Hygyenic-sanitary evaluation of flaxseed flour of the Metropolitan Region of Recife (RMR)

Avaliação higiênico-sanitária de farinha de linhaça da Região Metropolitana do Recife

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ABSTRACT
Flaxseed (*Linum usitatissimun* L.) is increasingly present in grain and cereal stalls in public markets, fairs and in the healthy food section in supermarkets. This is due to the growing demand for natural products, and a healthier nutrition appeal. These so-called healthier foods induce the expectation of reducing the risk of chronic non-communicable diseases, such as cancer, heart disease and those related to carbohydrate restriction. Given the importance that flaxseed is acquiring in the Brazilian market, with greater incorporation in the diet, it is necessary to carry out a study on the hygienic-sanitary quality of flaxseed flour. Ten samples of brown flaxseed flour sold in supermarkets in the Metropolitan Region of Recife (RMR) were collected, with the aim of evaluating the mandatory microbiological parameters, as well as the count of molds and yeasts, to check the sanitary quality and search for foreign material (dirt). It was observed that despite being in microbiological compliance with the legislation in force, the fungal concentration found is higher than the limit allowed by health standards. In the search for foreign material, cotton fibers were found. It is suggested that an identity and quality standard be created for flaxseed flour, and greater sanitary control during processing, to protect against the possibility of cross contamination.

Keywords: *Linum usitatissimun* L., healthy food, fungal contamination.

RESUMO
A linhaça (*Linum usitatissimun* L.) está, aos poucos, mais presente nas barracas de grãos e cereais dos mercados públicos, feiras livres e na seção de alimentos saudáveis em supermercados. Isso se deve à crescente procura por produtos naturais e ao apelo de nutrição mais saudável. Esses alimentos ditos mais saudáveis, induzem expectativa de reduzir o risco de doenças crônicas não transmissíveis, como câncer, doenças cardíacas e as relacionadas com restrição de carboidratos. Dada a importância que a linhaça está adquirindo no mercado brasileiro, com maior incorporação na dieta, se faz necessária a realização de um estudo sobre a qualidade higiênico-sanitária da farinha de linhaça. Foram coletadas dez amostras de farinha de linhaça marrom comercializadas em supermercados da Região Metropolitana do Recife (RMR), com o intuito de avaliar os parâmetros microbiológicos obrigatórios, assim como a contagem de bolores e leveduras, para verificação da qualidade sanitária e pesquisa de matérias estranhas (sujidades). Observou-se que apesar de estar em consonância microbiológica com a legislação em vigor, a concentração fúngica encontrada é superior ao limite permitido por normas sanitárias. Na pesquisa de matérias estranhas, foi encontrado fibras de algodão. Sugere-se que seja criado um padrão de identidade e qualidade para as farinhas de linhaça, e um maior controle sanitário durante o processamento, para proteção contra a possibilidade de contaminação cruzada.

Palavras-chaves: *Linum usitatissimun* L., alimentos saudáveis, contaminação fúngica.

1 INTRODUCTION

Flaxseed (Linum usitatissimun L.), plant of the Lineaceae family, is one of the oldest in history, having records that date back to more than 5000 B.C. Its use has always been related to food and medicinal use, in wounds. It is differentiated into two varieties: golden and brown, as they have more and less pigments on the external part, respectively. Although they do not differ much in their composition, the brown variety contains a greater amount of protein, while the golden variety has a greater amount of dietary fiber (OLIVEIRA et al., 2015; VIEIRA et al., 2020).

Its largest production is concentrated in Canada, 40 % of world production and 75 % of global flax seed sales. This seed began to be produced on a large scale in Brazil and finds its greatest production in Guarani das Missões, Rio Grande do Sul. In both cases, the destination of the product was not food, but textile manufacture, or the production of oil for paintings (OOMAH, 2001; BOMBO, 2006).

Recently, there has been an expansion in the offer of products with claims of health benefits. The so-called functional foods, the newest trend in the market (SZAKÁLY et al., 2019), being considered a border between food and medicine, aiming at reducing the risk of chronic diseases (GRISOTTI, 2010; IWATANI & YAMAMOTO, 2019).

Flaxseed falls into this category of food, as it has considerable content of essential polyunsaturated fatty acids ω-3, strong antioxidant and natural phytoestrogen, containing the precursors of lignan and isoflavone, responsible for reducing the risk of breast cancer, heart diseases and lowering cholesterol rates. In addition to these, the seed contains high rates of dietary fiber, responsible for regulating the gastrointestinal tract and also for reducing the absorption of sugars with a high glycemic index, thus maintaining glucose and cholesterol levels in a healthy way (NOVELLO & POLLONIO, 2012; CUPERSMID et al., 2012; CANELLA-RAWLS, 2003).

From the dissemination of the aforementioned concepts, the demand for flaxseed in the market increased as a means of supplementing the diet. In addition to its use in cooking with the use of whole seed, as a way to add fiber to various doughs, from breads and cookies to muffins. Flaxseed flour is also widely accepted, being consumed on top of foods such as fruits, milk and yogurts (OLIVEIRA et al., 2015).

As for the nutritional content, each 100 g of flaxseed contains 14.1 g of protein, 32.3 g of lipids, 43.3 g of carbohydrates and 33.5 g of dietary fiber (TACO, 2011). Because it has such a concentration of nutrients, this seed consists of substances necessary

for supplementing human food. There is a setback, however, for such a nutritional composition, which ends up serving as raw material for the proliferation of microorganisms, which can find favorable conditions for their multiplication in flaxseed.

With the addition of flaxseed to the menu to compose a healthy diet, an assessment of the safety of this food is necessary. Both public health and economic viability would be at risk, by admitting that the product could be contaminated, since some types of pathogenic bacteria could develop and cause harm to the health of the population, as well as the deterioration of the product would cause economic losses.

2 MATERIAL AND METHOD

2.1 SAMPLE ACQUISITION

10 packages of 300 g of each sample of industrialized brown flaxseed flour were obtained. Samples were purchased with closed and intact packaging, within the expiration date, and sold in supermarkets in Recife, Jaboatão dos Guararapes, Paulista and Olinda. The samples were coded to preserve the confidentiality of the investigated trademarks.

2.2 MICROBIOLOGICAL ANALYSIS

Microbiological tests were carried out in accordance with the requirements of the legislation that determines the microbiological standards of identity and quality for different food groups, RDC n° 331 (BRASIL, 2019). Despite the current legislation (BRAZIL, 2019), not requiring determination as to the standard count of molds and yeasts, research was carried out for these fungal groups, as these biological agents can cause damage to public health. The methodologies were used according to the Manual of Methods for Microbiological Analysis of Food (Silva et al., 2017).

25 g aliquots of aseptically homogenized samples were used in 225 mL of 0.1 % peptone water for serial dilution. For the quantification of aerobic mesophilic heterotrophic bacteria, the method of total counting of aerobic mesophilic plates was used using the culture medium Plate Count Agar (PCA), followed by pour plate inoculation with incubation at 35 °C for 24-48 h (SILVA et al., 2017).

The quantification of total coliforms was performed using chromogenic and fluorogenic substrate (Compact dry®) incubating at 35 °C for 24 h. Likewise, the quantification of thermotolerant coliforms was performed using chromogenic and fluorogenic substrate (Compactdry®), incubation at 42 °C for 24 h.

In the determination of filamentous fungi and mild duriform, the method of total counting of molds and yeasts in plates was used, using the spread plate technique with Acidified Dextrose Potato Agar (PDA-AC), incubation at 22-25 °C for 120 h (SILVA et al., 2010).

For the enumeration of Bacillus cereus, the direct plate counting method was used, 0.1 mL of serial dilutions of the flour homogenates were plated on a surface of Manitol Egg Yolk Polymyxin Agar (MYP). Incubation at 30 ± 2 °C for 20-24 h. Typical colonies were subjected to Gram stain and cultured on Brain and Heart Infusion Agar, incubated at 35 ºC for 24 h and a lecithinase test (SILVA et al., 2017).

The analysis of Salmonella spp. followed the method 967.26 of AOAC (2012). 25 g of each sample were weighed in a glass vial containing 225 mL of lactated broth, and subsequently incubated in a bacteriological oven at 35 ° C for 18 to 24 hours. For selective enrichment, 1 mL of each pre-enriched sample was transferred to a tube containing Tetrathionate Broth and another 1 mL aliquot to a tube containing Selenite Cystine Broth. These were sent to a water bath at 42 ° C for 7 hours, obtaining the enriched inoculum. Confirmatory analysis was carried out using tubes containing the enriched culture media, Selenite Cystine Broth and Tetrathionate Broth reserved, a handle was transferred to surfaces of XLD Agar (Xylose-Lysine Deoxycholate), Bismuth Sulphite Agar (BS) and Hektoen Agar (HE), previously prepared and incubated at 35 ° C for 24 hours.

All tests were performed in duplicate and the results were expressed by mean and standard deviation of the tests.

2.3 RESEARCH OF FOREIGN MATERIAL IN FLAXSEED FLOUR

It was adopted the mesh methodology with multiple meshes described by Fontes and Fontes (2005). 100 g of each sample were submitted a set of five meshes stacked on top of each other, arranged on an electromechanical shaker for 15 minutes at maximum frequency, with the largest mesh (opening) being maintained on top. The foreign material was separated and identified using a stereoscope and an optical microscope.

3 RESULTS AND DISCUSSION

Table 01 lists the results of microbiological and physical-chemical tests of 10 samples of industrialized brown flaxseed flour.

Table 1: Microbiological and physical-chemical tests on RMR flaxseed flour.

Sample	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10
Heterotrophic Mesophilic Aerobes (log_{10} CFU/g)	4.57 ± 0.05^{b}	4.83 ± 0.01^{b}	6.07 ± 0.12^{a}	3.48 ± 0.27^{c}	4.92 ± 0.16^{b}	4.51 ± 0.18^{b}	5.79 ± 0.11^{a}	4.63 ± 0.09^{b}	4.65 ± 0.12^{b}	4.79 ± 0.09^{b}
Bacillus cereus (log_{10} CFU/g)	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Total Coliforms (log_{10} CFU/g)	2.25 ± 0.30^{c}	3.26 ± 0.29^{b}	4.17 ± 0.11^{a}	2.16 ± 0.18^{c}	<1	<1	<1	4.25 ± 0.11^{a}	<1	<1
Coliforms at 45ºC (log_{10} CFU/g)	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Salmonella spp. (log_{10} CFU/25g)	Aus.	Aus.	Aus.	Aus.	Aus.	Aus.	Aus.	Aus.	Aus.	Aus.
Molds (log_{10} CFU/g)	1.94 ±0.14 ^{b}	1.68 ± 0.19^{b}	1.90± 0.05^{b}	<1	3.60 ± 0.12^{a}	2.00 ± 0.29^{b}	<1	1.99 ± 0.11^{b}	<1	2.93 ± 0.20^{a}
Yeasts (log_{10} CFU/g)	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1

The result of duplicates with a concentration greater than 1 log10 CFU/g was expressed as mean ± standard deviation. The letters overwritten on the same line with different letters (a, b, c), for each parameter, are significantly different.

When evaluating mesophilic aerobes (Table 1), it was observed that although there are no values in the current legislation to determine this biological parameter (BRASIL, 2019), their quantification is important, since high counts of these are related to inadequate hygiene in the production environment and the storage location (OLIVEIRA et al., 2015). According to Santos et al. (2005), a satisfactory concentration of heterotrophic mesophilic aerobes would have to be in the concentration below 4 log10 CFU/g and only one of the samples was found at this level. An unsatisfactory concentration of this group of microorganisms would be above 6 log10 CFU/g, and the concentration L3 was found in the sample at this limit. The other samples are in the acceptable concentration range, between 4 and 6 log10 CFU/g.

Bacillus cereus is an optional aerobic spore-forming bacterium, commonly found in soils, vegetables and in different processed and raw foods. This microorganism is capable of producing diarrheal and emetic enterotoxins (SAVINI, 2016; JAY, LOESSNER & GOLDEN, 2005), so its control is desired to guarantee the quality of the food, in which case, flaxseed flour is common to be consumed in natura. The results of the tests detected a concentration less than 1 log10 CFU/g, demonstrating to be in compliance with the hygienic-sanitary standards in Brazil (BRASIL, 2019).

Regarding the investigation of total coliforms (Table 1), despite not being mandatory in this food group, the presence of this bacterium in the food, indicates the level of environmental contamination to which the food is exposed (OLIVEIRA et al.,

2015). The total coliform concentration in the samples ranged from <1 to 4.17 log10 CFU/g. It was found higher levels than those obtained by Oliveira et al. (2015), with an average of 1.86 log10 CFU/g; Novello and Pollonio (2012), with an average of 0.89 log10 CFU/g and better results than those found by Vegi, Wolf-Hall and Hall. (2017) with an average of 4.1 log10 CFU/g.

For coliforms at 45ºC, the section 10 of ANVISA RDC No. 331/2019, which stands for the microbiologic parameters of flours, pasta, bakery products (industrialized and packaged), subsection a (starches, flours, starch and cornmeal, powdered or flaked) (BRAZIL, 2019), and determines the tolerance of 2 log10 CFU/g. The result obtained from the samples, prove that they are within the parameters determined by legislation, all samples were <1 log10 CFU/g.

According to the analyzes in Table 1, the samples are in compliance with the current legislation (BRASIL, 2019), however, with the contamination by fungi, it generates a worrying factor, since this cereal is usually consumed in natura. Molds can also cause food toxicity, since certain species, under deficient storage and conservation conditions, produce mycotoxins, as a secondary metabolite, with great carcinogenic, mutagenic and teratogenic potential (JAY, LOESSNER & GOLDEN, 2005; MOURA et al., 2014).

Table 2: Physical-chemical testing of flaxseed flour.

Samples	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10
pH	6,26 ± 0.16^{bc}	6,34 ± 0.14^{abc}	6,60 ± 0.11^{ab}	6,90± 0.25^{a}	6,10 ± 0.13^{bc}	6,40 ± 0.10^{abc}	6,18 ± 0.04^{bc}	6,02 ± 0.06^{bc}	5,97 ± 0.24^{c}	6,05 ± 0.08^{bc}

The result of duplicates was expressed as mean ± standard deviation. The letters overwritten on the same line with different letters (a, b, c), for each parameter, are significantly different.

The pH check is an important intrinsic parameter in the food, capable of limiting or favoring the capacity for the development of pathogenic microorganisms in the food (FRANCO, 2012; SOUZA et al., 2008). As can be seen in the results (Table 2), the pH of flaxseed flours is close to neutrality, ideal for favoring the growth of several species of decaying and pathogenic bacteria and fungi (BAPTISTA & VENÂNCIO, 2003).

Table 3: Amount of foreign material and weight retained in 100g of samples of flaxseed flour, captured by different mesh sizes.

Mesh (µm)		L1	L2	L3	L4	L5	L6	L7	L8	L9	L10
1700	Retained (g/100g)	1,3	0,07	0,09	4,95	2,80	3,87	1,03	1,76	0,96	1,45
	Foreign material (unit)	0	0	0	0	0	0	0	0	0	0
1000	Retained (g/100g)	12,0	0,90	0,94	24,75	16,07	26,82	3,07	8,41	2,75	7,35
	Foreign material (unit)	1	0	0	0	2	1	0	0	0	0
850	Retained (g/100g)	12,0	2,84	3,81	19,80	10,78	20,59	19,85	20,66	17,60	18,78
	Foreign material (unit)	0	0	0	1	0	0	0	1	0	0
600	Retained (g/100g)	19,2	12,02	15,02	16,53	19,48	14,18	15,15	29,07	16,79	31,50
	Foreign material (unit)	0	0	0	0	0	0	0	0	0	0
300	Retained (g/100g)	54,0	69,14	71,60	29,96	45,47	27,22	57,49	38,23	59,52	39,14
	Foreign material (unit)	0	0	0	0	0	0	0	0	0	0
Fundo	Retained (g/100g)	1,3	14,65	6,84	2,51	3,63	5,36	1,79	1,43	1,46	1,32
	Foreign material (unit)	0	0	0	0	0	0	0	0	0	0

Font: Authors, 2019.

The test for the detection of foreign material by Fontes and Fontes (2005) allowed the perception of a lack of standardization among the brands, regarding the milling of flaxseed flour. It is possible to notice the variation in particle size between the different samples (Table 3). Figure 1 shows the difference between the different grain sizes of the same sample. In some samples, whole flax seeds were found, being retained by a 1.7 mm opening mesh, as can be seen in Figure 2.

Figure 1: Flax seed sample with whole seeds

Font: Authors, 2019.

Figure 2: Flax seeds not ground in flour

Font: Authors, 2019.

According to Freire et al. (2014), granulometry can impact the result of preparations with flaxseed flour. The authors mention the interference in the formation of the gluten network by the fibers contained in flaxseed flour.

As for the test for foreign material, pieces of cotton were found, which indicate a failure in good flour manufacturing practices. No insects were found, nor fragments of insects. ANVISA's legislation nº. 14/2014 (BRASIL, 2014), which provides, among others, about foreign matter and its tolerance limits in food, does not include flaxseed flour in its standards. In the literature, no mention was made of the foreign material found in flaxseed flour samples, so the present study seeks to contribute to the quality standardization of this product.

4 CONCLUSION

Although the samples are in accordance with sanitary legislation, as for the microbial load found, the product must have greater hygienic rigor, to reduce the concentration of microorganisms found, since flaxseed flour is a product that can be consumed without applying heat, with indication of fresh consumption.

Flaxseed flour does not have standards for identity and quality, so there is no standard for physico-chemical, particle size tests and the presence of foreign material.

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