being breathed and in the daily meal, which, according to his estimate, would contribute up to 52,000 microplastics per year. If the presence of microplastics in regular foods continues to be identified and the records of microparticles determined so far are accumulated, enough quantities can surely be available to collect harmful amounts of toxic compounds that accompany them.

With regard to the packaging of the processed products, in the case of products packaged with plastic material, such as milk, refreshing beverages and some honey samples, the detachment of the packaging material contributed to the presence of MPs as recorded by Koelmans and Mason [29,33] in their study. This factor had no effect in the case of beer, as beer is usually packaged in glass bottles or aluminum cans.

5. Conclusions

The presence of microplastics was identified in samples of four different terrestrial foods produced in Ecuador, honey, milk, soft drinks and beer.

The number of microplastics found was between 10 and 100 MPs/L, with an average of around 40 MPs/L. These values are consistent with those reported by other research, Liebezeit for honey [25] and beer [26] and tap water carried out by Schymanki [35]. This latter author reports values between 28 and 39 microparticles per L of tap water or packaged drink.

The sizes of the particles found in the study are in the range of 13.45 and 6742.48 µm for the fibers and between 2.48 and 247.54 µm for the fragments.

From the composition analysis carried out with FTIR, we were able to confirm the presence of 12% of microplastics such as polypropylene, low- and high-density polyethylene and polyacrylamide—in proportions similar to those recorded in previous studies, 70% corresponding to PET and PP. Figure 5 shows the weighting of the presence of MPs in the analyzed foods.

A specific relationship between the presence of microparticles and the geographic area of processing could not be confirmed, although geographic characteristics, particularly the presence of high-speed winds, affect dispersal and reduce the presence of microparticles in production areas.

No significant correlations were found between the number of microplastics and the type of food processing, population or climate in the sampling areas. In general, a lower presence of MPs is observed in industrially treated products. Shruti mentions the influence of the production process and the contribution of water from the different sources of supply for industrial production, as well as the containers that contain food [47]. Our results confirm the low contamination with MPs in the studied foods, in consideration of the diverse and constant existing sources of contamination. However, the evident presence of this emerging pollutant requires continuity in its study, focused on identifying the causes and collaborating in actions to control this pollutant.

It was possible to observe important influence of the collection area on the content of microplastics only in honey, in rural areas with less contamination, its content was lower. The influence of the product container material was not evaluated.

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