European Food Research and Technology
https://doi.org/10.1007/s00217-024-04570-4

ORIGINAL PAPER

Gluten migration from biodegradable food contact materials poses a risk to celiac disease patients

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Received: 25 February 2024 / Revised: 25 April 2024 / Accepted: 27 April 2024

Abstract

Due to the ban of certain single-use plastics in the European Union, food contact materials (FCM) from biobased and/or biodegradable polymers are increasingly being used. Some FCM are made from wheat or rye and therefore contain gluten, which is a food allergen and known to cause celiac disease. Legislation currently does not require allergen labelling on FCM and there is only some first information that gluten from FCM can migrate into gluten-free foods. Our aim was to analyze the extent of gluten migration from six different FCM into a variety of liquid and solid foods to assess the risk of exposure for wheat allergy and celiac disease patients. We show that the extent of gluten migration depended on the properties of the material, the liquid or solid food it comes into contact with and contact time. There was no clear effect of temperature or pH value. Of the six FCM studied, wheat bran-based plates had the highest potential to release gluten with concentrations of up to 203.0 mg/L of gluten in deionized water after 30 min. To protect patients, it is important to raise awareness of the risk of gluten migration from such FCM and help patients identify and avoid gluten-containing FCM. Further, legislation needs to be adapted urgently to include mandatory labelling of allergens on these biodegradable FCM.

Keywords Biodegradable packaging · Celiac disease · Enzyme-linked immunosorbent assay (ELISA) · Gluten-free · Rye · Wheat

Introduction

The European Union (EU) banned certain single-use plastics including plates, cutlery, straws, drink stirrers and cups or containers made of expanded polystyrene from July 2021 to help combat plastic pollution of seas, rivers and oceans, to develop a circular economy and to promote a more sustainable future [1]. This decision accelerated the implementation of biobased and/or biodegradable polymers that are derived from biomass as a renewable resource. Biopolymers for use in food contact materials (FCM) can be synthesized from bioderived monomers (e.g., polylactic acid), produced by microorganisms (e.g., bacterial cellulose) or extracted directly from biomass (e.g., polysaccharides such as alginate, carrageenan, chitosan or pectin and proteins such as casein, gelatin, soy protein or gluten) [2]. The advantages of protein-based materials are their abundance, favorable film-forming properties and low cost, but they are more brittle, mechanically weaker and more permeable for water vapor compared to conventional plastics [3]. According to EU legislation, FCM must comply with good manufacturing practice in such a way that their constituents are not transferred to food in quantities that could harm human health, change the composition of the food in an unacceptable way or deteriorate the organoleptic properties under normal or foreseeable use [4]. These general requirements apply to intentionally added substances including stabilizers, plasticizers, antioxidants, solvents, catalysts and unreacted monomers as well as non-intentionally added substances including side or breakdown products [5]. Zimmermann et al. recently showed that 67% of the biobased and/or biodegradable materials tested contain a large number and diversity of toxic compounds [6].

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Published online: 15 May 2024

Protein-based materials from milk, egg, soy and wheat gluten are increasingly used in FCM [7]. Wheat gluten is isolated from wheat flour by washing with water during starch production. After drying to a powder, it retains its viscoelastic, cohesive and film-forming properties upon rehydration, which makes it particularly suitable for use in FCM [8, 9]. In case gluten proteins are present in foods, it is mandatory to label them on prepacked and non-prepacked foods to protect wheat allergy patients and those with celiac disease [10]. Further, gluten-free foods must not contain more than 20 mg/kg of gluten [11]. However, there is currently no regulation that requires the labelling of these allergens in FCM and this may put patients at a health risk.

Studies on the allergenic potential of these FCM and, more importantly, on the migration of allergens from such materials into the final food as consumed are still largely missing. Mauricio-Iglesias et al. found that 4–6%, 3% and 15% of the protein in gluten/montmorillonite materials migrated into water, acetic acid and ethanol, respectively, while migration into olive oil was negligible [12]. Awareness of this issue is only just beginning to emerge as more and more gluten-based FCM such as cups, plates, tableware and pasta-based straws are becoming widely available. There are several concerns for patients, because the raw material used to produce such FCM is often unknown, as is the production process and potential cross-contamination. In case the material is not stable during use, it could easily break down and be ingested by mistake.

In response to concerns by patients, the Italian, Dutch and Spanish celiac societies conducted some first investigations and showed that wheat bran-based dishes contaminated gluten-free soft cheese and lasagna, resulting in a gluten content of 45 mg/kg and more than 80 mg/kg, respectively, under real life use conditions [13]. These levels are well above the 20 mg/kg threshold for gluten-free food and may therefore be sufficient to cause symptoms in patients.

Based on this first confirmation that gluten can indeed migrate from FCM into gluten-free foods, our aim was to expand the collection of FCM and foods tested to provide a more comprehensive basis for assessing the risk of exposure for wheat allergy and celiac disease patients. We hypothesize that the extent of gluten migration from different materials depends on the material itself, the liquid or solid food it comes into contact with and contact time and conditions, such as temperature and pH value.

Materials and methods

Materials and food samples

All chemicals and reagents were of analytical grade or higher. Simulants A–D for testing migration of constituents from FCM were prepared based on Council Directive 85/572/EEC [14] and included deionized (DI) water (simulant A), 3% aqueous acetic acid (w/v, simulant B), 15% aqueous ethanol (v/v, simulant C) and olive oil (simulant D). Commercially available food samples were bought online and at a local supermarket. They included freshly brewed coffee (60 °C), pineapple juice, Coca-Cola, and sparkling water, as well as gluten-free bread, fish sticks, spinach ravioli, lasagna and pizza base.

Food contact materials

Six different FCM were tested. They included biodegradable plates, forks and knives (EatMit UG, Berlin, Germany), three straws (1 and 2: wisefood GmbH, Garching, Germany; 3: Thomas Hoof Produktgesellschaft mbH & Co. KG, Lüdinghausen, Germany) and a wafer cup (wisefood GmbH). The plates with a diameter of 24 cm were made from 100% edible wheat bran. The allergen was not marked on the packaging, but stated in personal communication with the manufacturer. Further information included the stability of the product, which stated that warm foods can be placed on the plate up to 15 min prior to consumption. The forks and knives were also made from wheat bran, but in combination with 90% polylactic acid. A reference to wheat as allergen was only stated on the website of the manufacturer. Straw 1 was edible and made from durum wheat semolina and wheat gluten which were indicated on the label as allergens. The stability of the product in liquid was stated to be at least 60 min. Straw 2 was made from durum wheat semolina, which was marked as an allergen. No time of stability was stated. Straw 3 was made from rye stalks and no further information was provided on the packaging. The wafer cup had a capacity of 110 mL and included oat bran and wheat flour, indicated as allergens on the label. The time of stability was stated to be 40 min. The FCM tested represent those commonly available in Germany, but these may be different in other countries, e.g., the United States, where stalk-based materials are used more frequently compared to bran.

Sample preparation and migration study

The migration of gluten from FCM was tested at conditions expected for normal usage of the materials. All liquid samples were tested in triplicates, while the solid foods were tested six times. A control sample was included for each simulant using paper cups and confirmed to be gluten-free (< 5 mg/kg of gluten). First, we studied the impact of contact time. The contact times were set to 15, 30, 45 and 60 min for the two straw samples (straw 1 and 2). For the plates, time steps of 10, 20, 30 and 40 min were selected, because they were not stable beyond 40 min. For the wafer cup, the contact times were set to 15, 30, 45 and 60 min. Straw 3 and the

cutlery were not included, because all results using constant contact times were below the limit of quantitation (5 mg/kg of gluten) of the ELISA (see below).

Second, the FCM were tested at one time point each considering their use and stability to liquid. In an effort to prevent false negatives, we decided to use the longest times until which the products were still mostly stable. Therefore, the cutlery was tested in pairs, including fork and knife, with DI water, 3% acetic acid, 15% ethanol and olive oil as well as gluten-free bread. The bread was cut into 25 pieces with the knife and fork. The samples for the three straws were prepared in narrow beakers with a volume of 200 mL and a contact time of 40 min with the liquids mentioned above. The plates were tested with simulants A–D as well as the solid foods [14]. The contact time for the liquids was 30 min and the volume was 100 mL. The gluten-free foods were prepared according to the instructions stated on the packaging. The contact time was set to 20 min. One slice of the gluten-free bread was placed on the plate and shaken for 5 min and 20 min on a multi-vortex mixer. The wafer cup was tested using the different liquid samples (DI water, 3% acetic acid, 15% ethanol, coffee (60 °C), Coca-Cola, pineapple juice and soda water) with a volume of 100 mL for a contact time of 40 min.

Gluten concentration

Gluten analysis by ELISA was performed using the RIDASCREEN Gliadin (R7001, R-Biopharm, Darmstadt, Germany) and RIDASCREEN Gliadin competitive test kits (R7021, R-Biopharm). The sample preparation was carried out according to the manuals of the test kits. To analyze the samples prepared with the plates, the samples were diluted 1:10 before the measurement. For Coca-Cola, pineapple juice, coffee and all solid foods, the sample preparation included the workup with skim milk powder or gelatin for the RIDASCREEN Gliadin or the RIDASCREEN Gliadin competitive, respectively, according to the manufacturer's instructions. The absorption was read at 450 nm using a plate reader Tecan i-control infinite 200Pro (2.0.10.0, Tecan, Männedorf, Switzerland). The results were calculated using the RIDASOFT Win.NET software (R-Biopharm) to receive gluten concentrations. The RIDASCREEN Gliadin test kit has a limit of quantitation of 5 mg/kg of gluten, which is why values below the calibration range are given as < 5 mg/kg in the following. The RIDASCREEN Gliadin competitive test kit has a limit of quantitation of 10 mg/kg of gluten.

Results and discussion

First, we analyzed the gluten content of the FCM directly to know how much gluten is present and could therefore migrate into foods and drinks. The cutlery and straw 3 were not analyzed, because the material was very hard and could not be homogenized or extracted. The gluten content of the other four materials ranged from 12.8 g/kg (straw 2) to 91.4 g/kg (plate) (Table 1), which confirms the presence of comparatively high amounts that could be transferred into foods and drinks. These results match those reported by the Spanish and Dutch studies that reported a gluten content of > 80 mg/kg (Spanish study) and of > 40 g/kg (Dutch study) for a wheat bran plate and of > 8 g/kg for an edible straw made of wheat bran, apple pulp and sugar (Dutch study), even if those values were only given as above a certain content [15].

Table 1 Gluten content of the food contact materials

Product	Raw material	Allergen labelling	Recommended contact time	Gluten content
			[min]	[g/kg]
Cutlery	90% polylactic acid and wheat bran	No[1]	n/a	n.t
Straw 1	Durum wheat semolina and wheat gluten	Yes	≥ 60	61.6 ± 1.2
Straw 2	Durum wheat semolina	Yes	n/a	12.8 ± 1.8
Straw 3	Rye stalks	No	n/a	n.t
Plate	100% edible wheat bran	No[2]	15	91.4 ± 12.0
Wafer cup	Oat bran and wheat flour	Yes	40	24.3 ± 1.8

Data are presented as mean ± standard deviation ($n = 3$, R5 sandwich ELISA)

n/a: information not available

n.t.: not tested, because the products could not be homogenized or extracted, because the material was very hard

[1] Wheat as allergen was only stated on the website of the manufacturer

[2] Wheat as allergen was only stated in personal communication with the manufacturer

Cutlery

Potential gluten migration from the forks and knives was tested with DI water, 3% acetic acid, 15% ethanol and oil after a contact time of 60 min. All values were < 5.0 mg/L, indicating that no detectable concentrations of gluten had migrated from the cutlery into the simulants. Cutting a gluten-free bread into 25 pieces using both fork and knife essentially produced the same result, because the gluten content of the bread was still < 5.0 mg/kg. Both tests indicate that gluten migration seems to be negligible from the specific brand of cutlery that we tested. Using those forks and knives seems to be safe, likely due to their hardness and composition of 90% polylactic acid, comparatively small contact areas and short contact times in case of pricking and cutting. However, further testing using a broader range of foods is advisable to confirm whether those forks and knives are indeed safe to use for celiac disease patients.

Straws

Gluten migration into liquids was tested for straws 1 and 2 with the simulants DI water, 3% acetic acid and 15% ethanol and real-life drinks. Coffee was selected, because it is acidic (pH ≈ 5) and served hot (60 °C) and the temperature might increase the release of gluten from the straws. Coca-Cola was chosen as a carbonated drink and its low pH value of ≈ 2.5. Pineapple juice was selected as a representative for juices and sparkling water (pH ≈ 5.5) was included as comparison to DI water (pH = 7). The experiment using different contact times until 60 min showed no detectable gluten migration from straw 1 into DI water and 15% ethanol, except for one value at 45 min using DI water, which was just above the LOQ (5.6 mg/L) (Table 2). In contrast, the gluten concentration increased to up to 20.2 mg/L using 3% acetic acid as simulant. Based on these results and stability of the straws, we selected 40 min as constant contact time to test all simulants again and the selected drinks. The results showed no detectable gluten migration from straw 1 into DI water, coffee, Coca-Cola, pineapple juice and sparkling water (Fig. 1A). Gluten concentrations of 15.8 mg/L and 28.1 mg/L were found in 3% acetic acid and 15% ethanol, respectively. While the result for 3% acetic acid matched those of the time series (Table 2), there was considerable variation in the results using 15% ethanol, as also evidenced by the large standard deviation (up to 112%). This can be explained by variations in soaking behavior of different straws, because, e.g., one straw was already soggy, while another one still stood stable in the liquid.

The results for straw 2 were quite comparable overall to those of straw 1. There was no detectable gluten migration from straw 2 into DI water and 15% ethanol until 60 min, but gluten concentrations up to 68.3 mg/L (60 min) were measured in 3% acetic acid (Table 2). The experiment using the different simulants and drinks for a contact time of 40 min confirmed these findings, because gluten migration from straw 2 was only detected using 3% acetic acid, which had a concentration of 42.8 mg/L (Fig. 1B). Since both straw 1 and 2 were made of durum wheat semolina, their comparable behavior is according to our expectations. Overall, further investigations will be needed to find out why gluten was detected in 3% acetic acid as a simulant, but not in any of the acidic beverages.

For straw 3 made from rye stalks we found no detectable gluten migration with either of the simulants or drinks at a contact time of 40 min, because all gluten concentrations were < 5.0 mg/L.

Table 2 Gluten migration from straws, plates and cups into different liquids depending on the contact time

Time [min]	Deionized water	Acetic acid 3%	Ethanol 15%	Oil
	Simulant A	Simulant B	Simulant C	Simulant D
		Gluten [mg/L]		
Straw 1				
0	< 5.00	< 5.00	< 5.00	n.t
15	< 5.00	7.5	< 5.00	n.t
30	< 5.00	13.8	< 5.00	n.t
45	5.6	20.3	< 5.00	n.t
60	< 5.00	20.2	< 5.00	n.t
Straw 2				
0	< 5.00	< 5.00	< 5.00	n.t
15	< 5.00	28.9	< 5.00	n.t
30	< 5.00	37.0	< 5.00	n.t
45	< 5.00	51.5	< 5.00	n.t
60	< 5.00	68.3	< 5.00	n.t
Plates				
0	< 5.00	< 5.00	< 5.00	< 5.00
10	114.7	103.3	56.1	< 5.00
20	161.4	121.0	69.5	< 5.00
30	275.9	182.2	70.6	< 5.00
40	278.5	109.9	123.7	< 5.00
Wafer cup				
0	< 5.0	< 5.0	< 5.0	n.t
15	15.3	6.3	< 5.0	n.t
30	13.1	5.9	6.1	n.t
45	22.1	8.7	12.8	n.t
60	29.4	10.6	14.3	n.t

n.t.: not tested, because these combinations do not represent common use

< 5.00 is the limit of quantitation of the ELISA kit and means that gluten was not detected

The recommended contact times were ≥ 60 for straw 1, not stated for straw 2, 15 min for the plates and 40 min for the wafer cup

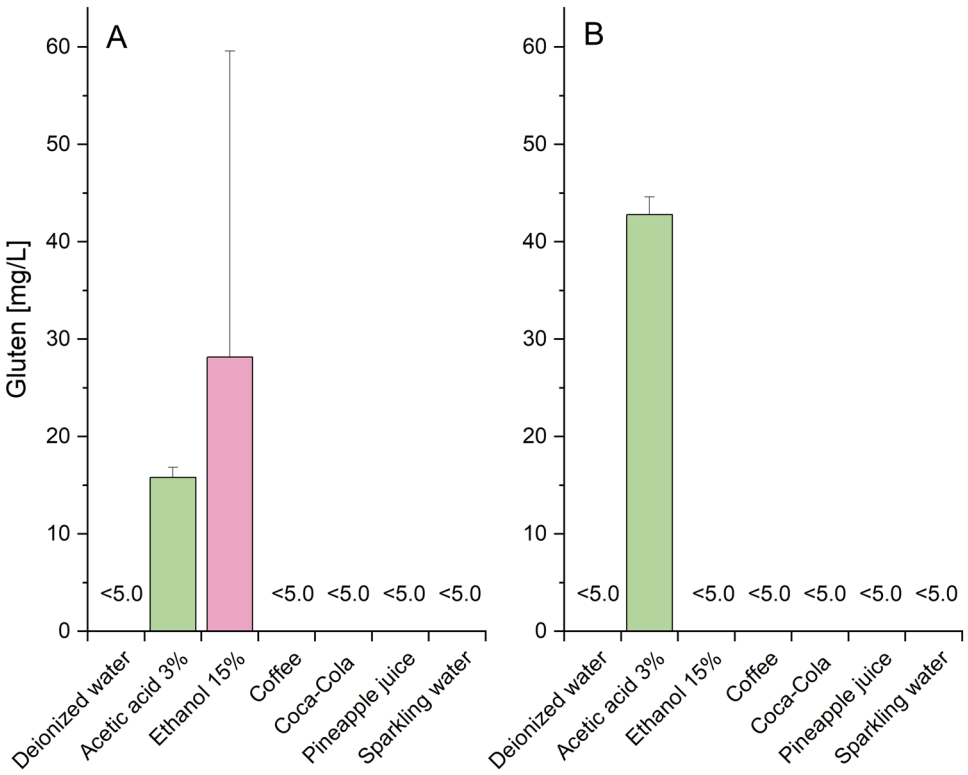

Fig. 1 Gluten migration into 200 mL of different liquids from straw 1 **A** and straw 2 **B** after a contact time of 40 min. Data are presented as mean + standard deviation ($n = 3$, R5 sandwich ELISA). There was no detectable gluten migration from straw 3 (all results < 5.0 mg/kg of gluten)

Our results are in line with those of the Dutch study that found a gluten concentration of 15 mg/L in milk that had passed through a wheat bran-based straw. Further, the Italian study also reported that straws from wheat stalks contained no detectable gluten, just as for our straw 3 made from rye stalks [15]. Therefore, straws made from stalks can be reasonably assumed to be safe for celiac disease patients, because gluten is only present in the grains, not in other parts of the wheat or rye plant. We found no evidence of grain dust being an issue, but stalks for use as FCM need to be properly cleaned after harvest to avoid cross-contact with gluten from the grains. In contrast, straws made from durum wheat semolina are definitely not safe.

Plates

Gluten migration from plates into simulants A–D at different time points clearly showed an increase of gluten concentrations up to 278.5 mg/L, 109.9 mg/L, and 123.7 mg/L in DI water, 3% acetic acid and 15% ethanol, respectively. Oil was the only simulant where we did not detect gluten (all values < 5 mg/L) (Table 2). At a constant contact time of 30 min, 203.0 mg/L, 194.9 mg/L, and 154.2 mg/L of gluten had migrated from the plates into DI water, 3% acetic acid and 15% ethanol, respectively, but < 5 mg/L of gluten into oil (Fig. 2). As already seen for straw 1, we had considerable variation (up to 25%) between the three replicates, because the plates behaved differently despite testing using the exact same conditions. For example, one plate was completed soaked after 30 min, while the second one was still stable and the third one had only lost some moisture through the bottom. This points to inhomogeneity within the plates and between different plates, even from the same lot.

To see whether gluten from the plates could also be transferred to solid foods, we placed one slice of gluten-free bread on the plate and shook it for 5 min or 20 min on a multi-vortex mixer. This resulted in more vigorous contact than could be expected in everyday use. After 5 min, the gluten content of the gluten-free bread was still < 5 mg/kg, but after 20 min it was 7.9 ± 1.0 mg/kg, showing that gluten from the plates could also contaminate dry products. When gluten-free fish sticks, pizza base, ravioli and lasagna were put onto the plates for 20 min, we did not detect gluten in the pizza base (all results < 5 mg/kg, n = 6). For fish sticks and ravioli, 5 out of 6 replicates also had < 5 mg/kg of gluten, while 1 replicate each had detectable gluten concentrations of 7.9 mg/kg (fish sticks) and 11.7 mg/kg (ravioli). The largest variability of results was observed for lasagna, where 4 out of 6 replicates had < 5 mg/kg of gluten, but the other 2 replicates had 10.4 to 1088.2 mg/kg of gluten. This points to an inhomogeneous distribution of gluten from the plates in the lasagna, likely dependent on the properties of the plate and the contact area of the lasagna.

Our results confirm those of the Dutch study that reported a gluten content of 24 mg/kg in gluten-free bread on a plate made from wheat bran. In addition, the Italian study showed

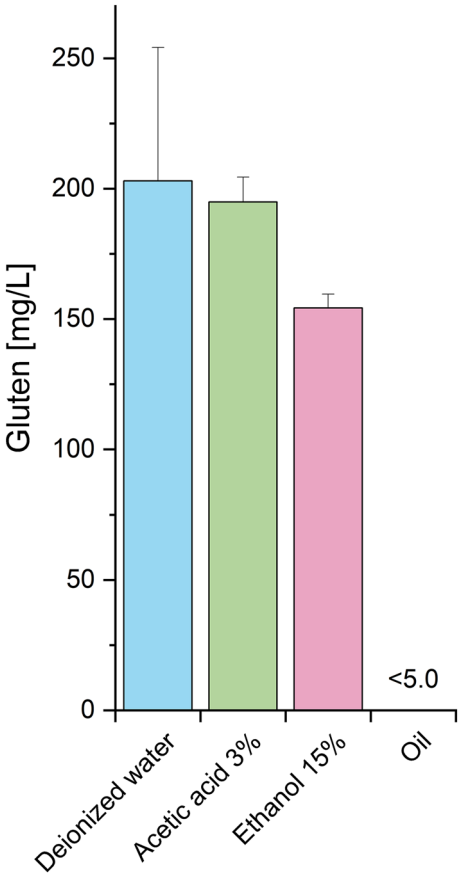

Fig. 2 Gluten migration into 100 mL of different liquids from the plate after a contact time of 30 min. Data are presented as mean + standard deviation (n = 3, R5 sandwich ELISA)

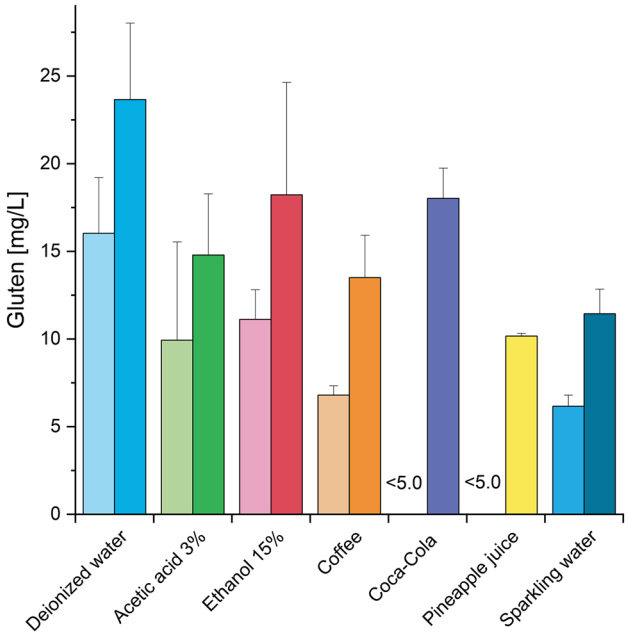

Fig. 3 Gluten migration into 100 mL of different liquids from the wafer cup after a contact time of 40 min. Data are presented as mean + standard deviation ($n = 3$). The left bar of each pair represents the result using the R5 sandwich ELISA and the right bar that using the R5 competitive ELISA, respectively

that more than 80 mg/kg of gluten were detected in lasagna that was placed hot on a wheat bran-based dish and this is also validated by our findings [15]. The fact that we did not detect gluten in oil as simulant might also be due to difficulties in extraction, as pure oil is not a recommended matrix for ELISA testing.

To sum up, considerable amounts of gluten migrated from wheat bran-based plates into originally gluten-free liquids and foods. Therefore, wheat allergy and celiac disease patients definitely need to avoid using those materials. As wheat was not indicated on the packaging of the plates, this is difficult to undertake in real life, e.g., at a food stall. On these occasions, patients can only be cautious themselves in case they receive a plate with the rather characteristic dark color and coarse-grained appearance of wheat bran.

Wafer cup

Simulants A–C were filled into wafer cups for different times and the gluten concentration reached up to 29.4 mg/L, 10.6 mg/L, and 14.3 mg/L in DI water, 3% acetic acid and 15% ethanol, respectively (Table 2). After a constant time of 40 min, gluten concentrations were < 5 mg/L for Coca-Cola and pineapple juice, but between 6.2 mg/L (sparkling water) and 16.0 mg/L (DI water) were detected using the R5 sandwich ELISA (Fig. 3). Since we thought that small fragments of gluten from the wafer cup could also be present, we additionally analyzed the liquids with the R5 competitive ELISA that only requires one antibody-binding site and therefore not only detects intact gluten, but also partially hydrolyzed gluten [16]. Using the R5 competitive ELISA, we detected gluten in all liquids and the concentrations ranged from 10.2 mg/L in pineapple juice to 23.7 mg/L in DI water (Fig. 3). The largest discrepancy between the results of both ELISA formats occurred for Coca-Cola and pineapple juice, where both values were < 5.0 mg/L when analyzed with the sandwich assay compared to 18.0 mg/L and 10.2 mg/L, respectively, when analyzed with the competitive assay. Even though it is known that the competitive assay usually provides higher values for the same sample compared to the sandwich assay, unless the sample has been heated [17, 18], these results still point to the presence of partially hydrolyzed gluten that escape detection in the sandwich assay.

One limitation of our study is that we did not re-run all the analyses for the other experiments with the cutlery, straws and plates, where we only have results from the sandwich assay, which is most commonly used unless the presence

of partially hydrolyzed gluten is expected, as in, e.g., beer or sourdough samples. Therefore, it might be possible that higher gluten concentrations would have been found using the competitive assay for these experiments, too. However, a comparison between the results of both ELISA formats was not the focus of our work, but rather a side aspect that warrants further investigations.

When comparing the different liquids, the highest gluten concentration originating from the wafer cup was detected in DI water. This is not necessarily according to our expectations, because gluten is defined as a protein that is not soluble in water, but rather in aqueous alcohols [11]. However, it is known that gluten is partially soluble also in water [19], and we observed gluten migration into DI water also from the plates. The gluten concentrations in 3% acetic acid and 15% ethanol were comparable and slightly higher compared to coffee and sparkling water. There was no clear effect of pH value or elevated temperature regarding the potential to enhance gluten migration and all results were in a rather narrow range overall. Despite the fact that all concentrations, except the value for DI water when measured with the competitive assay, were below the threshold of 20 mg/L for gluten-free products, we can only advise patients against using these cups. As these cups were sold as food and had wheat clearly indicated as an allergen on the label, avoidance could be easier compared to the plates that had no information on the packaging.

Conclusion

Of the six different FCM studied, we found that gluten was transferred into liquids from four materials, namely the durum wheat semolina-based straws 1 and 2, the wheat-bran based plates and the oat bran and wheat flour-based wafer cup. Further, gluten from the plates also migrated into gluten-free solid foods. The extent of gluten migration depended on the properties of the FCM itself, most importantly on its stability during use. It also depended on the liquid or solid food it came into contact with and here gluten from all four materials migrated in 3% acetic acid, from three materials in 15% ethanol and from two materials also in water and further liquids. Contact time was important with increasing gluten migration after longer times. There was no clear effect of further conditions, such as temperature and pH value.

There was no detectable gluten migration from the cutlery, most likely due to short contact times and small contact areas, and also not from the rye stalk-based straw 3, because the stalk does not contain gluten.

To protect patients, it is important to raise awareness of the risk of gluten migration from such FCM as the frequency of exposure is projected to increase with the move towards biodegradable packaging. In terms of legislation, Regulation (EU) No 1169/2011 requires allergen labelling, but only applies to food and not to FCM, while Regulation (EC) No 1935/2004 applies to FCM, but does not require allergen labelling. This leaves a regulatory gap that needs to be filled urgently involving celiac societies, the European Food Safety Authority and the European Commission. In the meantime, we can only advise patients to avoid using those FCM and to be cautious when travelling or eating out.

Conflict of interest

The authors have no relevant financial or non-financial interests to disclose.

Ethics Statement

This article does not contain any studies with human or animal subjects.

Acknowledgements This study was supported by the Deutsche Zöliakie-Gesellschaft e.V. (German Celiac Society). Open Access funding enabled and organized by Projekt DEAL.

Author contributions Conceptualization: Johanna Mossburger, Katharina Scherf; Formal analysis and investigation: Johanna Mossburger; Writing—original draft preparation: Johanna Mossburger, Katharina Scherf; Funding acquisition: Katharina Scherf; Resources: Katharina Scherf; Supervision: Katharina Scherf.

Funding Open Access funding enabled and organized by Projekt DEAL. Deutsche Zöliakie-Gesellschaft e.V.

Declarations

Data availability Data will be made available upon request.

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