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Microplastic identification in German beer – an artefact of laboratory contamination?

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Summary
Recent studies have detected microplastic fibres and particles in some food groups including honey and beer. The aim of this work was to replicate a method for microplastic analysis in beer. Several methodological pitfalls were detected in the literature method, including the staining agent rose bengal used in microscopic analysis, which false-negatively excludes some synthetic agents including the beer filtration aid polyvinylpolypyrrolidone (PVPP). False positive results may occur for non-plastic compounds such as starch or kieselgur. Other pitfalls in the analysis include the considerable background contamination, which did not allow differentiation between beer samples from blank samples in our laboratory. Specialized cleanrooms are required, but even then contamination may occur, because cleanroom classifications focus on small particles and may exclude the relevant sizes of microplastic particles. We judge the previous nonvalidated literature methods that reported positive findings in foods as unsuitable for the purpose of microplastic identification and believe that the results were artefacts due to contamination. Especially because beer production includes a microfiltration step to remove yeast cells, microplastic contamination due to raw materials is highly unlikely. So far, a validated methodology for microplastic detection in foods or beverages is unavailable.

Zusammenfassung
Aktuelle Studien haben über Funde von Mikroplastik-Fasern und -Partikeln in einigen Lebensmittelgruppen wie Honig und Bier berichtet. Das Ziel dieser Arbeit war die Wiederholung einer Methode zur Bestimmung von Mikroplastik in Bier. Dabei wurde eine Reihe von Schwierigkeiten festgestellt, u. a. die Verwendung des Färbemittels Bengalrosa für die mikroskopische Analyse, das falsch-negativ einige synthetische Fasern einfärbt, inkl. des Bierfiltrationshilfsmittels Polyvinylpolypyrrolidon (PVPP). Falsch positive Befunde können bei einigen Nicht-Plastikbestandteilen wie Stärke oder Kieselgur auftreten. Weitere Schwierigkeiten bestanden in der erheblichen Hintergrund-Kontamination, die eine Unterscheidung der Bierproben von Blindproben in unserem Labor unmöglich machte. Besondere Reinräume erscheinen für derartige Analysen notwendig, jedoch selbst unter diesen Umständen können Kontaminationen auftreten, da übliche Reinraum-Normen auf kleine Partikel ausgerichtet sind und die relevanten Teilchengrößen von Mikroplastikpartikeln nicht einschließen. Die in der Literatur publizierten, nicht validierten Methoden, die positive Befunde in Lebensmitteln berichteten, werden als für den Zweck der Mikroplastikidentifizierung nicht geeignet eingestuft und wir beurteilen die Befunde als Artefakte der Laborkontamination. Eine Mikroplastikkontamination ausgehend von den Rohstoffen ist insbesondere sehr unwahrscheinlich, da die Bierherstellung üblicherweise einen Mikrofiltrationsschritt zur Entfernung von Hefezellen beinhaltet. Eine validierte Methode zur Mikroplastikbestimmung in Lebensmitteln und Getränken ist derzeit nicht verfügbar.

Introduction

Microplastic is typically defined as particles of sizes between 1 µm and 5 mm; however, there is a lack of internationally accepted definitions or standardized methods for sampling and analysis [1]. The major research in the past focused around the environmental microplastic contamination, especially of the oceans and the accumulation in marine organisms [2–11]. Only recently, research was conducted on determining microplastics in other food groups besides sea foods. The occurrence was described in honey, sugar and beer by the group of *Liebezeit* & *Liebezeit* [12,13]. Because beer is one of our major areas of expertise as central government laboratory for beer analysis in the Southern German State Baden-Württemberg, this study was conducted to replicate and verify the findings of the 2014 beer study [13].

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Materials and methods

The analysis was conducted according to *Liebezeit* & *Liebezeit* [13]. A 25 mm polysulfone filter funnel with 200 mL capacity (*Pall Life Sciences,* Ann Arbor, MI/USA) was used, which was placed on a filter flask and connected to a vacuum pump. A millipore membrane filter (mixed cellulose esters, hydrophilic, 0.8 µm, 25 mm, black, gridded, *Merck Millipore,* Darmstadt/Germany) was placed into the funnel. Using a slight vacuum, 200 mL of a freshly opened full bottle of beer were filtered. 6 mL of rose bengal solution (*Sigma Aldrich,* Taufkirchen/Germany; 0.02 g in 100 mL bidistilled water) were then placed into the funnel. After 5 min of reaction time, the residue of the colouring agent was removed by filtration of 15 mL of bidistilled water. Afterwards, the filter was placed into a petri dish with lid until microscopy. Microscopy of the filters was done using an Axioskop 2 with reflected light illumination (*Carl Zeiss,* Jena/Germany). Particles were manually counted at fiftyfold magnification and classified according to *Liebezeit* & *Liebezeit* [13] into fibres, fragments and granules. To study laboratory contamination, 500 mL of bistilled water was subjected to the procedure instead of beer samples for several times.

Results

The results of the microplastic counts in blank samples compared to beer samples are provided in Table 1. Using t-tests and Wilcoxon tests, no significant differences ($p < 0.05$) were found between the counts in the beer samples compared to the blank samples. The high contamination in the blank samples (example in Figure 1) did not allow validating a positive contamination in any of the samples.

Discussion

Regarding the analysis of beer and other beverages, we were unable to completely replicate, reproduce and validate the methodology pointed out by *Liebezeit* & *Liebezeit* [13]. On the one hand, the exact repetition of the methodology was difficult because the materials and methods were pointed out insufficiently. For example, the manufacturer or supplier of the 0.8 µm grey, gridded cellulose nitrate filters and all other chemicals and devices (e. g. the microscope) were not specified, but specifically the filtration device and protocol was not specified at all, e.g. if the filtration was conducted at atmospheric pressure or under vacuum. We found that filtration at atmosphetric pressure is impractical because of very long filtration times. A faster filtration may also lead to less contamination.

We have detected problems about the use of rose bengal as dye to differentiate "natural organic particles" and to characterize non-stained materials as microplastic [13]. In some trials with agents that may be used in the filtration step during beer production, polyvinylpolypyrrolidone (PVPP) was found to be coloured from rose bengal, and PVPP fragments will therefore be false-negatively classified as not being microplastic (or false-positively classified as natural). Hence, PVPP filtration cannot explain the microplastic findings of *Liebezeit* & *Liebezeit* [13]. As kieselgur is not coloured by rose bengal in own trials, kieselgur particles would be false-positively misclassified as microplastic in the procedure of *Liebezeit* & *Liebezeit* [13]. Similar false-positive results would derive from starch and cellulose particles or fibres, which are also not coloured by rose bengal. We do not believe that "the synthetic nature of the contaminating particles" [13] can be established by staining with rose bengal.

Using a comparative procedure with data from *Hartmann* [14], *Liebezeit* & *Liebezeit* [13] claimed that kieselgur particles were not encountered and that this indicates that filtration with this material is no longer in use. However, according to our own observations in breweries in Southern Germany, *Braun* et al. [15] can be confirmed that kieselgur is still the most important and most widely used material. We believe that the absence of kieselgur particle findings would be proof for the appropriateness of the filtration technology, which completely removes yeast cells (3.5–8.5 µm [16]). As yeast cells are removed, all other larger particles will also be removed during beer filtrations. Haze formation in beer caused by filtration aids is a sign of inappropriate filtration technology [17], but is in our experience very seldom observed.

The fact that the breweries apply micro-filtration to remove yeasts also invalidates the opinion of *Liebezeit* & *Liebezeit* [13] that microfibers could derive from the production process (e.g. raw materials such as barley, hops, or water) prior to the filtration. If the raw materials are excluded, this leaves only the production process following filtration as relevant for re-contamination with microfibers. Basically, this is the short space in the filling line, when the open and cleaned bottles leave the washing machine and before they are capped in the filling machine. At the speed of modern bottle filling machines of 20,000–40,000 bottles/hour or more, the time span for potential contami-

Tab. 1 Putative* microplastic counts in beer compared to blank samples (the two groups were not significantly different: all counts are judged as being due to contamination)

Sample type	Fibres [n/L]	Fragment [n/L]	Granules [n/L]
Blank (bidistilled water, n = 10)	15 ± 9	20 ± 13	15 ± 12
Beer (n = 39)	16 ± 15	21 ± 16	27 ± 10

* See discussion for potential misclassification due to unsuitable staining agent

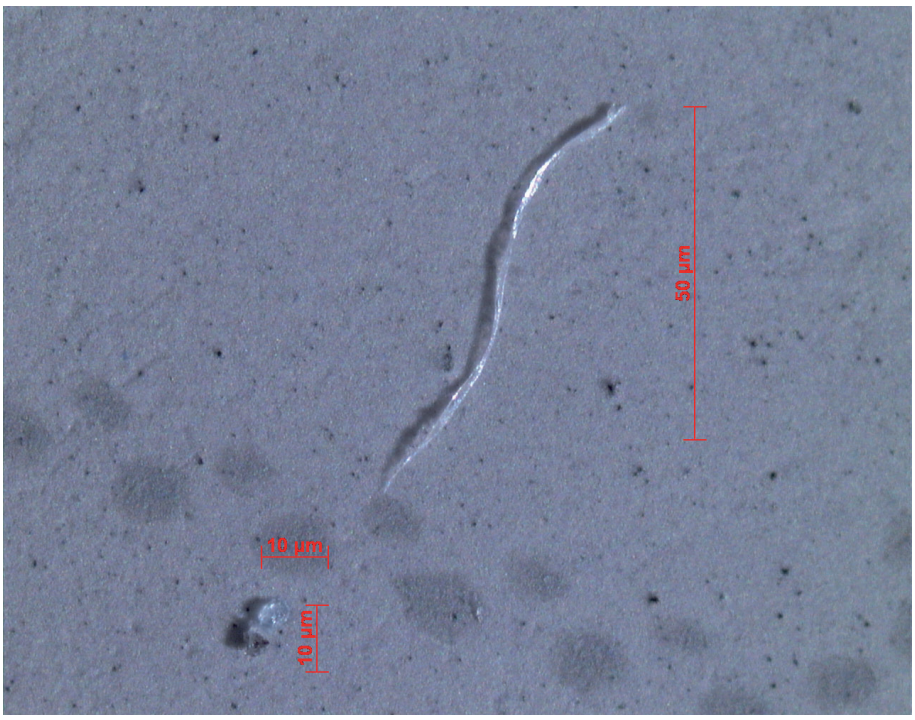

Fig. 1 Typical microscopic picture of putative microplastic particles detected in a blank sample due to contamination.

nation is very short and the filling line, where the bottles are openly exposed, is often covered to avoid external contamination. We believe it unlikely that the large contamination counts reported in *Liebezeit* & *Liebezeit* [13] may derive from such external contamination (e. g. by the workers' clothing) during bottle filling.

Besides the issue of misclassification due to the crude staining method, laboratory contamination might have led to the large counts of microplastic contaminants in the beer and honey samples [12,13]. In a recent article about contamination in microplastic analysis, *Woodall* et al. [18] remark that contamination is highly likely unless strict control measures are employed. Our results confirm a considerable influence of contamination. In the blank filtra-

tions of distilled water, we microscopically detected similar levels as in the beer samples. Therefore, it was not possible to confirm the intrinsic presence of microplastic particles in the beverages using the facilities of our standard beer laboratory, but special cleanrooms separated by air-locks are required, which are not normally available in official food control laboratories. However, even the strictest cleanroom measures would only allow a reduction of 90 % of fibre abundance due to contamination [18]. A problem is that international classifications of cleanrooms refer to the control of very small particles (0.1–5 µm), which are much smaller than the relevant microplastic particles [18].

In agreement with the opinion of the German Federal Institute for Risk Assessment [19], we do not believe that procedures are currently available to accurately quantify microplastic contamination, which would meet the criteria needed for use in governmental food control.

The reports of *Liebezeit* & *Liebezeit* [12,13] do not provide any information if cleanrooms were applied and to which category the cleanroom belonged (e. g. according to ISO 14644-1). *Liebezeit* & *Liebezeit* [12] reported a contamination of 33–49 fibres and 3–6 fragments for a 2 h filtration of laboratory air, and 24 fibres and 60 granular particles when a filter was exposed for 24 h. *Liebezeit* & *Liebezeit* [13] reported 2 fibres on a blank filter carried through the complete procedure and 2 fibres and 3 granular particles for a 1 h filtration of laboratory air. This confirms our own results of highly variable contamination from laboratory air. Nevertheless, *Liebezeit* & *Liebezeit* [13] suggested without providing any method validation data that "contamination from this source can be neglected" and conducted only some basic measures to avoid contamination (i. e. covering of filtration unit and glassware, exposure of filters for a maximum of 15 min). These measures would not be deemed sufficient by the standards of *Woodall* et al. [18] for microplastic identification. We therefore believe that there is a high possibility that the results of *Liebezeit* & *Liebezeit* [12,13] may be artefacts caused by laboratory contamination. Other researchers were also not able to replicate the results of *Liebezeit* & *Liebezeit* [12,13]. The BfR [19] reports about two laboratories that were not able to identify microfibers in honey, using microscopic analysis with magnifications up to 1000 times. The German Brewers Association [20] reported about results from independent laboratories including the Technical University of Munich, which were not able to detect microplastic fibres in beers.

Finally, we want to comment regarding the press coverage following the publication of the article about beer, which suggested to avoid German beer "when going to the Oktoberfest" (e. g., [21] and several other sources). Apart from the inappropriate polemics, we do not think that the conclusion from the press release was founded in science, because the results were based on a nonvalidated and highly unspecific method (e. g., no infrared spectroscopy was conducted, which is the most reliable method to identify microplastics [7]), but also because a risk assessment of microparticles in foods is currently not available or even possible [19]. Besides the general guidelines regarding alcohol consumption, we currently see no evidence to advise against beer consumption based on hypothetical microplastic contamination.

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