Microplastics in Lettuce: The Impacts of Packaging on Dietary Microplastic Exposure Through Produce

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Abstract

Microplastic contamination has become so ubiquitous as to pose a significant risk to the spheres of ecosystem and human health across the world. Dietary microplastic contamination has been of significant concern recently as a potential pathway for bioaccumulation of microplastics in humans. However, this body of literature has developed a bias towards the risk of contamination through seafood, as microplastic contamination in the ocean is particularly high. This research investigated microplastic levels in romaine lettuce intended for human consumption and investigate the role of plastic packaging in potential microplastic contamination in order to gain a new perspective on dietary exposure routes. In comparing packaged and unpackaged lettuce, no difference between the two groups was detected (p-value = 0.5839). This inspires further questions concerning the extent of laboratory microplastic contamination and the future of research into microplastics and produce.

1. Introduction

Microplastics, small fragments of plastics often undetectable to the naked eye, have become a consistent component of the environment of the entire biosphere. Plastics and their additives have been located throughout every major body of water and in many species, including humans (GESAMP 2016). The ubiquity of microplastics is of particular concern as many plastic additives, such as BPA, have been shown to be harmful to human health (Calafat et al. 2008), which has led to a growing concern around dietary microplastic exposure. One particular area of consumption which has not had significant scientific investigation is that of fresh produce. To address this gap, we evaluated romaine lettuce, a previously unresearched food item, and the impact which plastic packaging may have on its observed microplastic levels.

To date, no significant scientific investigation into produce has been conducted. Due to the density of microplastics in the ocean, investigation into microplastic proliferation has largely concerned oceanic products, such as seafood (Karbalaei et al. 2018). Additionally, several studies focus on these products well before they are intended for human consumption, including organs which usually are not consumed by humans, such as the gastrointestinal tract (Baalkhuyur et al. 2018, Rochman et al. 2015, Karlsson et al. 2017). The significant research conducted on non-marine dietary microplastics was largely focused on beverages and solubles, such as honey, salt, and beer (Karami et al. 2017, Iñiguez et al., Liebezeit and Liebezeit 2013, 2014). While microplastics were found in all of these, contamination was similarly pervasive (Lachenmeier et al. 2015). Among all of this research, no assessment of microplastics in fresh produce was conducted. By investigating an under-researched product, produce, at the stage of production nearest to consumption (in the grocery store), this research will create a more robust understanding of how all foodways impact human microplastic exposure, and how processing tactics, such as packaging, play a role in exposure.

For the purpose of this particular research, the overarching questions to investigate are: Are there detectable microplastics in romaine lettuce, a very common food item, intended for human consumption? If yes, do microplastic concentrations vary depending on whether or not they were packaged in plastics? Because of romaine's availability both in unpackaged heads and prepared plastic bags, it serves as the ideal produce through which to investigate how the phenomenon of how packaging impacts microplastic exposure. Using romaine lettuce, this research begins an investigation into the role of both produce and packaging in microplastic exposure.

2. Methods

2.1 Sample Preparation

This protocol is adapted from the methods of Loder et al. 2017, Maes et al. 2017, Wang and Wang 2018, Karlsson et al. 2017, and Quinn et al. 2018. Two unpackaged heads of romaine lettuce and two heads of romaine lettuce in plastic packaging were purchased from 5 nearby stores - Sprouts, Stater Bros., Cardenas, El Super, and Super King. Upon purchasing the lettuce, it was carried out in sealed individual paper bags to avoid contamination. In the lab, unbagged lettuce was thoroughly washed with deionized water, while bagged lettuce leaves were not washed, as per the recommended health guidelines for preparing unwashed and pre-washed lettuce (Palumbo et al. 2007). Lettuce leaves were inserted into a Hamilton Beach Glass Jar Blender Black (Model Number 54216) until it was filled to the top. 200 mL of Milli-Q deionized water were added to the blender. The solution was blended for 30 seconds on the slower "grind" setting (approximately 3,100 rpm), and then for 60 seconds on the faster "cream" setting (approximately 3,700 rpm). The contents of each beaker were strained through a stainless-steel filter with a mesh size of 5 mm to capture larger organic fibers while allowing all particles meeting the qualification of "microplastic" (<5mm) to pass through until 100 mL of solution was obtained. This solution was placed in a glass beaker covered with aluminum foil to avoid airborne contamination. 5 mL of the blended lettuce solution was extracted using a glass pipette and placed in a 100 mL glass beaker. Due to the large quantities of cellulose in lettuce, the enzyme cellulase from *Aspergillus niger* (purchased from Fisher Scientific) was used to digest the remaining organic material while leaving any microplastic particles intact. 5 mL of cellulase and 10 mL of a phosphate-buffered saline (PBS) solution (1 L prepared with 8 g NaCl, 200 mg KCl, 1.44 g Na_2HPO_4 and 240 mg KH_2PO_4 in deionized water, set to pH 5.0 using hydrochloric acid) were added. The beaker was covered with aluminum foil, and the solution was incubated at 50 degrees C for 4 days. These steps were repeated for every purchased head of lettuce.

When the incubation period was completed, NaCl in solution with deionized water (density=1.2g/mL, stirred for 10 minutes) was added to the incubated solution until the beaker had 100 mL of solution in it. The solution was left to settle for 30 minutes, then a vacuum system was used to collect the top 40 mL of each sample and any floating microplastics within it. 5 mL of 0.08 g/mL Nile red dye solution was added to this extracted solution and allowed to stain for 30 minutes. This extracted and stained solution was run through vacuum filtration with an entirely glass apparatus to separate stained fibers from their liquid matrix. The resulting filter paper was placed in a glass beaker, covered with aluminum foil, and allowed to dry overnight. R was used to generate random coordinate points. A grid was created on the dried filter paper, and four random points were plotted onto this grid on each paper. The digital Revolve microscope with fluorescent light (4x zoom lens, TXRED fluorescent/FL overlay at 100% brightness, ~110 ms capture) and ocular techniques were used to count the number of fluorescent particles present at each marked location on the paper. These steps were repeated with each incubated solution.

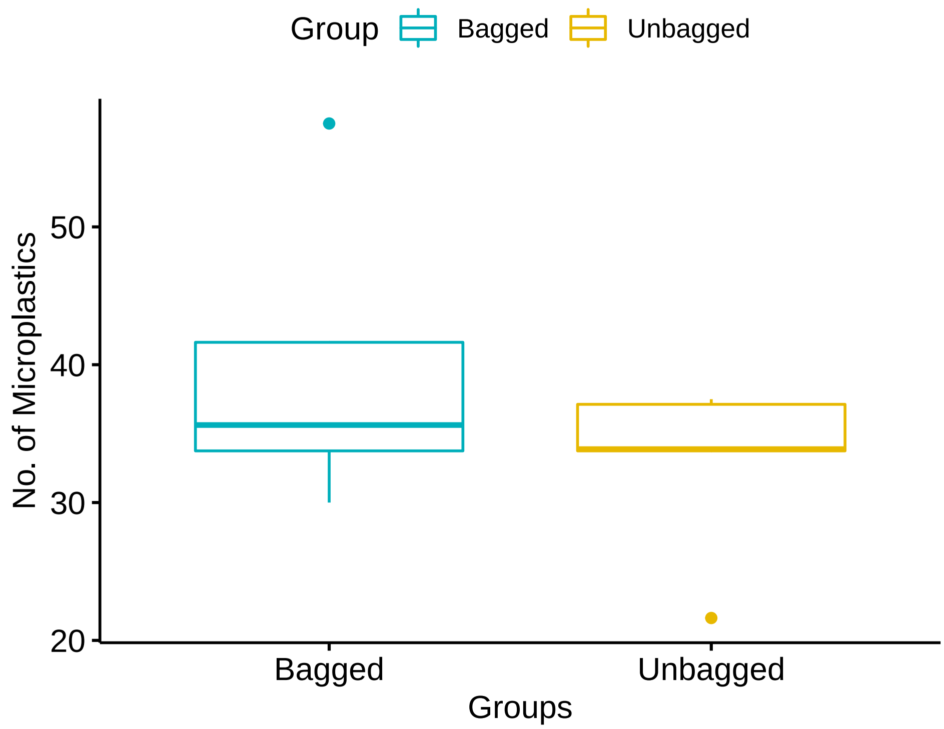
To mitigate the impacts of contamination or procedural error, the results were corroborated through the use of blanks. 3 water "samples" went through this protocol alongside the experimental samples, but with 100 mL of deionized water rather than solution material. All equipment used was washed thoroughly before and after touching any sample, and all water used to wash was deionized so as to avoid contamination from the water.

2.2 Statistical Analysis

The data collection yielded five replicates and five pseudo-replicates for both bagged and unbagged lettuce, as two bagged and unbagged heads were purchased at five different stores. In order to more accurately reflect these replicates, the number of microplastics found on each head of lettuce was averaged with its pair. For example, the number of microplastics found on each bagged lettuce head purchased at Cardenas were averaged with one another. This experiment was designed to test the null hypothesis of "there is no difference in microplastic quantities in romaine lettuce with or without plastic packaging." In order to determine whether or not to reject the null hypothesis, whether or not the data fell within normal distribution was first assessed, using Q-Q Plots and the Shapiro-Wilk normality test (See Appendix II). The data for the unbagged lettuce was found to diverge from the normal distribution, so a Paired Samples Wilcoxon Test was used to assess whether the difference between the bagged and unbagged lettuce were statistically significant.

3. Results

The averages showed that bagged lettuce generally had slightly more microplastics than unbagged lettuce, as is shown in the graph below. However, the Paired Samples Wilcoxon Test yielded a p-value of 0.5839, indicating no statistically significant difference between bagged and unbagged lettuce.



5. Discussion

Although statistical significance was unable to be determined, the raw numbers of microplastic particles observed in the samples indicates that further research into the presence of microplastics in produce is warranted. At least 5, and up to 103, microplastics were observed at each point on each piece of filter paper which was analyzed. The filter papers were generated from just 5 mL of "lettuce slurry," and the points observed on the filter papers were just 400 micrometers across. This indicates that they typical number of microplastics consumed when eating a salad, for example, would likely be much higher than the numbers observed in this experiment. Additionally, the numbers which were observed in this experiment were not dissimilar from those found in other, similar studies - even those involving notoriously contaminated seafood. For instance, De Witte et al. found between 4.3 and 6.1 plastic fibers per 10 grams of mussel material in a study conducted in 2014 on bivalves intended for public consumption. The relatively high numbers found in this study indicate that microplastics in produce may be an issue of significant concern which should be investigated further.

Author	Matrix	Unit	Minimum	Maximum
Fossi et al. (2015)	Fin Whales	number microplastics/meters cubed	0.0	9.67
Baalkhuyur et al. (2018)	Red Sea Fishes	number microplastics/gastrointestinal tract	0.0	3.00
Rochman et al. (2015)	Fish and Bivalves	number microplastics/gastrointestinal tract	0.0	21.00
Cauwenberghe and Janssen (2014)	Bivalves	number microplastics/grams wet weight	0.0	0.63
Devriese et al. (2015)	Brown Shrimp	number microplastics/grams wet weight	0.0	1.23
Kolandhasamy et al. (2017)	Mussels	number microplastics/grams (by organ)	1.0	10.00
De Witte et al. (2014)	Blue Mussels	number microplastics/10 grams	2.6	5.10
Iñiguez et al. (2017)	Spanish Table Salt	number microplastics/kg	43.0	283.00
Karami et al. (2017)	Commercial Salts	number microplastics/kg	0.0	10.00
Liebezeit and Liebezeit (2013)	Honey and Sugar	number microplastic fibers/500g	20.0	330.00
Liebezeit and Liebezeit (2014)	German Beer	number microplastics/L	16.0	254.00

That being said, there was a significant risk of contamination in this experiment. Three blanks were used to evaluate laboratory microplastic contamination, Although the average of the three blanks was lower than averages found for all but three of the experimental samples, most of these differences were quite small. As was noted by Lachenmeier et al. (2015), methodologies for finding microplastics have consistently struggled to avoid contamination, as air and water oftentimes contains microplastic particles. Although great care was taken to ensure that samples were covered at all times, and only deionized Milli-Q water was used in association with this experiment, laboratory contamination was not avoided in this experiment either. It is possible that the deionized water had some remaining microplastics in it, particularly as the Milli-Q apparatus is made out of plastic. Although water is a very necessary element of experiments such as these, future experiments may consider testing the deionized water itself for microplastics before conducting the experiment, or further purifying the water by running it through the glass vacuum apparatus used in the experiment, so that any particles in the water large enough to be captured by the filter would be suspended before the water was used in the experiment. Another major contamination concern is airborne contamination; however, airborne contamination is much more difficult to avoid. This being said, some further precautions may be taken. A reduction of the plastics present in the lab prior to the conduction of this experiment, an investment in cotton lab coats and gloves (polyester and latex were used to complete this experiment), and perhaps the completion of the entire experiment under a fume hood may help to reduce airborne microplastic contamination. Additionally, as airborne contamination is a large concern, testing the air in the laboratory for microplastics may help to provide some context as to where the contamination is coming from, and perhaps indicate more concretely how that contamination may be minimized.

Additionally, some methodological issues may have contributed to potential error in the data obtained. One particular issue which was encountered was that, in an attempt to liquefy the lettuce in as little time as possible, the original solution was strained using a metal sieve. Although this allowed the liquids to be separated from remaining solids, the fibrous remains largely accumulated on top of the sieve and did not filter into the solution used to produce the papers which were observed. This may have lead to the underestimation of how many observed plastics were in the lettuce, as many particles may have become trapped in this pile and not made it into the experimental solution. Another issue which was encountered was the difficulty to determine which particles viewed under the microscope were synthetic, and which were simply undigested lettuce fibers. A misinterpretation of remaining biotic material may have lead to an overestimation of the microplastics present in the samples. One way which both of these issues could be resolved is through a more thorough enzyme digestion, similar to the one described by Loder et al. (2017).When taking the time to do a complete enzyme digestion, the blended lettuce would not have to be strained, as the approximately three week-long process would thoroughly break down the biomatter on its own. Additionally, the longer digestion process would make it less likely that biotic fibers would remain in the solution and perhaps be mistaken as microplastic particles. If the time constraint is too significant to complete a full digestion process, a combination of boiling and nitric acid could also be used to eliminate biotic material, as per the methods of Rochman et al. (2015), albeit risking potential damage to and subsequent underestimation of microplastics in the material.

One additional improvement which may be made to similar research in the future may be found in the number of replicates used. The statistical power of this particular experiment with 10 replicates per group was found to be 0.41, indicating that the probability of making a Type II error and mistakenly not rejecting a null hypothesis which should be rejected is greater than 50%. In this experiment, it was found that in order to obtain statistical power of 0.8, 26 samples from each group would be needed, for a total of 52 experimental samples, plus blanks. On the box plot, there appears to be a slight trend towards more microplastics being present on bagged rather than unbagged lettuce, however, with the number of replicates which were used in this research, that trend is not statistically significant. As statistical significance was not obtained, the null hypothesis of "there is no difference in microplastic quantities in romaine lettuce with or without plastic packaging" could not be rejected, and the role of packaging in microplastic quantities in produce could not be affirmatively determined. In order to determine whether or not packaging does or does not play a role in microplastic quantities on romaine lettuce, this or a similar experiment would likely have to be repeated with many more replicates.

Due to the magnitude of work necessary to reproduce these results with more replicates and more thorough enzymatic digestion, this particular experiment likely should not be repeated without a much longer time frame available for its completion. If this experiment could be re-conducted over the course of at least two months, or even a semester, then it would be interesting to investigate whether or improving the enzymatic digestion and increasing the number of replicates produced statistical significance. In lieu of such a time commitment, the inconclusive results of this experiment may still serve as a starting point for further research into the presence of microplastics in produce. As this experiment observed microplastic quantities similar to those observed in other, more researched dietary arenas, further research into whether or not these values are of concern, or likely due to contamination, are warranted. Experiments comparing microplastics found on various produce with background levels found in laboratory air or water samples may be useful in concluding whether or not microplastics in produce are a genuine concern. Additionally, further research investigating the impacts of plastic packaging and airborne contamination individually may be more illuminating than a study comparing the two, as levels of microplastics in both scenarios are likely high. Although the high microplastic levels observed may be due to contamination, further research is necessary to affirmatively answer this question.

6. Conclusion

As statistical significance was not obtained in this experiment, it is difficult to speak conclusively as to what may be learned from this research. In many ways, this experiment simply provides a foundation on which more targeted questions may be built. However, the relatively high levels of microplastics found in this experiment indicate that produce microplastic exposure may be comparable to areas more traditionally considered to be high-risk, such as seafood. This reality underscores the necessity, discussed by Rist et al. (2018), of expanding and contextualizing the debate around microplastics. Microplastics have become entirely omnipresent across essentially all global environments, so much so that no laboratory protocol has been designed which may entirely eliminate the risk of microplastic contamination. While it is of course important to understand the risks posed by a threat as universal as microplastic exposure, dietary pathways are still just one of many routes through which individuals are exposed to microplastic pollution every day. While this indication that microplastics may be present in produce as well as other more traditionally high-risk foods is certainly unnerving, it remains just one piece in a large web of constant microplastic exposure. The microplastics observed in lettuce samples, as well as the high risk of laboratory contamination, indicates how universal microplastics are in almost all environments today. As this report indicates, areas which were previously considered to be unlikely sources of microplastic exposure may be found to have substantial contamination, underscoring the need for further research into the scope of microplastic exposure.

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Appendix I: Raw Data

X	Store	U.B....Bagged	Sample..	Point.1	Point.2	Point.3	Point.4	Average
Cardenas U.B. #1	Cardenas	UB	1	5	10	12	13	10.00
Cardenas U.B. #2	Cardenas	UB	2	32	52	38	11	33.25
Cardenas B. #1	Cardenas	BG	1	70	62	84	103	79.75
Cardenas B. #2	Cardenas	BG	2	37	32	30	42	35.25
El Super U.B. #1	El Super	UB	1	76	29	24	18	36.75
El Super U.B. #2	El Super	UB	2	38	44	32	36	37.50
El Super B. #1	El Super	BG	1	37	31	36	33	34.25
El Super B. #2	El Super	BG	2	53	52	52	39	49.00
Sprouts U.B. #1	Sprouts	UB	1	31	34	19	32	29.00
Sprouts U.B. #2	Sprouts	UB	2	54	20	66	15	38.75
Sprouts B. #1	Sprouts	BG	1	36	45	51	52	46.00
Sprouts B. #2	Sprouts	BG	2	28	19	20	34	25.25
Stater Bros U.B. #1	Stater Bros	UB	1	33	45	37	51	41.50
Stater Bros U.B. #2	Stater Bros	UB	2	47	24	34	29	33.50
Stater Bros B. #1	Stater Bros	BG	1	21	58	28	20	31.75
Stater Bros B. #2	Stater Bros	BG	2	28	22	36	27	28.25
Super King U.B. #1	Super King	UB	1	10	26	23	34	23.25
Super King U.B. #2	Super King	UB	2	57	21	33	66	44.25
Super King B. #1	Super King	BG	1	22	24	26	27	24.75
Super King B. #2	Super King	BG	2	46	41	45	39	42.75
Blank #1	Blank	Blank	1	18	10	55	31	28.50
Blank #2	Blank	Blank	2	28	27	8	12	18.75
Blank #3	Blank	Blank	3	24	46	25	13	27.00

Appendix II: Normality Tests

In order to determine whether or not to reject the null hypothesis, whether or not the data fell within normal distribution was first assessed, using Q-Q Plots and the Shapiro-Wilk normality test.

For bagged lettuce data, the Shapiro-Wilk normality test yielded a p-value of 0.2768, indicating that it is within the bounds of normal distribution. The Q-Q plot, shown below on the left, also demonstrates that most points fall within the expected bounds.For unbagged lettuce data, the Shapiro-Wilk normality test yielded a p-value of 0.04526, indicating that it is not within the bounds of normal distribution. The Q-Q plot, shown below on the right, also demonstrates that, while most points fall within the expected bounds, some are much further out of normal distribution than they were for the bagged lettuce data.