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Evaluation of the presence of gluten in oat flour labeled "gluten free" sold in Brazil

Avaliação da presença de glúten em farinhas de aveia rotuladas como "sem glúten" comercializadas no Brasil

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ABSTRACT

Gluten is a protein found in grains such as wheat, rye, and barley, and also in oats when cross-contaminated. While safe for most people, gluten can cause serious health issues in individuals with celiac disease, non-celiac gluten sensitivity, dermatitis herpetiformis, and gluten ataxia. This study evaluated the presence of gluten in oat flours labeled as "gluten free" marketed in Brazil, using a qualitative immunochromatographic strip test with a detection limit of 5 mg·kg ^{1}. Nine brands of oat flour were analyzed using lateral flow tests, performed in triplicate. The test device is designed to detect gluten in raw materials, finished food products, beverages, and environmental samples. Results showed that two brands tested positive for gluten in all three replicates (22.2% of samples). Two other brands tested positive in only one out of three replicates, which was considered a negative result, totaling 77.8% of samples classified as gluten free. These findings reveal that, despite "gluten free" labeling, there is a risk of cross-contamination in some products, which may compromise the safety of individuals with gluten-related disorders. The study emphasizes the need for stricter quality control in the gluten free food industry and the implementation of more sensitive and standardized methods to detect trace amounts of gluten, thus promoting greater consumer safety and confidence.

Keywords: immunochromatography; food analysis; celiac disease; gluten cross-contamination; food safety.

RESUMO

O glúten é uma proteína presente em grãos como trigo, centeio e cevada, além da aveia quando contaminada. Embora seja seguro para a maioria das pessoas, pode causar sérios problemas em indivíduos com doença celíaca, sensibilidade ao glúten não celíaca, dermatite herpetiforme e ataxia por glúten. Este estudo avaliou a presença de glúten em farinhas de aveia rotuladas como "sem glúten", comercializadas no Brasil, utilizando o método qualitativo por fitas imunocromatográficas, com limite de detecção de 5 mg·kg ^{1}. Foram analisadas nove marcas de farinha de aveia por meio de teste de fluxo lateral, realizado em triplicata. O dispositivo utilizado é indicado para detectar glúten em matérias-primas, alimentos prontos, bebidas e superfícies ambientais. Os testes mostraram que duas marcas apresentaram glúten em todas as replicatas (22,2% das amostras). Outras duas marcas testaram positivo em apenas uma das três replicatas, sendo consideradas negativas, totalizando 77,8% de amostras com resultado negativo. Os achados indicam que, mesmo com a rotulagem "sem glúten", há risco de contaminação cruzada em alguns produtos, o que compromete a segurança de pessoas com condições relacionadas ao glúten. O estudo destaca a importância do controle rigoroso na produção de alimentos sem glúten e o uso de métodos sensíveis e padronizados para garantir a segurança do consumidor.

Palavras-chave: imunocromatografia; análise de alimentos; doença celíaca; contaminação cruzada por glúten; segurança alimentar.

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INTRODUCTION

Gluten is a protein complex that occurs in cereal grains such as wheat, rye, and barley. In the case of oats, the presence of gluten usually happens due to cross-contamination during the cultivation, processing, or transportation stages (Biesiekierski, 2017). The main constituent proteins of gluten are gliadin and glutenin. Hydrated gliadins provide extensibility and viscosity to the dough, while hydrated glutenins are responsible for characteristics such as cohesion, resistance, and elasticity. Genetic improvement strategies have sought to strengthen these properties, such as amplifying the number of copies of the high molecular weight glutenin gene to improve gluten elasticity (Roszkowska *et al.*, 2019).

Gliadin-like proteins are also present in other cereals, such as secalin (rye), hordein (barley), and avenins (oats), all of which are classified as gluten fractions. These proteins have a high content of amino acids, such as proline and glutamine, which are difficult for enzymes to digest in the gastrointestinal tract (Ludvigsson *et al.*, 2013). In predisposed individuals, peptide fragments that are resistant to digestion can cross the intestinal barrier and trigger an immune response, resulting in clinical manifestations such as wheat allergy or gluten-related autoimmune diseases, such as celiac disease. These conditions can cause gastrointestinal and systemic symptoms of varying severity (Ludvigsson *et al.*, 2013; Biesiekierski, 2017).

The only treatment for people with gluten-related disorders is to follow a completely gluten free diet, including celiac disease, non-celiac gluten sensitivity, dermatitis herpetiformis, gluten ataxia, and wheat allergy. However, it is almost impossible to maintain a gluten free diet, as gluten contact is prevalent in foods (Vargas *et al.*, 2024). Some products specifically targeted at people with these disorders may contain small amounts of gluten proteins, above the 20 ppm gluten limit specified by the Codex Alimentarius. This is due to cross-contact with originally gluten free cereals during milling, storage, and handling, or also due to the use of wheat starch as the main ingredient in some food formulations (Codex, 2008; Ludivgsson *et al.*, 2013; Roszkowska *et al.*, 2019).

Gluten cross-contact is defined as the transfer of traces or particles of gluten from one food to another, directly or indirectly, occurring during planting, harvesting, storage, processing, industrialization, and the transportation and marketing of products (Vargas *et al.*, 2024). There are many negative consequences for people who have gluten-related disorders if they do not adhere to the diet correctly and take due care with this type of cross-contamination (Falcomer *et al.*, 2018).

Individuals with celiac disease can consume gluten free oats, although some studies recommend excluding oats from the diet of these patients, which causes debate on the subject (Spector Cohen *et al.*, 2019; Wieser *et al.*, 2021). This controversy is attributed, in part, to the genetic variability of avenins present in different oat cultivars, which may present various degrees of immunoreactivity in celiac individuals (Kosová *et al.*, 2020). However, oats should be introduced into the diet with caution, and patients should be closely monitored for evidence of any adverse reactions (strong recommendation). They can be gradually reintroduced after a year. The vast majority of clinical studies report that moderate amounts of pure oats are well tolerated by most patients with celiac disease, with only a smaller number of patients (less than 1 %) suffering from the harmful effects of oat consumption. However, pure oats must meet the criteria of the legislation for gluten free foods (Spector Cohen *et al.*, 2019; Wieser *et al.*, 2021).

According to the Codex Alimentarius, foods intended for people with gluten-related disorders must contain a maximum of 20 mg of gluten per kilogram of food (20 mg·kg^{-1}, or 20 ppm). Although Law 10.674 of 2003 in Brazil requires food industries to label their products as "CONTAINS GLUTEN" or "GLUTEN FREE", this is not regulated. However, it does not provide a maximum amount of gluten permitted for labeling as "gluten free" (Brazil, 2003). The National Health Surveillance Agency (ANVISA) emphasizes that, for those with food allergies, there is no safe level of exposure to gluten. Therefore, any product containing traces of gluten must have the warning "contains gluten", regardless of the quantity. Products that are labeled "gluten free" must demonstrate in laboratory tests that they do not contain detectable traces of the substance (Brazil, 2017; Brazil, 2022).

There are several types of gluten detection methods in food. One is rapid tests with immunochromatographic strips, which detect gluten quickly and easily using antibodies bound to colored latex beads. An extracted sample migrates through the strip, where the presence of gluten is indicated by a colored band, providing a positive or negative result. This method is accessible to laboratories with little infrastructure and is helpful for rapid screening, but it does not allow the quantification of gluten concentration. They have a detection limit between 5 – 20 mg·kg-¹, varying according to the manufacturer (González *et al.*, 2007).

Other existing gluten detection methods are the ELISA based on the R5 antibody, widely accepted by the Codex Alimentarius as a Type I Method, and the ELISA based on the G12 antibody, which has a sensitivity comparable to R5, with detection limits around 2 mg·kg-¹ of gluten (Barbosa *et al.*, 2007; Hochegger *et al.*, 2015). The G12 antibody recognizes epitopes in the 33-mer peptide of α2-gliadin, which is highly immunotoxic to celiac patients and is widely used for rapid screening. However, studies show that this antibody may present cross-reactivity with specific avenin epitopes in some oat cultivars, which reinforces the importance of considering this factor when interpreting the results (Kosová *et al.*, 2020; Dvořáček *et al.*, 2022). Real-time PCR allows the detection of specific DNA from cereals that contain gluten, such as wheat, rye, and barley, offering high sensitivity, but does not guarantee the presence of functional gluten and can be influenced by manufacturing processes that degrade DNA (Barbosa *et al.*, 2007; Hochegger *et al.*, 2015).

Therefore, this study aims to evaluate the presence of gluten in oat flour marketed in Brazil labeled "gluten free" using the qualitative method of immunochromatographic strips. The unique feature of this research is its exclusive focus on Brazilian products, a context in which regulation and control of cross-contamination in gluten free foods are still limited, requiring more frequent and updated assessments. To date, no specific studies have been found to investigate gluten contamination in oat flour in Brazil, which makes this research a pioneer in the analysis of the safety of this product in the national market.

Furthermore, immunochromatographic strips represent a practical, rapid, and accessible approach to qualitative gluten detection. Although this method is already commercially available, its use in systematic evaluations of products labeled as "gluten free" in Brazil has still not been explored. Thus, its application in this study contributes significantly to monitoring the presence of gluten in oat-based foods, promoting advances in cross-contamination control, and ensuring food safety for celiac or gluten-sensitive consumers.

MATERIALS AND METHODS

A qualitative experimental study was carried out to evaluate the presence or absence of gluten in oats labeled as gluten free.

Materials used

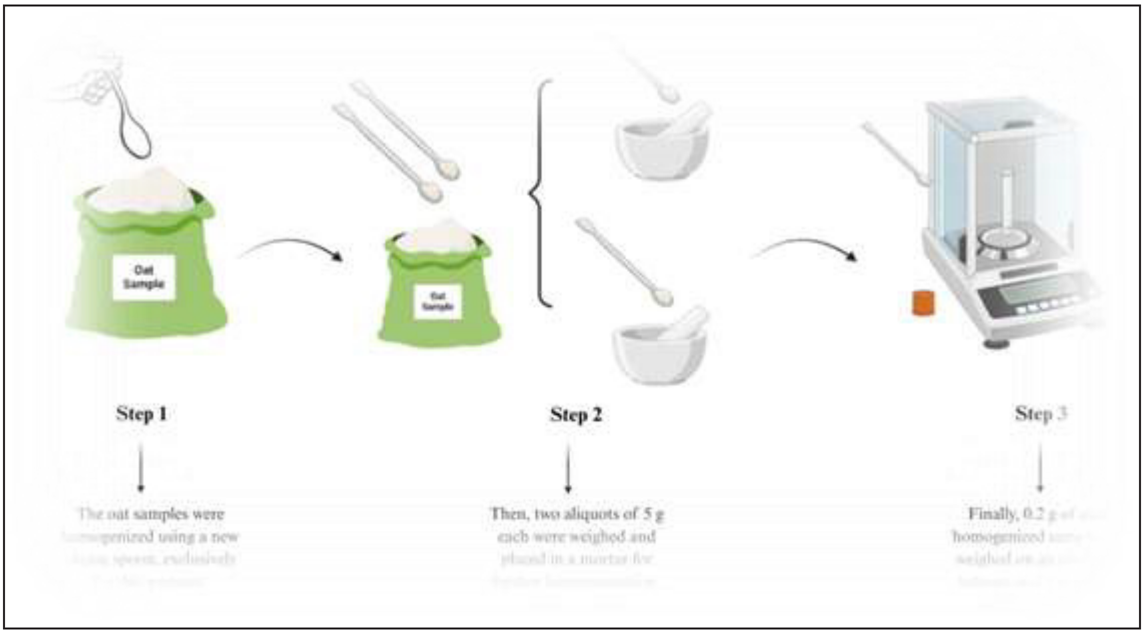
Gluten G12 test kits (kit lot 1: 1000069431, kits 2 and 3: 1000058958) from Romer Labs were used, which are certified by the AOAC Research Institute for tested performance methods (Certificate No. 061403) (Romer Labs, 2025). Nine different brands of oat flour labeled and marketed in Brazil as "gluten free" were purchased from supermarkets, health food stores, and online. Wheat flour was also purchased for blank analysis/quality control. All of them were analyzed, and the brand names were not disclosed for ethical reasons. Romer Labs guaranteed that this test could detect gluten in oat flour.

Mortars, pestles, spatulas, analytical scale (Núcleo Equipamento LTDA), beakers, and gloves were used to prepare the samples. Detergent, 70 % v/v alcohol, vinegar, salt, a dishwashing sponge, and paper towels were used to clean the laboratory to ensure the absence of gluten on surfaces and utensils before performing the tests, avoiding cross-contamination, according to the procedure described by Vargas *et al.* (2024). pH strips were used for sample control, as the samples must have a pH in the range of 6 – 8.

Sample preparation

A sample of each of the nine brands of oat flour was obtained and standardized using a mortar and pestle. The procedure involves careful preparation of oat flour samples; flour extractions were performed according to the manufacturer's recommendations, ensuring homogeneity and avoiding cross-contamination between different brands. First, each package of oat flour was mixed with a new and exclusive plastic spoon, ensuring the integrity of each sample. Then, two 5.0 g portions of flour were removed from each package, transferred to the mortar, and homogenized. Finally, 0.2 g of each homogenized sample was weighed on an analytical balance and transferred to an extraction tube, ensuring the necessary precision for subsequent analyses, as shown in Figure 1. this substance in food. In addition, this value is widely used by most industries in the country. Afterwards, the dilution tube was closed and shaken vigorously by hand for 15 s.

Figure 1 - Steps of the preparation of oat flour for gluten detection.



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Analysis procedure

The gluten detection analysis of oat flours was performed using the AgraStrip Gluten G12 test kit, in triplicate, according to the manufacturer's manual, and through the following steps, briefly described (Romer Labs, 2023).

The extraction tube containing the sample was filled (Bottle A) to the bottom of the neck of the tube with extraction buffer (bottle A, not supplied by the manufacturer to its specifications). Then, the tube was closed with the cap and shaken vigorously by hand for 1 min. After shaking, the cap was removed and replaced with a new dropper. Three drops were then transferred to a dilution tube.

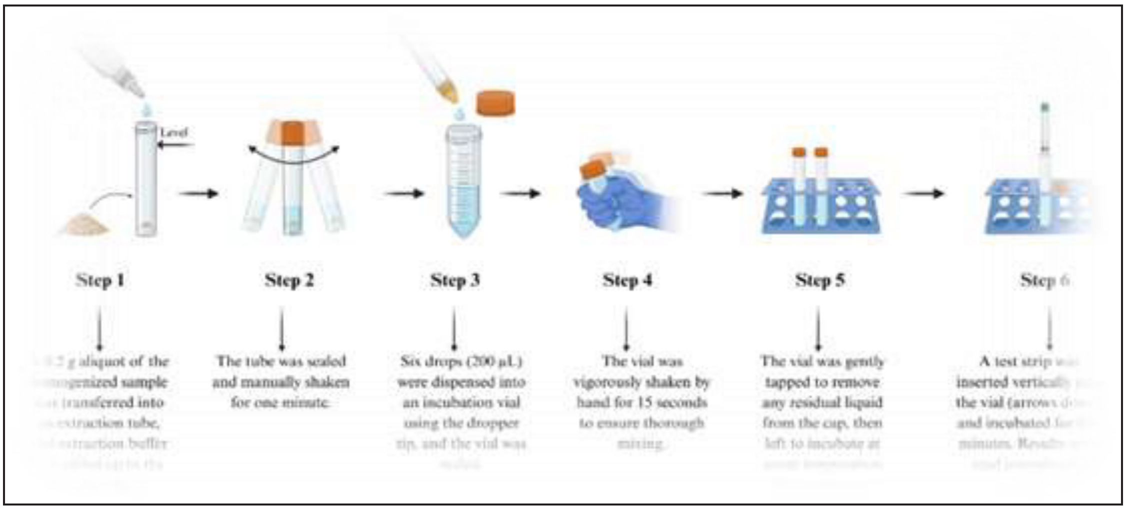
The dilution buffer (bottle B, not supplied by the manufacturer according to its specifications) was transferred to the dilution tube containing the extract. The dilution tube had three graduated marks, allowing the desired cutoff value for the sample to be quickly and easily defined. For this procedure, the cutoff value of 5 mg·kg^{-1} was developed since Brazilian legislation does not establish a specific limit for the presence of

The cap was removed, and the test strip was placed vertically in the dilution tube, with the white arrows pointing downward. This procedure allowed the liquid to flow down the strip onto the wick block. This process took approximately 45 s.

After the liquid had flowed onto the wick, the test strip was removed from the dilution tube and placed upright (with the arrows pointing down) in a slot on the vial rack provided. The strip was left for 10 min to develop the test and the result was read immediately after this period. The reading of the result on the strip could be concluded as negative, positive, or invalid. According to the manufacturer, strips that presented weak coloration were considered positive results, thus indicating gluten levels close to the detection limit. Figure 2 represents the steps of the analysis.

For samples that presented divergent results between the three tests, it was considered the result that offered the highest number of results. Quality control was also performed with a wheat flour sample to have a positive analysis standard, which was performed separately from the other tests to avoid cross-contamination by gluten.

Figure 2 - Steps of the LFD test to detect gluten in oat samples.



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RESULTS AND DISCUSSION

The results of this research, with nine different brands of oat flour labeled as "gluten free" and sold in Brazil using the lateral flow immunochromatography method, presented the following results, as shown in Table 1. It is worth noting that all nine samples presented a pH in the range of 6 – 8, a requirement indicated according to the manufacturer of the test kit.

Of the nine brands of oat flour labeled as "gluten free", two (samples 4 and 7) presented a positive qualitative result for gluten in the three test samples, totaling 22.2 % of the nine samples. Two brands presented a positive qualitative result, but in only one sample of the three tests, considering the overall negative result for these two brands (samples 6 and 8), since the result of the test that appeared most often was considered. Five brands of oat flour (samples 1, 2, 3, 5, and 9) presented

Table 1 - Results of analyses of oat flour labeled as "gluten free" sold in Brazil.

Sample	Result			Final result considered
	Test 1	Test 2	Test 3	
1	negative	negative	negative	negative
2	negative	negative	negative	negative
3	negative	negative	negative	negative
4	**positive**	**positive**	**positive**	**positive**
5	negative	negative	negative	negative
6	positive	negative	negative	negative
7	**positive**	**positive**	**positive**	**positive**
8	negative	negative	positive	negative
9	negative	negative	negative	negative
Wheat flour	positive	positive	positive	positive

Source: authors (2025).

a negative result for the three samples tested. Thus, 77.8 % of the oat flours presented a negative qualitative result for gluten.

Therefore, the two brands of oat flour with positive results are probably due to gluten cross-contamination, which can occur from the planting to the harvesting of the oats and in their manufacturing process, as well as in the marketing of the product (Vargas *et al.*, 2024). An example of gluten cross-contamination that can occur during marketing is in supermarkets and warehouses. Often, the company stocks foods labeled as "gluten free" next to packages of wheat flour, which usually releases flour dust easily from the packages, thus allowing cross-contamination to occur. However, publications on gluten contamination of oats have been limited in recent years.

In 2006, Spain and the United States reported that approximately 75 % of products labeled gluten free were contaminated. In Canada and India, 88 % and 85 % of products showed high gluten contamination levels in oats. Therefore, handling gluten free oats is not so simple since gluten contamination in oats can occur at different stages and in various ways. This can happen when cereal crops (wheat, barley, and rye) are rotated with legumes such as chickpeas, lentils, or beans. It is a frequent application as it helps fix nitrogen in the soil. In addition, they are harvested with the same equipment, stored, processed, transported, and distributed together (Rodríguez *et al.*, 2022).

A pilot study of gluten contamination in grains, seeds, and flours in the United States that included samples of rolled and steel-cut oats (Thompson; Lee; Grace, 2010) showed that nine of 12 containers used, representing four different lots from each of three distinct brands (Quaker, Country Choice, and McCann's), had gluten levels ranging from 23 $mg \cdot kg^{-1}$ to 1,807 $mg \cdot kg^{-1}$. Another study conducted in 2011 showed that approximately 88% of 113 samples of Canadian commercial oats were contaminated with gluten above 20 $mg \cdot kg^{-1}$, and there was no difference among the types of oats tested (Koerner *et al.*, 2011). Additionally, studies indicate that specific oat cultivars present epitopes that can react with anti-gluten antibodies, such as G12, even without cross-contamination, due to the structure of their avenins (Dvořáček *et al.*, 2022).

In Canada and the United States, this "purity protocol" (which includes good manufacturing practices and hazard analysis of critical materials and control points) was created, which aims to ensure the prevention of gluten contamination during the stages of cultivation, harvesting, storage, processing, handling, and transportation. This protocol tests the gluten content throughout the production processes and in the final product. This protocol is auditable for gluten free certification and carries strict labeling. One requirement is that the oats should be planted on land that has not consumed cereals containing gluten in the last three years. The farmer must remove any stray gluten grains when it is close to harvest time. This is possible due to the difference in structure of oats compared to other cereals (Rodríguez *et al.*, 2022). However, this protocol is not applied everywhere. This may explain, at least in part, the reason for gluten contamination in a large portion of oat products in 2022 (Rodríguez *et al.*, 2022; Thompson; Keller, 2023).

A study of products containing oats in their composition showed that 11% tested for quantifiable gluten equal to or greater than 5 $mg \cdot kg^{-1}$. The percentage of oat tests with quantifiable gluten varied yearly, from a minimum of zero in 2014, 2017, 2019, and 2021 to a maximum of 35% in 2022. However, the drought that occurred in 2021 impacted both Canada and the United States, where oats were grown. Some products in this study had the purity protocol, tested for oats with quantifiable gluten, a recent development. Six of the seven packages of oat products with the purity protocol tested for quantifiable gluten occurred in 2022/2023, and one occurred in 2016 (Thompson; Keller, 2023). Considering that the companies probably did not follow the purity protocol, hence the large number of packages of oats contaminated with gluten.

Another study was conducted with food samples with the description "does not contain gluten" and foods with the description "contains gluten" and were analyzed using the Gluten Assay Kit (Tepnel BioSystems, UK). The foods described as "does not contain gluten" did not present gluten in the samples, except for the pasta. All foods with the description "contains gluten" tested positive. Oat flour was also analyzed in this study, but with the description "contains gluten", and the result for oat flour was confirmed, presenting 40 $mg \cdot kg^{-1}$ of gluten. This was not expected since data in the literature indicate that monoclonal antibodies for ω-gliadin would not be compatible with oat prolamins. The publication also recommended that investigation studies with PCR became necessary to confirm the presence of gluten in oats (Barbosa *et al.*, 2007).

The gluten results obtained in the analyzed food products demonstrate the viability of using the immunoassay method and highlight the need for strict food control. This occurs because, during the industrialization process, the ingredients may become contaminated, or, on the other hand, gluten may be omitted from the list of ingredients on the product label (Barbosa; Abreu; Zenebon, 2007).

Another place where gluten cross-contamination can occur is during the packaging stage of the product. Allred et al . (2017) determined that packaging also plays an important role, given that some producers use very fragile materials for packaging. In the case of regular oats, 1% contamination with other grains is generally allowed, but it must be controlled.

A study by Silvester *et al.* (2020) provides essential insights into the difficulty people with celiac disease face in maintaining a completely gluten free diet. Even following a restricted diet, two-thirds of the participants were exposed to gluten at some level, as evidenced by new tests for detecting gluten in food, urine, and feces. These findings suggest that although a gluten free diet is the main recommendation to avoid complications associated with gluten ingestion, achieving absolute adherence can be challenging. Cross-contamination and the presence of small amounts of gluten in foods considered safe are potential sources of involuntary exposure, indicating that many celiacs maintain a low-gluten diet, rather than a completely gluten free diet. A gluten free diet involves a combination of economic, social, and cultural factors that vary according to each individual's context. Issues such as cost, food availability, social acceptance, and dietary traditions can facilitate or hinder this choice. Thus, public policies, nutritional education, and a more accessible market are essential to make this diet viable for those who need to follow it.

Analytical errors in laboratory tests are widespread. Therefore, inadequate sample homogenization may be a possible cause for the occurrence of two samples that only presented a positive result once. Thus, the components or substances being tested may not be evenly distributed. This means that different parts of the sample may have varying concentrations of the components in question. According to Thompson and Keller (2023), a test portion with a result that differs from the others may occur due to the heterogeneous nature of gluten cross-contamination and the difficulty in homogenizing some samples.

Most companies that produced the oat flour samples we contacted stated that they do not process gluten-containing foods in their facilities. All companies ensured strict quality control, performing tests to detect gluten in their products or receiving supplier reports. Two of the brands use foods containing gluten in their facilities but ensure that the oat flour is stored and processed in separate areas; however, these were not the brands that tested positive for gluten. Some brands did not disclose their oat suppliers; however, some did mention whether they were imported or domestic. Among the six brands that provided this information, four are domestic suppliers, one is international, and another works with domestic and international suppliers.

The oat brands purchased were contacted to verify how these companies control gluten cross-contamination and confirm whether the oats are of national or international origin, as shown in Table 2.

All companies ensured strict quality control, performing tests to detect gluten in the oats or receiving supplier reports. Most companies provide limited information on the export of gluten free oats; only brand number 6 stated that it exports its products to other countries. However, we do not know whether the supplier of any of these brands exports gluten free oats to other countries. With this information, we could compare the results with data available in the literature.

Theoretical frameworks from other countries on gluten detection help to contextualize the study, as different markets have specific regulations and advanced analysis methods. Techniques such as the one used in this study, the ELISA and PCR techniques, are widely used to ensure compliance with maximum contamination limits (usually 20 $mg{\cdot}kg^{-1}$ depending on the law of each country). In addition, new methods, such as mass spectrometry and biosensors, increase the accuracy of the tests. Considering these international references allows us to understand how companies can adapt to

Table 2 - Information on the oat flour companies used.

Brand code oatmeal	Supplier (national or International)	Works with gluten-containing products as well
1	National	No
2	International	Yes
3	National	Yes
4	National	No
5	National	No
6	National and International	No
7	Not provided	No
8	Not provided	No
9	Not provided	No

Source: Authors (2025).

global standards, ensuring the reliability of the export of gluten free oats.

The final stage of the work consisted of alerting individuals with celiac disease about possible cross--contamination and errors in labeling gluten free oats. This alert was disseminated through social media and in partnership with the Brazilian Celiac Association - Rio Grande do Sul (ACELBRA-RS).

Failure to comply with the legislation in Brazil can result in economic and legal sanctions for companies. An example of this occurred in a lawsuit filed by a consumer with celiac disease against a granola manufacturer. The plaintiff purchased a product labeled with the prominent indication "GLUTEN FREE, LACTOSE-FREE"; however, the warning "May contain traces of gluten" was included in smaller letters. This inconsistency constituted inadequate labeling, violating the consumer's right to information and the company's duty of transparency, in disagreement with the Gluten Law (Law No. 10.674/2003). The process resulted in a decision in favor of the author since concrete damage to his health was proven after ingesting the product, which triggered the symptoms of celiac disease (Rio Grande do Sul, 2018).

Despite the advances obtained in this study, some limitations should be considered. The main limitation is related to the use of immunological tests based on the G12 antibody, which, although widely used for rapid gluten screening, may present cross-reactivity with epitopes present in specific oat cultivars, leading to possible false positives even in the absence of cross-contamination (Kosová *et al.*, 2020; Dvořáček *et al.*, 2022). In addition, the heterogeneity in the distribution of gluten in the samples may compromise the representativeness of the results. It is also noteworthy that budget constraints limited the number of samples and replicates and the application of complementary analytical methods, such as ELISA and LC-MS/MS, which could have provided greater specificity and sensitivity in detecting toxic peptides. These factors should be considered when interpreting the data and reinforce the need for complementary studies to deepen the understanding of the presence and detection of gluten in oat-based products.

Future Perspectives

Given this study, it is clear that further research is needed using not only oat flour but also oat flakes and bran. In addition, it is recommended to use other methods of detecting gluten in foods, such as the quantitative ELISA method. Studying these foods with more sensitive techniques, such as mass spectroscopy and Western Blot, would also be interesting. Another proposal is to apply these methods to different types of foods and in larger samples. It is also important to conduct a study to identify the process variables that can give positive or negative results, such as products used in washing, type of food shared on the conveyor belt, and number of washes, among others.

CONCLUSION

This research revealed that, although most of the nine samples of oat flours labeled as "gluten free" in Brazil analyzed were within the legal contamination standards, 22.2 % presented detectable traces of gluten. These levels can be significant for individuals with celiac disease and gluten sensitivity. Therefore, it is recommended that manufacturers implement stricter quality controls throughout the production chain, from cultivation to packaging, to minimize the risk of cross-contamination. In addition, more effective monitoring and more transparent practices in the gluten free product market are necessary to ensure food safety and maintain the trust of Brazilian consumers.

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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