

Gluten contamination in oat varieties and gluten-free oat-only products from them

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Abstract

Gluten contamination from wheat, rye, and barley was examined in oats at initial and final stages of the production-consumption line, i.e., in seed form in field and processed, packaged form in market. The study focused on 23 oat seed varieties and 15 gluten-free oat-only products from these seeds. The oat seeds included all varieties registered in Turkey (23), cultivated in an experimental field during 2021–2023 growing seasons and harvested. 15 gluten-free oat-only products represented all such products available on market. A gluten-free protocol was followed throughout harvesting, handling, and analysis processes. Contamination levels in samples were determined using R5 antibody-based sandwich ELISA method, based on 5 ppm and 20 ppm gluten concentration limits. None of oat seeds had a gluten concentration greater than 5 ppm. However, 11 of 15 gluten-free oat-only products (73%) had a gluten concentration greater than 5 ppm, and 10 gluten-free oat-only products (67%) had a gluten concentration greater than 20 ppm. The contamination rate in seeds was significantly lower than global average, while in gluten-free oat-only products, it was considerably higher. This trend reflects the high prevalence of contamination worldwide in both oat seeds and gluten-free oat-only products, with contamination rate being higher in gluten-free oat-only products, suggesting that increased interventions lead to higher contamination levels.

Keywords: contamination; gluten, gluten-free; oat.

Practical Application: Oats are gluten-free (GF), however gluten-free foods made of them are mostly contaminated with gluten in Turkey.

1 INTRODUCTION

Oats are growing increasingly popular among gluten-free food (GFF) consumers. They hold significant potential to address the nutritional shortcomings of processed GFFs (Gobbetti et al., 2018; Marciniak et al., 2021) and are recognized for improving their flavor and functional properties (Hoffmanová et al., 2019).

Wheat, barley, and rye are universally acknowledged to contain gluten naturally. However, there is no unanimous agreement regarding oats. Some researchers argue that oats naturally contain gluten (Benoit et al., 2017), while others suggest that oats are inherently gluten-free but may be contaminated with gluten from external sources such as wheat, barley, or rye (Fritz & Chen, 2020).

Both perspectives are reflected in regulations concerning GFFs. Mainstream legislation (Canada, 2015; European Union [EU], 2014; Food and Drug Administration [FDA], 2013) allows the use of oats provided the gluten concentration (GC) in the final product does not exceed 20 ppm. In contrast, some regulations, such as the Australia New Zealand Food Standards Code (Australia, 2014), prohibit the inclusion of oats in GFFs. The former approach could pose a risk to GFF consumers, particularly in the long term if oats naturally contain gluten.

The latter approach, however, might unnecessarily deprive GFF consumers of the benefits of uncontaminated oats if they are inherently gluten-free.

As seen in Turkey, the potential of oats has garnered significant attention globally (Smulders et al., 2018). For example, a survey conducted between 2015 and 2017 revealed that domestically manufactured GFFs containing oats—either as the sole ingredient or as part of the recipe—were not available in Turkey (Atasoy, Gokhisar, & Turhan, 2020). Since then, a notable number of domestic GFFs featuring oats have entered the market. However, gluten contamination in oats remains an unresolved issue, continuing to concern GFF consumers worldwide (Guenouni et al., 2022).

Oats are used without their husks and are referred to as groats. Groats are typically processed into rolled oats, flakes, steel-cut oats, flour, and similar products, with or without bran. These can serve as the sole ingredient in oat-only products or as one of the components in oat-based products. The bran can also be consumed either as a standalone ingredient or as part of a product. Oat-only products can also act as raw materials for oat-based products. If oat-only products contain exogenous gluten, the GC in oat-based products will depend on the proportion of oat-only products in their formulations. Understanding the relationship between

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groats and oat-only products concerning exogenous gluten is a crucial first step in identifying gluten contamination in oat products.

Several studies have examined gluten contamination in ordinary oat-only products (OROs) and GFFs containing oats as an ingredient (Koerner et al., 2011; Raju et al., 2020). However, research on gluten contamination in oat seeds (OSs) (Gell et al., 2021) and gluten-free oat-only product (GFOs) (Rodríguez et al., 2022; Thompson & Keller, 2023) remains limited. To the authors' knowledge, no studies have specifically investigated exogenous gluten in OSs and the GFOs from them. Furthermore, no research has yet addressed gluten contamination in OSs or GFOs in Turkey. This study aims to evaluate gluten contamination in various oat varieties and the GFOs produced from them.

1.1 Relevance of the work

Oats play an important role in gluten-free foods due to their high nutritional value. As the usage of oats increases in GFF production globally, so do contamination concerns. Understanding gluten contamination in oat grain would address contamination in GFFs containing oats (GFOs). Research on gluten contamination in GFOs is limited, and no simultaneous studies on contamination in both oat seeds and their GFOs have been identified. Currently, there are no studies in Turkey addressing it in oats (seeds, products). Our manuscript aims to detect gluten contamination in OSs grown in Turkey and their GFOs, with potential strategies to mitigate/eliminate contamination.

2 MATERIALS AND METHODS

2.1 Samples

In total, 23 registered and commercially available oat varieties were cultivated at the experimental field of the Trakya Agricultural Research Institute in Turkey during the 2021–2023 growing seasons and harvested accordingly (Table 1). The experimental field was located near other experimental wheat fields at the Institute. To prevent gluten contamination, a gluten-free protocol was followed during the harvesting process. Panicles of each variety were manually collected by experienced staff wearing gloves. The staff changed gloves and clothes between collections of different varieties. The oat panicles were placed in material-proof paper bags and then sealed. Additionally, 15 packaged oat products (11 rolled, 4 flour) labeled as gluten-free, with oats as the only ingredient, were purchased from the market. All samples were stored at 4°C until the GC analysis.

Gluten concentration analysis

GC analysis was conducted in a traffic-limited, gluten-free laboratory, where a strict gluten-free protocol was followed to prevent potential contamination from staff or the environment. Panicles from each oat variety were separated into spikelets and whole grains, with the husks of each whole grain removed by hand using gloves. The packages of oat varieties and GFOs were treated with 40% ethanol before and after entering the laboratory. New gloves and coats were used, and benches were cleaned with ethanol between each sample preparation. GC analysis was performed twice for each sample. The analyses were carried

Table 1. Oat varieties used in the work.

	Variety	Registry
1	Sebat	Trakya Agriculture Company, Tekirdag, Turkey
2	Yeniçeri	Bahri Dagdas International Agricultural Research Institute, Konya, Turkey
3	Kahraman	Trakya Agricultural Research Institute, Edirne, Turkey
4	Kırklar	Trakya Agricultural Research Institute, Edirne, Turkey
5	Sarı	Aegean Agricultural Research Institute, Izmir, Turkey
6	Fetih	Aegean Agricultural Research Institute, Izmir, Turkey
7	Albatros	Ata Tohumculuk Company, Ankara, Turkey
8	Bc Marta	BC Institute Company, Ankara, Turkey
9	Dirilis	Bahri Dagdas International Agricultural Research Institute, Konya, Turkey
10	Arslanbey	Sutcu Imam University, Faculty of Agriculture, Kahramanmaraş, Turkey
11	Küçükayla	Trakya Agricultural Research Institute, Edirne, Turkey
12	Kehlibar	Som Un Company, Kırklareli, Turkey
13	Kayı	Aegean Agricultural Research Institute, Izmir, Turkey
14	Kupa	BC Institute Company, Ankara, Turkey
15	Halkalı	Trakya Agricultural Research Institute, Edirne, Turkey
16	Somun Yıldızı	Som Un San. ve Tic. Ltd. Şti. Company, Kırklareli, Turkey
17	Kazan	Bahri Dagdas International Agricultural Research Institute, Konya, Turkey
18	Manas	Aegean Agricultural Research Institute, Izmir, Turkey
19	Yazır*	Bahri Dagdas International Agricultural Research Institute, Konya, Turkey
20	Avar	Aegean Agricultural Research Institute, Izmir, Turkey
21	Kınalı	Trakya Agricultural Research Institute, Edirne, Turkey
22	Kaymaklı	Trakya Agricultural Research Institute, Edirne, Turkey
23	Elmas	Trakya Agricultural Research Institute, Edirne, Turkey

*Yazır is naked and others are husked.

out using the Ridascreen Gliadin R 7001 Sandwich ELISA test kit (R-Biopharm, Darmstadt, Germany), which utilizes the R5-Mendez method, an R5 antibody-based enzyme-linked immunosorbent assay (ELISA). The procedure provided by the supplier for oat samples was followed.

The R 7006 Cocktail solution (R-Biopharm, Darmstadt, Germany) was used for extraction as per the guideline. For each analysis, a 200 g sample was homogenized using a grinder. From the homogenized sample, 1 g was weighed into a vial and 10 mL of cocktail solution was added. The mixture was vortexed for at least 60 s, and then incubated for 40 min at 50°C. After cooling, 30 mL of 80% ethanol solution was added, and the sample was shaken in a rotator (MX-RD-Pro, DLAB, Beijing, China) for 1 h at room temperature. The samples were then centrifuged at 14,000 rpm for 10 min (Zentrifugen D-78532, Hettich, Tuttlingen, Germany). The supernatant was transferred to a screw-top vial and stored in the dark at room temperature until use the following day.

The supernatants obtained the previous day were diluted according to the guidelines. Extracts (100 µL) were added to the wells and incubated for 30 min at room temperature. After the incubation, the wells were washed three times with the washing buffer. Diluted conjugate (100 µL) was then added to the wells, followed by another 30-min incubation and subsequent washing, as described earlier. Substrate (50 µL) and chromogen (50 µL) were added to the wells and incubated in the dark for 30 min. Afterward, stop solution (100 µL) was added, and the plate was gently shaken. Finally, the absorbance was measured at 450 nm using an ELISA Reader (Multiskan Go, Thermo Scientific, Massachusetts, USA).

The performance of each analysis cycle, consisting of eight samples, was monitored using a FAPAS Quality Control Material, Cake Mix (T27298BQC) (Fera Science, UK). A calibration curve was prepared for each analysis cycle using standard gluten solutions (0, 5, 10, 20, 40, and 80 ppm) provided with the test kit, following the procedure outlined above (Figure 1). The GC of the samples was determined from the calibration curve based on their absorbance.

3 RESULTS

Benoit et al. (2017) quantified avenin in several oat varieties using the same kit employed in this study (Ridascreen Gliadin R 7001) and validated the results with SDS-PAGE. The kit manufacturer specifies that it is designed for the quantitative analysis of prolamins from wheat (gliadin), rye (secalin), and barley (hordein) in both raw products (such as oats) and processed foods (R-Biopharm, 2021). However, the manufacturer does not mention prolamins from oats (avenin, the endogenous gluten). Consequently, the gluten detected in the samples in this study has been attributed to gluten contamination from wheat, rye, or barley.

The GC of the test material used in this study (FAPAS Quality Control Material) was reported in its datasheet as 14.2 ppm, with a Z-score range of 7.1–21.3 ppm. This study determined its GC was 14.3 ppm, with a Z-score range of 8.2–17.9 ppm. A third-order polynomial function was used

to fit all calibration curves, yielding a coefficient of determination (R^2) greater than 0.9990 and closely aligned regression constants. The calibration curves nearly overlapped (Figure 1). The similarity between the GC concentrations, along with the high R^2 values and overlapping calibration curves, demonstrates the consistency of the results and the reliability of the GC analysis conducted.

Calibration curves for GC levels of 0, 5, 10, 20, 40, and 80 ppm were generated in this study. The test kit used has a reported limit of detection (LOD) of 1 ppm GC and a limit of quantification (LOQ) of 5 ppm GC. Absorbance readings between 0 ppm and 5 ppm GC do not necessarily indicate the presence of gluten in a sample, as they may result from matrix effects and are susceptible to false positives and negatives. False absorbance is significant below 5 ppm GC, while it becomes negligible above this threshold. Consequently, the LOQ is deemed adequate considering analytical sensitivity, technological limitations, regulatory standards, cost, accessibility, cross-reactivity, specificity, and the matrix effect (R-Biopharm, 2021). The kit manufacturer recommends reporting the GC of samples with absorbance lower than the standard 5 ppm GC as “< LOQ” rather than providing a specific value.

Different perspectives exist regarding the acceptable GC in GFFs. Mainstream regulations set the contamination limit at 20 ppm GC (Canada, 2015; EU, 2014; FDA, 2013), while others, such as the Australia New Zealand Food Standards Code (Australia, 2014) and Chile Health Regulation (Chile, 2022), adopt the LOQ of the analytical method, typically 5 ppm. The preference for using LOQ instead of 20 ppm has been gaining traction (Monachesi et al., 2021), and the GC results in this study were reported for both limits.

The absorbance of all 23 oat seed samples corresponded to GC < 1 ppm in the calibration curves (Figure 1). As recommended by the kit's manufacturer, the GC for all OSs was therefore reported as < 5 ppm (LOQ). Among the 15 gluten-free oat-only products (GFOs) analyzed, the absorbance of 11 samples exceeded the 5 ppm threshold and 10 samples exceeded the 20 ppm limit.

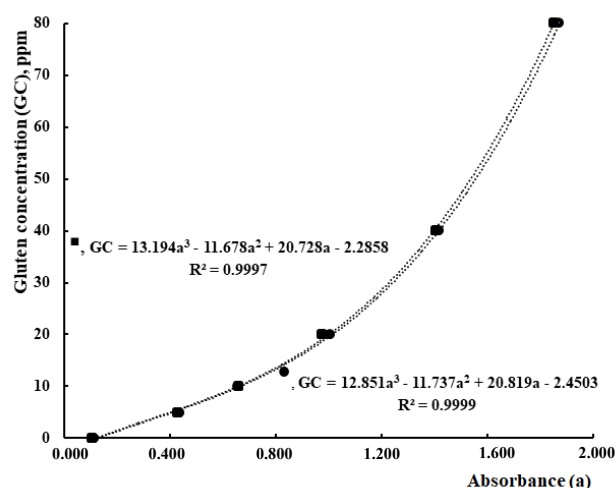


Figure 1. Sample calibration curves used to determine gluten concentration in samples.

4 DISCUSSION

4.1 Contamination in oat seeds and gluten-free oat-only products

All necessary precautions were taken from field harvesting to laboratory analysis to ensure adherence to a gluten-free protocol, minimizing the risk of contamination in OSs. None of the oat varieties analyzed had a GC exceeding 5 ppm (Table 1), indicating that both hulled and dehulled seeds remained uncontaminated, even when cultivated near wheat fields. Since the gluten was assessed in the groat form, this finding suggests that the outermost layer, the husk, was also free from exogenous gluten. If the husk had been contaminated, it would have transferred gluten to the groats during dehulling, which would have been detected in the GC analysis.

The naked seed variety, Yazır, also had a GC < 5 ppm (Table 1), further supporting that the surfaces of the oat varieties were not contaminated with exogenous gluten. Based on both the 5 ppm and 20 ppm thresholds, the contamination rate of the seeds was determined to be 0%. This absence of exogenous gluten demonstrates the effective application of the gluten-free protocol throughout the process, from field to laboratory, under the specified working conditions.

The findings of this study are comparable to previous research on gluten contamination in OSs (Table 2). Hernando et al. (2008) reported that none of the 25 oat varieties harvested in Spain had a GC > 5 ppm. In contrast, Ballabio et al. (2011) and Benoit et al. (2017) found contamination in OSs from Italy and the USA, respectively. Ballabio et al. (2011) analyzed 36 oat varieties and detected that 32 varieties (89%) had a GC > 5 ppm, while 7 varieties (19%) had a GC > 20 ppm. Similarly, Benoit et al. (2017) reported that 3 out of 20 oat varieties (15%) had a GC > 20 ppm. Vilmane et al. (2015) studied 6 oat varieties from Latvia and found all had a GC > 20 ppm. Gell et al. (2021) reported that 44 and 13% of 32 oat varieties from Australia were

contaminated according to the 5 ppm and 20 ppm thresholds, respectively. For Hungary, Gell et al. (2021) found contamination in 11 and 10% of 35 oat varieties based on the 5 ppm and 20 ppm criteria, respectively.

Globally, contamination was observed in 66 (37%) and 30 (17%) of 177 oat varieties based on the 5 ppm and 20 ppm thresholds, respectively (Table 2). The absence of contamination in this work is significantly lower than the global averages, likely due to the manual harvesting process employed. Unlike this study, the cited works in Table 2 do not specify their oat sampling methods.

Preventing contamination of OSs with exogenous gluten during the journey from harvesting to GC analysis has not always been successfully achieved in the literature. Excluding the work of Hernando et al. (2008) and the present study, OSs from different countries were contaminated with exogenous gluten (Table 2), highlighting the lack of proper implementation of gluten-free protocols. The journey of OSs from harvest to analysis is relatively short and straightforward compared to the more complex route from harvesting through processing, handling, and packaging. If contamination cannot be prevented under the more controlled conditions of the former, minimizing or eliminating it under the harsher conditions of the latter appears highly challenging.

As in other countries (Burden et al., 2015; de Koning et al., 2024; Lambert & Ficken, 2016), GFF issues in Turkey have been documented by Turhan (2017) and Atasoy, Gokhisar, and Turhan (2020). One of the study's key findings, which covers the years 2015–2017, was the absence of GFOs and GFFs containing oats as an ingredient (Atasoy et al., 2019). Since then, the number and variety of these products have increased significantly, following the global trend (Smulders et al., 2018). In this study, 15 domestically produced GFOs (11 rolled oats, 4 flour) were detected in the market. However, the qualitative

Table 2. Number and rate of gluten contamination in oat seeds and gluten-free oat-only products.

Reference and country	Total	Number, rate (%)	
		> 5 ppm	> 20 ppm
Seed			
Hernando et al. (2008), Spain	25	0, 0	0, 0
Ballabio et al. (2011), Italy	36	32, 89	7, 19
Vilmane et al. (2015), Latvia	6	6, 100	6, 100
Benoit et al. (2017), USA	20	NA*	3, 15
Gell et al. (2021), Australia	32	14, 44	4, 13
Gell et al. (2021), Hungary	35	11, 31	10, 29
This work, Turkey	23	0, 0	0, 0
Overall	177	66, 37	30, 17
Overall (excluding this work)	154	66, 43	30, 19
GFO			
Gélinas et al. (2008), Canada	5	NA	2, 40
Rysová et al. (2019), Czech Rep.	3	0, 0	0, 0
Rodríguez et al. (2022), Chile	25	10, 40	9, 36
This work, Turkey	15	11, 73	10, 67
Overall	48	23, 48	21, 44
Overall (excluding this work)	33	12, 36	11, 33

*GFO: gluten-free oat-only product; NA: not available.

progress in terms of contamination has not been as promising as the quantitative growth. Most of the GFOs are contaminated with exogenous gluten. Notably, 11 of the 15 samples (73%) have a GC greater than 5 ppm, and 10 of them (67%) have a GC greater than 20 ppm (Table 2). The presence of exogenous gluten in GFOs, despite the absence of the seed harvested in the field, indicates the lack of an effective gluten-free protocol during manufacturing. Even more concerning is the absence of mandatory gluten analysis in the final products before they are released to the market.

This study, along with a few similar works, highlights common trends in gluten contamination of GFOs (Table 2). In Canada, 2 out of 5 GFOs (1 bran, 2 meal, 2 rolled) were found to be contaminated according to the 20 ppm criterion (Gélinas et al., 2008). In the Czech Republic, 3 GFOs (all flakes) were free from gluten contamination according to the 5 ppm criteria (Rysová et al., 2019). In Chile, 10 and 9% of 25 GFOs (8 flour, 13 rolled, 4 instant) were contaminated with gluten based on the 5 ppm and 20 ppm criteria, respectively (Rodríguez et al., 2022). Globally, 23 (48%) and 21 (44%) of 48 GFOs were contaminated based on the 5 ppm and 20 ppm criteria, respectively (Table 2). Turkey's contamination rate is well above the global average and has significantly increased the overall global rate (Table 2).

Globally, both OSs and GFOs exhibit a high prevalence of contamination. However, the contamination rate is higher in GFOs, indicating that increased intervention may raise the prevalence. Nonetheless, Hernando et al. (2008) and the present study demonstrated that it is possible to keep OSs free from exogenous gluten at the harvesting stage, while Rysová et al. (2019) showed that the same can be achieved for GFOs post-harvesting and during manufacturing (Table 2). If this can be achieved even once, it could be consistently maintained.

4.2 Comparison of the contamination in GFOs, GFFs, and OROs

GFOs generally exhibit a higher prevalence of gluten contamination than GFFs. For domestic GFFs in Turkey, the contamination rates were reported to be 18.5 and 17.5% based on the 5 ppm and 20 ppm criteria, respectively (Atasoy et al., 2019). In this study, for GFOs, the contamination rates were 73% for the 5 ppm criterion and 67% for the 20 ppm criterion (Table 2). The rate for GFOs is nearly four times higher than that for GFFs. A similar disproportionality was observed in Canada, where the contamination rates were 40% for GFOs and 9% for GFFs (a four-fold difference) according to the 20 ppm criterion (Gélinas et al., 2008). The significantly higher gluten contamination in GFOs may be due to manufacturers' false assumption that oats are inherently gluten-free, leading them to skip the final analysis for exogenous gluten in the GFOs. As long as regulations do not require manufacturers to verify the gluten-free status of their final products, the gluten contamination issue in both GFOs and GFFs is unlikely to be resolved.

The contamination trends in OROs are nearly identical to those in GFOs. Gélinas et al. (2008) analyzed 3 OROs (1 bran, 2 meals) from Spain, and all were found to be contaminated according to the 20 ppm criterion. Hernando et al. (2008) collected 109 OROs (including rolled oats, flakes, meals, flour,

bran, and whole grains) from various countries in Europe, the USA, and Canada, determining that 72 and 64% of OROs were contaminated based on the 5 ppm and 20 ppm criteria, respectively. Koerner et al. (2011) found that 98 and 93% of 133 OROs (steel-cut, rolled, flake, meal, quick oats, bran) from Canada were contaminated according to the 5 ppm and 20 ppm criteria, respectively. Rysová et al. (2019) concluded that 94 and 83% of 36 OROs (including 20 flakes, 1 instant meal, 2 flours, 3 brans, 2 naked seeds, and 1 sprouted oat) from the Czech Republic contained exogenous gluten based on the 5 ppm and 20 ppm criteria. Rodríguez et al. (2022) identified that 67% of 27 OROs (6 flour, 13 rolled, 8 instant) from Chile had exogenous gluten according to the 20 ppm criterion. Storsrud et al. (2003) reported a contamination rate of 15% for 49 OROs (40 rolled and 9 bran) in Sweden based on the 20 ppm criterion.

The contamination rate in GFOs is almost identical to that in OROs. While the contamination in OROs may be more understandable, as they do not carry the gluten-free claim, it is unacceptable in GFOs, which are marketed as gluten-free, potentially misleading consumers. The similar contamination rates in both GFOs and OROs point again to the false assumption that oats are naturally gluten-free and the lack of verification for the gluten-freeness of the final product.

Thompson and Keller (2023) shared data on the gluten status of 213 oat products collected in the USA between 2011 and 2023. Of the 213 samples, 24 had a GC > 5 ppm. Oats were the only ingredient in 6 of these 24 samples, all of which underwent a gluten-free protocol and were certified. In the report, it is observed that the protocol and certification are not always utilized effectively. The gluten-free oat protocol has been proven effective in combating gluten contamination in oats (Allred et al., 2017) and was created in response to the widespread awareness of gluten contamination in oats. It can only minimize the risk of contamination if GFOs are legitimately proven to be gluten-free. The protocol alone does not ensure gluten-freeness, rather, it is the verified gluten-free status of the product that validates the protocol.

5 CONCLUSION

Gluten contamination in OSs and GFOs is a widespread issue. Although OSs are initially free of exogenous gluten, contamination can be significant due to interventions occurring after harvesting and before packaging. The prevalence of contamination is higher in GFOs compared to GFFs and is nearly the same as in OROs. This can be attributed to the false assumption that oats are naturally gluten-free, leading to a lack of GC analysis in the final product.

According to both previous and current studies, oats become contaminated with exogenous gluten at some point between harvesting in the field and packaging at the plant. Implementing decisive measures could address the contamination issue and prevent it. It is generally understood that contamination occurs through contact with exogenous gluten (Vargas et al., 2024). However, exogenous gluten is technically expected to intermingle with the oats rather than just make contact (Atasoy, Ulutas, & Turhan, 2020).

Gluten contamination in GFFs has persisted since the concept of GFFs emerged, and they have almost become an inseparable duo. Contamination can be minimized and potentially eradicated by requiring proof of gluten-freeness for every batch of GFFs produced. It is incomprehensible why such proof has not been required for years, particularly for the benefit of GFF consumers and responsible producers who can achieve clean production. For voluntary GFF consumers, they are paying for a product they do not wish to consume. For mandatory GFF consumers, the stakes are even higher, as their health is at risk in addition to their financial loss. Authorities should seriously consider regulations that demand proof of gluten-freeness, prioritizing public health and fair trade.

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