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Juglans regia and *Carya illinoinensis*: safety, chemical composition, and preference

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This study aimed to investigate the global scientific production of Chilean walnuts and pecans, analyze the efficiency of hygienic-sanitary treatments, compare both nuts for chemical composition and preference, perform chemical analyses of the pecan nutshell, prepare food bars with nuts and shells, and test their acceptability. Studies were consulted in the Scopus database and then directed to bibliometric analysis. Hygienic-sanitary treatments were performed by placing the nuts in the shell in hot water, cold water with hypochlorite, hot water with hypochlorite, and microwave. The proximate composition followed the official methods, and the sensorial analysis of the nuts was performed through a bilateral paired affective test. Food bars were prepared, tested by an affective acceptability test, and analyzed for chemical composition. After refining the research, we observed more publications for the walnut and its satisfactory hygienic-sanitary treatment with hot water of mesophilic aerobic bacteria, molds, yeasts, *Salmonella*, *Escherichia coli,* and mycotoxins. Regarding the physicochemical composition, the results differed significantly except for the ether extract; the untrained tasters preferred pecans. The pecan nutshell showed expressive amounts of carbohydrates and fiber, and the food bars were accepted by the tasters.

Keywords: nut; *Juglandaceae*; high-performance liquid chromatography.

Practical Application: Pecan nuts were submitted to different hygienic-sanitary treatments; all hygiene treatments were efficient for pecan nuts in the shell; pecan nut shell is a source of carbohydrate and crude fiber; only the ethereal extract showed no significant difference between the nuts.

1 INTRODUCTION

The botanical family of walnut trees (*Juglandaceae*) comprises 10 genera and over 60 species of native trees, which are important for their fruit and wood quality. The most widely cultivated species are *Juglans regia* (Chilean walnuts) and *Carya illinoinensis* (pecans), with an increasing expansion of the cultivated area in South America (Savian et al., 2021). English or Chilean walnut production has been increasing rapidly, with China being the leading producer, followed by the United States. Chile has also become a major producer and the world's third largest walnut exporter (Jimenez Luna et al., 2022); therefore, it is an important crop in Chile and surpassed only by the grapevine (Moya-Elizondo et al., 2021).

Pecans, nonetheless, are native to North America and were introduced to Brazil in 1870 in São Paulo State. Cultivation has also been increasing significantly recently, especially in Brazil (EMBRAPA, 2018).

The Chilean walnut presents a "brain" shape, wrinkled shell, intense flavor, and light color, whereas pecans have an elongated shape, smooth shell, sweet flavor, and dark colors. However, both are similar in physicochemical composition and concerning the beneficial effects on health, which has increased nut popularity and demand (Magnuson et al., 2016).

Nevertheless, despite being highly nutritious, there has been an increase in the incidence of diseases in these two main species in recent years, mostly scabies and anthracnose, consequently compromising crop profitability and product quality (Savian et al., 2021).

This has been influenced by several aspects, including environmental factors, different cultivars, inadequate temperatures during processing (Kluczkovski, 2019), and carelessness regarding good agricultural, production, and pre- and post-harvest processing practices (Kharel et al., 2018). In-shell nuts are even more susceptible to microbiological contamination and require satisfactory hygienic-sanitary treatment such as hot water (Kharel et al., 2018).

Storage is a delicate stage for pecan nuts since they are rich in unsaturated fatty acids, leading to greater oxidation. Descalzo et al. (2021) recommended storing them at temperatures of up to 2°C, and Zhang et al. (2016) recommended microwave pre-treatment for better preservation. In 2018, Zhang et al. (2018) also dried pecan nuts in a microwave with variable power and pre-treatment by immersion in sodium hypochlorite to enhance their hygienic-sanitary quality.

In this context, coupled with the population's growing interest in foods that bring health benefits and higher nut productivity and commercialization, the demands of the national and international markets have intensified. The list of microbiological standards for foods recommends mold and yeast count/g for bars of nuts, almonds, chestnuts, peanuts, leguminous seeds,

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and edible seeds, added or not with other ingredients, in addition to the investigation of the presence of *Salmonella*/25 g and *Escherichia coli*/g count for nuts, almonds, and edible seeds (BRASIL, 2022).

Molds such as *Aspergillus*, *Penicillium*, and *Fusarium* are highly relevant regarding product safety and produce toxic compounds such as mycotoxins, among them aflatoxins, which are of great health interest due to their toxigenic power identified as B1, B2, G1, and G2 (Silva et al., 2017). This leads to toxicity in humans and animals, as manifested by infections or diseases arising from the invasion of living tissues, allergies or hypersensitivity reactions, and poisoning resulting from ingesting food containing toxic metabolites, in addition to leading to substantial economic losses (Kluczkovski, 2019).

Thus, the entire nut production chain deserves more attention to fulfill its nutritional role, since it contains high unsaturated fatty acid levels, low saturated fatty acid levels, high vitamin E levels, polyphenols, flavonoids, arginine, and fiber. Walnuts have hypocholesterolemic and hypotensive effects, lower rates of cardiovascular disease, and antioxidant properties (Siebeneichler et al., 2023). Thus, one can suggest that they may delay the aging process, stimulate the immune system, and protect against heart disease and certain types of cancer (Costa & Jorge, 2011).

With these strong arguments, the US Food and Drug Administration recommends eating 43 g/day of any of the following oilseeds: almonds, hazelnuts, walnuts, pecans, pistachios, and peanuts to prevent heart disease (*apud* Florowski et al., 2019). There are different varieties of nuts, and their culinary uses are quite versatile; they can be consumed raw (*in natura)* or toasted, as an ingredient in various sweet and savory dishes, sold with or without shells, in halves, in pieces of different sizes, or even as flour. With this exponential consumption, the market is expanding and opening space to produce oil and by-products such as husk and defatted flour (Oro et al., 2009).

The shell, an abundant residue from agroindustry, can become a by-product with nutritional potential as it is a source of carbohydrates and crude fiber (Dorame-Miranda et al., 2019). This was confirmed in a study in Mexico in 2016, in which researchers identified 30.16% of carbohydrates in the shells of '*Wichita*' pecans and 31.36% in '*Western Schley*'; concerning fiber, the authors found 58.82% in '*Wichita*' and 57.91% in '*Western Schley*' (Flores-Córdova et al., 2016).

Given the above, this study sought to investigate the world's scientific production of Chilean nuts and pecans and to analyze the efficiency of hygienic-sanitary treatments used in shelled pecan nuts, compare both nuts for chemical composition and preference, perform chemical analyses of the pecan nutshell, prepare food bars with nuts and shells, and test acceptability.

2 MATERIALS AND METHODS

2.1 Type of study

This study focused on bibliometrics (Aria & Cuccurullo, 2017), which, according to Tague-Sutcliffe (1992), concerns a quantitative study of scientific production with the dissemination and publication of results measured according to the statistical models and standards. In addition to the experimental part, research was conducted transversally, descriptively, and quantitatively (Gil, 2002) and carried out from May to October 2022 after approval by the Ethics and Research Committee of the Universidade Federal de Santa Maria.

2.2 Data collection

Data were collected on the Scopus database to select documents that addressed the descriptors "pecan," "*Juglans regia*," and "*Carya illinoinensis*" in the title, abstract, or keywords. The search was then refined by only targeting articles published between 2012 and 2022. Subsequently, the selected articles were directed to bibliometric analysis using the tool Bibliometrix from RStudio® (version 7.6) to better present the data worked out, elucidating the annual scientific production, countries that publish the most, and a word cloud generated through the 50 most cited words in the keywords.

2.3 Samples

Chilean walnuts were purchased in a store specialized in natural products in the city of Santa Maria (Rio Grande do Sul, southern Brazil). The pecans were harvested in the production cycle of 2022 and kindly provided by Santa Leocádia Ltda (Figure 1). The microbiological, chemical, and sensory analyses were performed from May to August 2022.

2.4 Hygienic-Sanitary treatment of in-shell nuts

The in-shell pecans provided by the agroindustry were subjected to hygienic-sanitary treatments to count mesophilic aerobic bacteria, *Salmonella*, *E. coli*, mold, and yeast. The hygienic-sanitary treatments were as follows: soaking the nuts in hot water; immersion of the nuts in cold water with sodium hypochlorite; immersion of the nuts in hot water with sodium hypochlorite; and microwave exposure of nuts.

Figure 1. Image of (A) Chilean walnuts and (B) pecan nuts, 2022.

All treatments were performed in the Laboratory of Dietetic Technique of the Franciscan University (Rio Grande do Sul State, southern Brazil), with 100 g samples of in-shell pecans. For treatment 1, a sample was immersed in water at 90°C for 5 min. For treatment 2, the sample was immersed in 500 mL of cold water with 1 mL of hypochlorite for 10 min. Treatment 3 consisted of immersing the sample in water at 82°C and adding 1 mL of hypochlorite in 500 mL of water for 5 min. For the treatment 4 (microwave treatment), the sample was exposed for 1 min in 140 W and, 1 min in 373 W in a commercial microwave oven.

2.5 Microbiological analyses

Mesophilic aerobic bacteria, molds, and yeasts were counted in pecans after being subjected to the different hygienic-sanitary treatments mentioned above. After selecting the hot water immersion method, the analyses recommended in the list of microbiological standards for food and mycotoxin determination continued.

2.5.1 Aerobic mesophilic bacteria, mold, and yeast determination

After different sanitization treatments of the in-shell pecans, mesophilic aerobic bacteria, molds, and yeasts were counted by the plating technique of Silva et al. (2017) by using plate count agar for mesophilic aerobic bacteria and potato dextrose agar acidified with 10% tartaric acid as the medium to identify mesophilic aerobic bacteria, molds, and yeasts.

2.5.2 Salmonella determination

To determine the presence/absence of *Salmonella* in 25 g after hot water treatment, we utilized ISO method 6579, which is applied to all foods intended for human and animal consumption and samples from food manufacturing and/or handling environments (Silva et al., 2017).

2.5.3 Escherichia coli determination

For *E. coli* count, the most probable number (MPN) technique was used. This consists of a quantitative analysis method that enables one to determine the MPN of the target microorganisms in the sample by inoculating aliquots of that sample in a series of tubes containing a liquid culture medium suitable for their growth (Silva et al., 2017).

2.6 Mycotoxin determination

Finally, to further ensure the use of the nutshells in preparations that are desirable by the donor agroindustry, they were submitted to mycotoxin analysis in the Laboratory of Mycotoxicological Analysis (LAMIC) of the Universidade Federal de Santa Maria to detect aflatoxins, namely, B1, B2, G1, and G2 (Figure 2). The analysis was performed using the method adapted from Sulyok et al. (2007) and described by Mallmann et al. (2020). The sample was ground, homogenized, and an aliquot weighed for subsequent extraction of the mycotoxins with acetonitrile and water solution. The extract was then filtered and

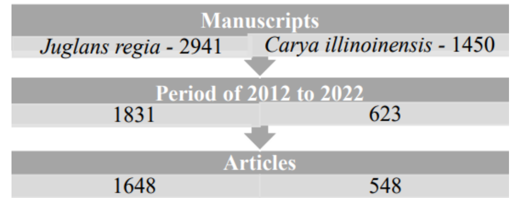

Figure 2. Publications on *Juglans regia* and *Carya illinoinensis*, 2022.

diluted in a flask with methanol-water plus the internal standard of each mycotoxin, and finally, the analyses were performed by high-performance liquid chromatography coupled to triple quadrupole mass spectrometry (HPLC-QqQ-MS/MS), with an electrospray ionization source. This procedure was performed in two triplicates.

To validate the method, the following parameters were estimated: selectivity, the limits of quantification and detection, linearity, accuracy, robustness, and reproducibility based on the standard of the Brazilian Association of Technical Standards (ABNT, 2005) of the National Institute of Metrology, Quality, and Technology, in addition to the matrix effect. Further details are not provided by LAMIC regarding the methods, procedures, and chromatograms due to the confidentiality policy required by ISO 17025.

2.7 Chemical analysis

The proximate composition of the two species of nuts and pecan shell was performed in the physicochemical laboratory of the Universidade Federal de Santa Maria (DTCA/UFSM/RS/BR), in triplicate, according to official methods, and expressed in percentage. Total lipids were cold extracted by organic solvents using the method described by Bligh and Dyer (1959) and the official techniques of the Association of Official Agricultural Chemists (2005).

Protein content was determined by the micro-Kjeldahl method (AOAC 950.48). The crude fiber was determined according to the recommendations of the Instituto Adolfo Lutz (1985). Moisture analysis was calculated by oven drying at 105°C (AOAC 925.10), ash by calcination in a muffle furnace at 550°C (AOAC 923.03), and carbohydrates by difference.

2.8 Sensory analysis of Chilean nuts and pecans

The sensory analysis was performed through a bilateral paired affective test (used in preference evaluation) after approval by the Ethics and Research Committee (CAAE no. 60712322.8.0000.5346). The test was conducted in the Dietetic Technique laboratory and the Sensory Analysis room at the Universidade Franciscana, in individual booths, under fluorescent light, and with untrained evaluators over the age of 18 years.

The evaluators randomly received two samples in a tray coded with three digits selected from a random number table; each sample consisted of a Chilean nut and a pecan. Both samples were offered at room temperature on a small white porcelain plate with a glass of water to clean the palate between samples, a paper napkin, a pen, and a sensory evaluation form on which the evaluator chose their favorite sample and provided comments, if necessary, in addition to the signature on the informed consent form (two copies). The tabulated data were expressed as percentages.

2.9 Food bar preparation

A recipe for a food bar was formulated using pecan nut flour, whey protein, powdered pecan nutshell, chestnut, apricot or raisin, previously fractionated honey, and glucose syrup. The ingredients were mixed until they formed a firm mass, which was placed in aluminum molds and pressed with a rolling pin until it reached a thickness of 1 cm, obtaining bars with a standard size weighing 30 g. The dough remained at rest for 2 h in the refrigerator for later unmolding. The main ingredients were cracked nuts and fruit (Table 1).

2.10 Sensory analysis of food bars

The sensory analysis of the bars was carried out in the Dietetic Technique laboratory and the Sensory Analysis room at the Universidade Franciscana (UFN/RS/BR), in individual booths, under fluorescent light, with untrained evaluators, who randomly received two samples (randomly coded with three digits) on a tray, being a sample of pecan nut bar with apricot and another sample of pecan nut bar with raisin, both offered at room temperature and in a small disposable white plate, next to a glass of water to clean their palate between tastings, a paper napkin, a pen, the informed consent form (two copies), and a sensory evaluation form that contained a seven-point hedonic scale ranging from "I liked it very much" to "I disliked it very much." The evaluated attributes were appearance, texture, odor, and flavor (Dutcosky, 2013).

2.11 Statistical analysis

Regarding the chemical analysis, the data were organized in an Excel spreadsheet (Office 365) and analyzed using the IBM SPSS software (version 25). Descriptive analyses included mean and standard deviation, and a comparison between means was performed using the Student's t-test for unpaired data, as all variables met the normality requirement according to the Shapiro-Wilk test. All tests were conducted with a significance level of 5%.

The sensory analysis data were evaluated using the Microsoft Excel® 2010 software and presented as mean and standard deviation (mean ± SD). To calculate the acceptability index (AI) of the food bars, we used Equation 1:

AI (%) = A ´ 100/B(1)

Where:

A: the average score obtained for the product;

B: the maximum score given.

The AI was carried out for the attributes and calculated considering the maximum score achieved for the product analyzed as 100% and the average score in percentage. According to Dutcosky (2013) and Monteiro (1984), the product is accepted when it exceeds at least 70%.

Table 1. Ingredients used in the formulation of walnut bars with apricots or raisins, 2022.

Ingredients	**Quantity (g)**
Pecan flour	190
Whey protein	30
Apricots/raisins	150/150
Chestnut	100
Nutshell powder	1
Glucose syrup	10
Honey	30

3 RESULTS

After consulting the Scopus database, 43,496 manuscripts were found referring to nut, 2,941 manuscripts to *Juglans regia*, and 1,450 manuscripts to *Carya illinoinensis* (Figure 2).

Of these articles, a more expressive number for the Chilean nut can be observed, although with an exponential increase of publications for both nuts, with China standing out as the country with the most studies on the Chilean nut, in contrast to Brazil, with only 28 articles. Regarding the pecan, the largest number of publications was reached by the United States, China, and Brazil, respective (Figure 3 and Table 2). The most cited words are presented in Figure 4.

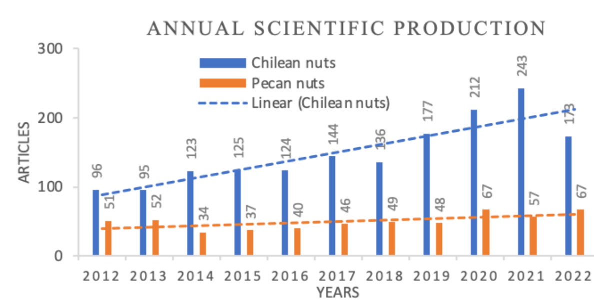

Figure 3. Annual scientific production of the Chilean nut and pecan nut.

Table 2. Countries that most publish about Chilean nuts and pecan nuts.

Chilean nut		**Pecan nut**	
Region	Frequency	Region	Frequency
China	1014	USA	608
Iran	560	China	236
USA	386	Brazil	228
Turkey	342	Mexico	179
India	288	Spain	30
Italy	247	Italy	23
Pakistan	149	Argentina	17
Spain	130	Tunisia	16
France	128	India	15
Romania	103	UK	14

Regarding the most cited Wordcloud, in both, the scientific name is evident, followed by articles, nuts, and some countries. However, when reading the articles, one can divide them into three main themes: microbiological safety, composition, and acceptability. In this context, we started to question the safety of pecans donated by the Santa Leocádia agroindustry, showing promising preliminary microbiological results since there was no contamination in in-shell pecans by mesophilic aerobic bacteria, molds, and yeasts after different hygienic-sanitary treatments.

Thus, continuing with the method already employed in the donor agroindustry, immersion of in-shell walnuts in hot water (90°C) and complying with the list of microbiological standards for food of ANVISA (1986), the presence of *Salmonella*/25 g and *E. coli* count/g was determined. Although *Salmonella* is considered by the World Health Organization the main global agent of foodborne diseases, with tens of millions of cases per year worldwide, in the case of in-shell walnut, the result was the absence of 25 g of sample, with a favorable result for thermotolerance: < 3 MPN/g.

As already discussed, in the absence of mold and yeast contamination, the presence of aflatoxins, namely, B1, B2, G1, and G2, was not detected in the nutshells submitted to hot water, which also depend on propitious factors for their production, including temperature and humidity and the physical integrity of the substrate during storage. The limit of quantification was set at 1 μg/kg for the four aflatoxins, with a coefficient of variation between 80 and 95%.

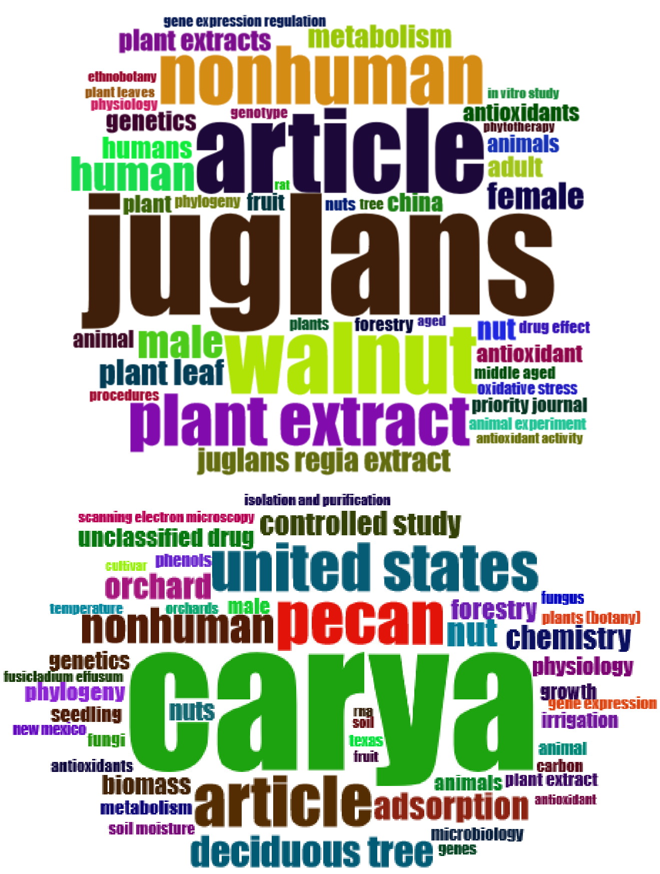

Figure 4. Wordcloud of Chilean nuts and pecan nuts.

For the chemical analysis, the results were expressed in g 100 g^{-1} in the total dry mass, where a high caloric value was observed as well as the superiority of the ether extract compared to the other macronutrients in both nuts (Table 3), with a moisture content of 3.53% (SD ± 0.05) in the Chilean walnuts and 2.89% in the pecans (SD ± 0.00).

At a 5% significance level, there was a significant difference for all nutrients except for ethereal extract ($p > 0.05$). Regarding the moisture, the difference was represented by $p = 0.002$.

After microbiological analysis of the pecans in the shell and the chemical composition of Chilean walnuts and pecans, in terms of preference, non-trained tasters participated in the sensory analysis, among them academics, professors, and other employees of the Franciscan University, excluding those younger than 18 years old. In total, there were 82 tasters, of whom 55 (67%) were females and 27 (33%) were males.

According to the results, 76% (62) preferred the pecans and only 24% (20) chose the Chilean walnuts, with a significant difference between the samples at the 0.001% probability level (American Society Testing & Materials, 1986). In the comments, some evaluators made manifestations regarding the coded numbers, transcribed already with the sample identification: "the pecan nut is tastier," "the Chilean nut is drier," "the Chilean walnut is more bitter," "the pecan has a lighter taste and does not have bitterness in the end," "the pecan is slightly sweetened," "the pecan, despite the different texture, was perceived as being sweeter," "I liked them both, although the Chilean is more bitter," "I found the pecan pastier," "I felt less fat in the pecan," "Both are very good, but the pecan has a more pronounced taste," "the pecan is softer and tastier," "the Chilean walnut is tastier," "the Chilean walnut has a bitter taste in the beginning," "the pecan is more bitter and greasier," and "the Chilean is sweeter and drier apparently."

Regarding the composition of the pecan nutshell, it proved to be a source of carbohydrates and fiber (Table 4), with a moisture content of 14.2% (SD ± 0.06).

The sensory analysis of the food bars was conducted through an affective acceptability test involving 70 participants above 18 years old, including 51 females (73%) and 19 males (27%) (Table 5).

Table 3. Chemical composition of the Chilean walnuts and pecans, 2022.

Analyses	Results (in g%)		*p*-Value
	Chilean walnuts	Pecans	
	Mean ± SD	Mean ± SD	
Ash	1.78 ± 0.02	1.73 ± 0.01	0.030
Ether extract	68.55 ± 0.34	70.40 ± 1.43	0.094
Crude protein	15.57 ± 0.19	9.77 ± 0.32	0.000
Crude fiber	3.76 ± 0.04	2.27 ± 0.05	0.000
Carbohydrates	6.81 ± 0.41	12.94 ± 1.30	0.001

Table 4. Chemical composition of the pecan nutshell, 2023.

Analyses	Results (in g %)
	Mean ± SD
Ash	1.78 ± 0.006
Ethereal extract	0.41 ± 0.05
Crude protein	2.16 ± 0.11
Crude fiber	28.15 ± 0.08
Carbohydrates	53.3 ± 0.28

Table 5. Results of the sensory analysis of the apricot and raisin bars, 2022.

Preparations / Attributes	Apricot bar SD Mean±SD	AI (%)	Raisin bar Mean (SD)	AI (%)
Appearance		74	5.44 ± 1.18	77
Texture	5.70 ± 1.05	81	5.87 ± 0.88	84
Odor	5.51 ± 1.14	78	5.57 ± 1.12	79
Flavor	5.97 ± 0.98	85	6.39 ± 0.79	91
AI total%		80		82

4 DISCUSSION

4.1 Hygienic-sanitary treatment of in-shell nuts

As already mentioned, four hygienic-sanitary treatments were performed with in-shell pecans, since the agroindustry intends to work with sustainability, in this case, using the shells in food preparations to reduce environmental impact and add nutritional value to their products.

Thus, the procedure already used in the agroindustry was tested, immersion of the nuts in hot water (i.e., a sample was immersed in water at a temperature of 90°C for 5 min), since in the study of Kharel et al. (2018), both prior treatments with hot water in in-shell nuts at 70°C for 8.6 min, 80°C for 6.6 min, or 90°C for 4.6 min showed promise for achieving a minimum 5-log reduction of several bacterial pathogens of public health interest, not changing physicochemical characteristics. However, as the temperature increased, so did consumer preference, even though they did not differ significantly.

In treatment 2, the sample was immersed in cold water with hypochlorite, following the recommendations of Ordinance No. 78 (Rio Grande do Sul, 2009) and Collegiate Directive Resolution No. 216 (Brasil, 2004), which provide for the hygiene of horticultural food products using the following criteria:

- Selection of the food, removing parts or products that have deteriorated and are not in proper condition;
- Careful washing of food with drinking water;
- Disinfection with immersion in chlorinated solution with 100–250 ppm of free chlorine for 15 min or other appropriate products, registered at the Ministry of Health, released for this purpose and according to the manufacturer's indications;
- Rinse with drinking water.

As some products differ in dilution and immersion time, we followed the manufacturer's recommendation: "Disinfection of fruits and vegetables: to disinfect fruits and vegetables, dilute one tablespoon (10 mL) of sanitary water Girando Sol® in 5 liters of water. Rinse well after 10 minutes." Thus, the unshelled nuts remained in 500 mL of water with 1 mL of hypochlorite for 10 min.

For treatment 3, the sample was immersed in water at 82°C along with adding chlorinated solution with 100–250 ppm of free chlorine for 5 min, according to the recommendation on the label (i.e., 1 mL of hypochlorite in 500 mL of water).

Regarding the microwave treatment, the known reference was Zhang et al. (2016), who left the nut samples for 2.5 min at 2455 MHz, 650 W power (1.7 W/g was employed in the first 150 s, followed by cooling for 30 s, and 2.6 W/g was applied in the final 120 s). It was chosen to be exposed for 1 min at 140 W and 1 min at 373 W in a commercial microwave oven.

As all the procedures were effective, we decided to continue with the one already used in the Santa Leocádia agroindustry since it is already routine and does not cause any environmental impact.

4.2 Microbiological analyses

Molds and yeasts constitute a large group of microorganisms usually originating from soil or air, with molds being more versatile than yeasts as they can assimilate any carbon source from food. This is different from some yeasts that are unable to assimilate nitrate and complex carbohydrates, requiring vitamins, and others that are unable to utilize sucrose as a sole carbon source, limiting the range of foods susceptible to spoilage by yeasts (Silva et al., 2017).

As the nuts from the Santa Leocádia agroindustry do not have direct contact with the soil and are stored below 10°C, there was probably no development of mold and yeast, which find optimum growth temperature in the range of 25–28°C; additionally, bacteria in which the favorable range of growth goes up to around 40°C (Silva et al., 2017).

Hence, as there was no difference between the methods, we decided to continue with the treatment already in place and implemented in the agroindustry (i.e., with hot water) to perform the other analyses contained in the list of microbiological standards for foods. A consistent argument for maintaining the use of hot water for packaging in-shell nuts was suggested by McKay et al. (2022), who confirmed the hypothesis that this treatment improves shelling efficiency, measured by the number of whole halves and large pieces, probably resulting from moisture absorption by the shell, making it more pliable and less prone to fracture. Similarly, they realized that higher water temperature improves microbial inactivation, even in a shorter time, while a lower water temperature increase facilitates the ease of cracking/peeling, but for longer exposure time; thus, they cautiously suggested the use of water above 85°C for a time longer than 5 min (McKay et al., 2022).

Despite the low moisture of walnuts, Kharel et al. (2018) reported various articles proving *Salmonella* and *E. coli* outbreaks because of nut ingestion. They then decided to investigate the feasibility of using hot water treatment as an elimination step and subjected in-shell nuts to hot water at 70, 80, and 90°C for 1, 2, 3, 4, and 5 min. They found that *Listeria monocytogenes* was the most susceptible to heat treatment and reduced by 4.6 ± 0.35 log CFU/g at 70°C for 5 min. In contrast, 3–5 min at 80 and 90°C was necessary to achieve a similar level of reduction (5 log) for enteric *Salmonella* and *E. coli*.

Regarding the *E. coli*/g count, the MPN technique was used because it is a highly versatile technique that allows one to enumerate different groups or species of microorganisms. The culture media and incubation conditions varied, and the main applications were the counting of total coliforms, thermotolerant coliforms, and *E. coli* in water and food (Silva et al., 2017).

4.3 Mycotoxins

Hidalgo-Ruiz et al. (2019) corroborated the use of QqQ-MS/MS because of the advantages this analyzer offers, such as reliability, low sample preparation cost, and robust analysis. Regarding chromatography, aflatoxins, namely, B1, B2, G1, and G2, are identifiable by their blue fluorescence for aflatoxins B1 and B2, and green fluorescence for aflatoxins, G1 and G2, when observed under ultraviolet light at 365 nm. The G-series differs chemically from the B-series by the presence of a 3-lactone ring in place of the cyclopentenone ring. A double bond is found as a vinyl ether on the furan terminal ring only in aflatoxins B1 and G1. These structural variations are also related to their activities, with aflatoxins B1 and G1 being carcinogenic and considerably more toxic than B2 and G2 (Jaimez et al., 2000).

The hypothesis that pecans from the Santa Leocádia agroindustry are free of aflatoxins and any other contamination is likely due to the care taken from harvesting the nuts without direct contact with the soil using shade cloth or the inverted umbrella technique, sanitization of in-shell nuts in hot water, reduction of the humidity to less than 3%, vacuum packing, and storage in a chamber at a temperature of up to 10°C until shipping. However, according to the Instituto Adolfo Lutz (2008), although countries are importing and exporting more food, increasing the responsibility of industries and regulatory bodies, it emphasizes that in almost all raw materials, besides nuts, rice, corn, barley, and milk, one or more types of mycotoxins have already been detected.

These contaminations are mostly due to fungal strains such as *Aspergillus*, one of the most important species in food spoilage and mycotoxin production, which grow very well in temperatures of 37°C or higher, increased humidity in storage and/or transportation, and inadequate hygiene conditions (Kluczkovski, 2019). Kluczkovski (2019) reported on studies conducted in Brazil and Portugal in which no mycotoxins or aflatoxins were detected in pecans, in contrast to samples from Mexico, in which 12.5% were contaminated with aflatoxin B1. Thus, the European Commission has established a limit of 15 and 10 μg/kg of aflatoxin total and a maximum of 8 and 5 μg/kg for aflatoxin B1 in peanuts and tree nuts, respectively, if the samples are treated before human consumption, and a limit of 4 and 2 μg/kg for aflatoxin total and aflatoxin B1, respectively, if they are intended for direct human consumption (Comissão Europeia, 2006).

In this context, Kluczkovski (2019) reinforced the importance of prevention through proper harvesting and storage conditions by maintaining temperatures between 4 and 15°C, grain moisture content around 2.5%, relative humidity of 40–60%, oxygen concentration below 2.5%, and in the dark. Nevertheless, if remediation is needed, aflatoxin decontamination can be performed through physical and chemical methods, which can affect the nutritional properties of food or be unsafe for human consumption. Gamma radiation and ozone applications have shown great potential to detoxify aflatoxins in some food matrices, while biological methods based on removing or degrading aflatoxins by bacteria and yeast have shown good prospects. Moreover, improved packaging materials can also minimize post-harvest aflatoxin contamination, in addition to using roasting to mitigate aflatoxins (Karlovsky et al., 2016).

4.4 Chemical analysis

Not very different data were found in a study conducted in 2016 in Mexico with '*Wichita*' and '*Western Schley*' pecans, which showed caloric values of 704 and 702 kcal, 2.94 and 3.70% of moisture, 1.60 and 1.40% of ash content, 68.67 and 69.96% of lipid content, 7.32 and 9.99% of protein content, and 5.23 and 6.75% of crude fiber content, respectively (Flores-Córdova et al., 2016). Descalzo et al. (2021) reinforced that pecans are a source of vitamins and minerals, especially vitamin E, folic acid, calcium, magnesium, phosphorus, potassium, several B vitamins, and zinc, in addition to excelling in unsaturated fatty acids. They are also an interesting source of proteins (6.88–9.26%), with low carbohydrate content (4.92–17.33%) and fiber (5.55–15.94%). The lipid content of pecans ranges from 60 to 75% (Oro et al., 2009).

Another study conducted with 11 cultivars of pecans produced in southern Brazil found lipids ranging from 52.69 to 69.76%, carbohydrates with a greater oscillation between 4.92 and 17.33%, protein from 6.88 to 9.26%, moisture content from 4.56 to 5.50%, ash content differed among the cultivars and ranged from 1.10 to 1.69%, and dietary fiber had an average of 10.8% (Ribeiro et al., 2020).

Another study also with pecans from southern Brazil but with a larger sample (n = 60) showed greater differences in morphological and genetic characteristics than in the chemical ones, meaning the chemical composition of the species did not diverge significantly, revealing an average of 70.3% for ethereal extract and 10.1% for protein (Poletto et al., 2020).

In addition to being a source of carbohydrates and fiber, the pecan nutshells also have phenolic compounds, such as phenolic acids, flavonoids, proanthocyanidins, and condensed tannins, which provide therapeutic effects (Hilbig et al., 2018). Corroborating this, Dorame-Miranda et al. (2019) also found significant amounts of carbohydrates and mainly crude fiber (especially cellulose and lignin) in walnut shells grown in Mexico. They found 29.48% of carbohydrates (reducing sugars represented 34.63% of the carbohydrate content, including starch, glucose, sucrose, fructose, and dextrose), 55.65% crude fiber, 1.30% crude protein, 0.30 of ether extract, 1.76% ash content, and 11.50% moisture. Hence, the amount of ash is usually low, as reported herein (i.e., 1.78%), while in a study in Mexico, it was even lower: 1.5% (Torres-Pérez, 2019).

4.5 Sensory analysis

A disagreement of judgments was noticed, which is normal in any sensory analysis, especially when using affective testing, as it is a subjective method that considers the opinions of individuals in the interpretation of the effects of sensory stimuli (single or multiple) according to the impressions perceived by the organs that will generate the interpretations (Instituto Adolfo Lutz, 2008).

Nevertheless, when it comes to sensory analysis, regardless of preference or acceptability, various authors have proved the approval and higher consumption of nuts of the most diverse varieties, whether raw or roasted (Gong et al., 2018; Magnuson et al., 2016), added as ingredients in elaborated products (Florowski et al., 2019; Reyes-Padilla et al., 2018), harvested early (Resurreccion & Heaton, 1987), with the use of hot water as a microbial intervention strategy (Kharel et al., 2019), stored under refrigeration (Descalzo et al., 2021), pre-treated in a microwave (Zhang et al., 2016; Zhang et al., 2018), and treated with gamma irradiation (Taipina et al., 2009).

In this study, the pecan nut obtained the highest preference, likely due to its sweeter flavor, as confirmed by the physicochemical analysis, in which it showed significant superiority in the number of carbohydrates.

Regarding the sensory analysis of the food bars, we observed that both the walnut bar with apricots and the raisin bar received a score above 5 (I liked it slightly) in relation to all attributes, indicating a good acceptance by the tasters and corroborating the AI above 70%, showing the viability of adding walnut shells to food preparations.

5 CONCLUSION

A growing interest in world publications regarding nuts was observed, although with a higher concentration of Chilean walnuts. In this study, all the hygienic-sanitary treatments were efficient for in-shell pecans, although we decided to continue with the method already implemented in the Santa Leocadia agroindustry of immersing the nuts in hot water because its efficiency has been proven, is already part of the routine, and does not harm the environment. No mycotoxins were found, and the microbiological analyses showed no contamination.

Regarding the physicochemical composition, a significant difference was noticed among the nuts except for their major constituent (the ether extract). The nut preferred by the tasters was the pecan, and this is likely because they perceived a softer and sweeter flavor, as confirmed by the physicochemical analysis since a higher concentration of carbohydrates was found.

Future research may further explore the use of pecan nutshells in preparations, considering that, if properly cleaned, they will not present risks to the consumer, add nutritional value to food products, and contribute to the environment. Despite various studies encouraging and proving the benefits of adding pecan nutshells to food preparations, no examples and sensory tests of such preparations were found in the literature, thus highlighting the research gap in this field.

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