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Effectiveness of triple washing in removing gluten from surfaces and utensils in domestic kitchens

Eficácia da tripla lavagem na remoção de glúten em superfícies e utensílios de cozinhas domésticas

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Abstract:

Objectives: The aim of this paper was to analyze the effectiveness of triple washing in removing gluten from kitchen surfaces and utensils. Methods: The technique used consists of three stages. First stage: clean all surfaces and utensils with detergent and water using a new sponge, rinse with a disposable cloth and water. Second stage: cleaning with a second sponge and a solution of vinegar and salt (3%), rinsing with a disposable cloth and water. Third stage: 70% alcohol with a paper towel. The sandwich enzyme-linked immunosorbent assay (ELISA) method was used to analyze gliadin in swab samples. Results: By carrying out the three stages of sanitization, gluten can be satisfactorily removed, leaving surfaces and utensils with less than 10 ppm of the protein, which is considered safe for most people with celiac disease. Conclusion: Triple washing is effective in removing gluten from utensils and surfaces in domestic kitchens.

Keywords: celiac disease; ELISA test; gluten; protein; removal.

Resumo:

Objetivos: Analisar a eficácia da tripla lavagem para remoção de glúten de superfícies e utensílios de cozinha. Métodos: A técnica utilizada é composta de três etapas. Primeira etapa: limpar todas as superfícies e utensílios com detergente e água com o auxílio de uma esponja nova, enxaguar com pano descartável e água. Segunda etapa: limpeza com uma segunda esponja e solução de vinagre e sal (3%), com um enxágue utilizando pano descartável e água. Terceira etapa: álcool 70% com o auxílio de papel toalha. Foi utilizado o método imunoenzimático tipo sanduíche (ELISA) para analisar a gliadina em amostras de swabs. Resultados: Realizando as três etapas da higienização, consegue-se remover satisfatoriamente o glúten, deixando as superfícies e utensílios com menos de 10 ppm da proteína, o que é considerado seguro para a maioria das pessoas com doença celíaca. Conclusão: A tripla lavagem é eficaz para remover glúten de utensílios e superfícies de cozinhas domésticas.

Palavras-chave: doença celíaca; glúten; proteína; remoção; teste ELISA

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INTRODUCTION

Celiac Disease (CD) is a chronic condition of the small intestine, characterized by an immune response triggered by the ingestion of gluten in the diet, occurring in people with a genetic predisposition. The organ most affected by CD is the small intestine, where its villi atrophy, impairing the absorption of nutrients from food and, as a consequence, other clinical manifestations [1, 2, 3].

It is estimated that the worldwide prevalence of celiac disease is 1% of the population, and this figure could be as high as 6% due to underdiagnosis. Celiac disease occurs in genetically predisposed people, and usually appears in childhood, but can develop in adulthood and also in the elderly, where the mechanism may be some traumatic or stressful factor that triggers autoimmunity in these predisposed people. It is more common in women[4]. When a gluten-free diet is started, a rapid improvement in symptoms is noted and, in most cases, the intestinal mucosa regenerates within a period of up to two years[5, 6].

Gluten, a protein found in various cereals, is a combination of the proteins prolamin and glutamine. In CD, the form of gluten that most triggers autoimmunity is wheat gluten, composed of alcohol-insoluble glutenins and alcohol-soluble gliadins, with a high content of glutamine and proline. The gliadin fraction, in particular the 33-mer -2-gliadin peptide, is recognized by T lymphocytes via the HLA DQ gene, which is present in 90-95% of CD cases[7]. Other cereals, such as barley, whose storage proteins are known as hordeins, rye, called secalins, and oats, referred to as avenins, can also induce autoimmunity in individuals with CD. Collectively, these proteins are classified under the term “gluten” [8].

The only safe treatment for CD is strict adherence to a gluten-free diet, and all precautions regarding cross-contamination. Despite the associated difficulties, such as dietary restrictions, high costs and contamination risks, strict adherence to treatment is imperative to avoid the development of other conditions due to the ongoing autoimmune aggression[9].

One of the challenges faced by individuals with CD is the occurrence of cross-contamination by gluten on utensils and surfaces during food preparation in domestic kitchen environments previously used to handle products containing gluten[10]. Cross-contamination is defined as “The presence of any food allergen not intentionally added to the food

as a result of the cultivation, production, handling, processing, preparation, treatment, storage, packaging, transportation or preservation of food, or as a result of environmental contamination." [11, 12].

In Brazilian coeliac groups on social media, a procedure called triple washing is recommended. This technique was proposed by Professor Flávia Anastácio de Paula in 2007, due to the need to decontaminate her kitchen after her diagnosis and that of her three children. As cross-contamination by gluten is a challenge for coeliacs, triple washing can help remove gluten from surfaces and utensils. Its sanitizing steps consist of solubilizing the fractions of the gluten molecule using alkaline ingredients, acids and 70% alcohol, a solubility discovered by Osborne in 1924 [13, 14, 15].

Therefore, this study aimed to analyze the effectiveness of home cleaning to remove gluten from kitchen surfaces and utensils, in order to completely remove gluten.

MATERIAL AND METHODS

Surfaces and utensils were cleaned using the homemade triple wash technique. The triple wash technique consisted of washing the surfaces and utensils in three stages, the first stage being a wash with common detergent, which also removes larger soiling. The second stage consisted of washing with a vinegar and salt solution (100mL of vinegar + 3g of salt). In the third stage, 70% alcohol was used with a paper towel.

The tests were carried out in the Nutrition and Dietetics Laboratory at La Salle University and followed the following stages, divided into two days. On the first day, preparations were made using wheat flour as the source of gluten. The preparations were: homemade pasta, carrot cake and homemade bread. The following places and utensils were analyzed and identified: stainless steel worktop (A1), marble worktop (A2), stainless steel pan (A3) and aluminum pan (A4). These places and utensils were in direct contact with the gluten for 24 hours.

On the second day, samples were taken and cleaned. The samples were taken at each stage of the technique and identified as: E0, the sample was taken before the washing procedure; E1, the first stage of the procedure, detergent and water were used, with the aid of a sponge, which was duly discarded after use, and the rinsing was carried out with

the aid of a disposable cloth; E2, the second stage of the procedure, was carried out with a vinegar and salt solution, a sponge was used, which was duly discarded after use, and the rinsing was carried out with the aid of a disposable cloth. E3, the third stage of the procedure, was carried out using 70% alcohol with the aid of a paper towel.

The samples were collected at each washing stage using a swab and were immediately placed in an extraction solution that came with the kit. After collecting the samples, they were prepared for the Elisa test. The preparation followed these steps: Using a 100µL micropipette, the samples were added to the spaces on the plate, together with the calibration curve, in duplicate, for 20 minutes at room temperature. After 20 minutes, the samples were discarded and the microwells were washed with washing solution five times and dried with absorbent paper. Using a 100µL micropipette, the Conjugate solution was added to the microwells and incubated for 20 minutes at room temperature. After 20 minutes, the samples were discarded and the microwells were washed five times with washing solution and dried with absorbent paper. Using a 100µL micropipette, the Substrate solution was added to the microwells and incubated for 20 minutes at room temperature and in the dark, using aluminum foil. After this time, the color turned blue. Finally, the STOP solution was added to the microwells using a 100µL micropipette. The color changed from blue to yellow. The plate was placed in the ELISA Microplate Reader, Kasuaki brand, model DR-200Bs-NM-BI, and the samples were read at a wavelength of 450nm.

The test method used was a sandwich immunoenzymatic assay (ELISA - Enzyme Linked ImmunoSorbent Assay) which quantitatively analyzed gliadin in swab samples. The ELISA test kit used was AgraQuant® Gluten G12® from Romer Labs. The antibodies present in the tests are synthesized in a similar way to what triggers symptoms in humans and react with the gluten present in food and on surfaces, forming complexes that can be detected. They target the 33-mer peptide, which is resistant to enzymatic digestion and heat denaturation and is the fragment of the gliadin fraction of gluten to which coeliacs react, making it a reliable analytical marker [16].

DATA ANALYSIS

All the analyses were carried out in duplicate. The determination of gluten in the sam-

ples was carried out using the spreadsheet AQ Allergens Spreadsheet_Original_96888, supplied by Romer Labs®. Simple calculations and the creation of graphs were carried out using the Excel 2019 program (Microsoft, WA, USA). The reduction in gluten after the sanitization methods was expressed as a percentage (%), according to the following equation:

$$Reduction\ (\%) = \frac{CGa - CGd}{CGa} \times 100$$

CGa represents the gluten concentration (ppm) before sanitizing and CGd represents the gluten concentration (ppm) after sanitizing.

RESULTS AND DISCUSION

The results were expressed as gluten concentration (ppm) before and after each stage of triple-wash sanitization. Materials that had less than 10 ppm of gluten after the three stages of triple-wash cleaning were considered effective [17,18] (Table 1)

Table 1. Elisa test results and gluten reduction percentage after the cleaning steps in different utensils and surfaces.

Material	Id. sample	Mean Gluten Concentration (ppm) and Standard Deviation (±SD)	Reduction percentage (%)
Stainless steel bench	E0 A1	67.70 ± 0.82	
	E1 A1	19.40 ± 3.88	
	E2 A1	10.01 ± 1.11	
	E3 A1	6.42 ± 0.34	90,52
Marble countertop	E0 A2	93.42 ± 26.32	
	E1 A2	25.21 ± 9.61	
	E2 A2	10.77 ± 0.64	
	E3 A2	6.74 ± 0.29	92,79
Stainless steel pan	E0 A3	134.69 ± 14.02	
	E1 A3	17.06 ± 1.25	
	E2 A3	13.89 ± 0.07	
	E3 A3	5.94 ± 0.47	95,59
Aluminum pan	E0 A4	63.13 ± 5.85	
	E1 A4	20.32 ± 1.55	
	E2 A4	11.75 ± 2.09	
	E3 A4	6.58 ± 0.37	89,57

Due to its low solubility in aqueous solvents, gluten represents a residue that requires specific approaches for its effective removal during cleaning procedures[19, 20]. The composition of wheat protein consists of approximately 5% of its proteins soluble in alkaline solution and salt, around 40 to 45% soluble in acid and 33 to 45% soluble in 70% alcohol [13, 14, 15, 21].

As shown in Table 1, by carrying out the three washing steps, a very satisfactory result was achieved for all the materials analyzed, since the surfaces and utensils were left with less than 10 ppm of gluten, which is considered safe for most celiacs[17, 18]. Another study, carried out in Spain, showed that washing with ordinary detergent alone is also effective, but using detergents with enzymes improved cleaning efficiency six times over[22]. Fuciños et al.[20] also demonstrated the effectiveness of using detergents with enzymes, where it used this cleaning method in a food industry in Spain. In Argentina, the Guide to Recommendations for Preparing a Safe Gluten-Free Menu proposes that an initial sanitizing stage be carried out by applying detergent, followed by the subsequent use of a 200 ppm chlorine solution, and finally the use of 70% alcohol, to disinfect utensils and surfaces that have come into contact with products containing gluten [23].

In this study, sponges were changed at each stage of sanitization by triple washing. Due to the characteristics of the sponge materials, they have the capacity to retain gluten particles, which can result in the possible transfer of these particles to other utensils during washing. Hashimoto et al.[24] in Japan revealed that using the same sponge on wheat-contaminated utensils and uncontaminated utensils resulted in a transfer of approximately 80% of wheat allergens. Even after rinsing, total removal of the allergens was ineffective. This highlights the importance of avoiding sharing sponges when cleaning kitchen utensils to reduce the risk of cross-contamination in food preparation environments, even in the direct absence of the allergen.

Wheat is known to be a very important cereal for the industry, especially in the bakery sector, as it contains the best quality and quantity of the proteins needed to form gluten, where a satisfactory sensory standard can be achieved in pasta production[7, 25] . But, for the safety of people with CD, whose only effective treatment is a gluten-free diet and cross-contamination, it is important to have effective sanitization procedures to remove gluten from surfaces and utensils, so that the individual with CD can have a quality of life

in their home [26].

CONCLUSION

This study demonstrated the effectiveness of triple-washing for domestic kitchens, using easily accessible and inexpensive products, since by carrying out the three steps, the gluten content was reduced to less than 10 ppm, which is considered safe for most individuals with Celiac Disease.

More studies need to be carried out to test the effectiveness of other sanitizing procedures, since triple washing, although effective, is very labor-intensive and involves the disposal of sponges, which increases the cost of the procedure and problems for the environment if they are not disposed of properly. Its effectiveness should also be tested in restaurants and industrial kitchens, as these places generally use other types of sanitizing products, so that effectiveness can perhaps be achieved without the need to triple-wash.

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