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Non meat-based *alheiras*– a safer novel trend?

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Non meat-based *alheiras*– a safer novel trend?**Abstract**

In response to nutritional and health concerns, the food industry has begun to offer a wider variety of products that reflect changing consumer preferences. In addition to traditional *alheiras*, made with pork and/or poultry meats, other varieties of *alheiras* (“innovative”) made from codfish, mushrooms, tofu, soy and vegetables were launched on the Portuguese market. The objective of this study was the characterisation of these products, giving particular attention to their microbiological and chemical safety. Therefore, fourteen different products were analysed. *Enterobacteriaceae*, *Enterococcus*, lactic acid bacteria, yeasts and moulds, were the prevalent microbiota of “innovative” *alheiras*. Sulphite reducing *Clostridium* spores, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella* spp. or *Staphylococcus aureus* were not detected in any sample. No differences were observed between traditional and “innovative” *alheiras* concerning pH and water activity values while nitrites, nitrates and biogenic amines were found to be within accepted limits for these kinds of products. In terms of organic acids, lactic acid was found in all analysed samples while malic and succinic acid seemed to be predominant only in “innovative” *alheiras*. In conclusion, unlike traditional *alheiras* which often contain pathogenic agents, no harmful organisms nor chemical hazards were found in these new products, even though produced by the same companies.

Keywords: Food safety, foodborne pathogens, biogenic amines, consumers’ trend

1. Introduction

Consumers' interest in traditional and local foods has increased worldwide in recent years, especially in Europe (Albayrak & Gunes, 2010; Balogh, Békési, Gorton, Popp, & Lengyel, 2016; Guerrero et al., 2009; Kühne, Vanhonacker, Gellynck, & Verbeke, 2010; Pieniak, Verbeke, Vanhonacker, Guerrero, & Hersleth, 2009; Roosen, 2009). It is believed that local products are more authentic, nutritious, fresher and tastier, and buying them from local producers will support and contribute to the local and national economy (Chambers, Lobb, Butler, Harvey, & Bruce Traill, 2007). Also, consumers are increasingly aware that food is connected with their health status and this growing awareness associated with progress in nutrition science provides the food industry with opportunities to develop a range of new products (Hung, Verbeke, & de Kok, 2016; Marcos, Viegas, de Almeida, & Guerra, 2016; Ojha, Kerry, Duffy, Beresford, & Tiwari, 2015).

Alheiras are traditional, smoked, naturally fermented meat sausages, produced in the North of Portugal. In recent years, different formulations of *alheiras* made with ingredients other than pork and/or poultry meats, such as fish, veal, lamb, mushrooms, tofu, soy and vegetables (in this manuscript denominated “innovative” types) have become available, as a way to improve competitiveness through innovation.

Even though there is already extensive scientific knowledge on fermentation and behavior of pathogens in traditional *alheiras* (Barbosa, Gibbs, & Teixeira, 2010; Felício, Hogg, Gibbs, Teixeira, & Wiedmann, 2007; Ferreira et al., 2011), to our knowledge detailed characterization of these “innovative” products is not available in the scientific literature. Despite traditional fermented meat products are considered relatively safe due to their low pH, low water activity (a_w), high salt level, presence of nitrate and nitrite, competition with endogenous microbiota, addition of spices, herbs

and smoke compounds, several studies demonstrated that they can harbor pathogenic bacteria (Anal et al., 2020; Ferreira et al., 2007a; Ferreira et al., 2007b; Ferreira et al., 2006; Siriken, Pamuk, Özakin, Gedikoglu, & Eyigör, 2006; Prado, Sampayo, González, Lombó, & Díaz, 2019; Talon, Leroy, & Lebert, 2007). Foodborne pathogens like *Listeria monocytogenes*, *Escherichia coli*, *Salmonella* spp., *Clostridium botulinum* and *Staphylococcus aureus* can overcome hurdles imposed during processing and cause serious human infections and intoxications and some outbreaks have been reported worldwide (Arnedo-Pena et al., 2016; Castellano, Holzapfel, & Vignolo, 2004; Cocolin et al., 2007; Colak, Hampikyan, Ulusoy, & Bingol, 2007; Ethelberg et al., 2009; Kuhn, Torpdahl, Frank, Sigsgaard, & Ethelberg, 2011; Sartz et al., 2008; Schimmer et al., 2008).

“Innovative” *alheiras* are produced by the same companies that produce the traditional ones, so it is predictable that many of the problems previously associated with these traditional products, could also be associated with these new products. Therefore, the main objective of this study was the microbiological and chemical characterization of “innovative” *alheiras* with particular reference to factors that might influence product safety.

2. Materials and methods

2.1 Sampling

A group of 14 “innovative” *alheiras* (Table 1) available on the Portuguese market were selected. On each occasion, whenever possible, a traditional *alheira* from the same producer was also selected. This study was conducted between September 2017 and May 2018, during which two *alheiras* from each type were bought from supermarkets on two different occasions (Lot A and B). Samples were transported to the laboratory in

portable, insulated cold-bags and stored at 4 °C until analysis, normally between 1 and 2 days after collection. From each lot, two *alheiras* were divided into various pieces and manually mixed.

2.2 Physicochemical analysis

The pH value was determined directly with a Crison MicropH 2002 pH-meter (Crison, Barcelona, Spain) equipped with an InLab 427 puncture electrode (Mettler Toledo, Columbus, OH, USA).

Titrateable acidity (TA) was measured by titration from the pH jump in endpoint mode (by default pH value is set to 8.1 – 8.2) with 0.1 NaOH (Merck, Darmstadt, Germany). Samples were analyzed in triplicate and titrateable acidity was calculated as a percentage of lactic acid (Zaika, Zell, Smith, Palumbo, & Kissinger, 1976).

Water activity was assessed using the AquaLab Series 3 Water Activity Meter (METER Group, Inc. USA) equipment with a probe measuring over the range 0-1 a_w with temperature control.

2.3 Nitrite and nitrate concentrations

For each sample, 200 g were used for analysis of nitrite and nitrate concentrations following Portuguese standards NP 1846:2006 and NP 1847-1:2009, respectively. A standard curve was established for both analyses. Two readings were performed for each test.

2.4 Biogenic amines determination

Ten grams of each sample of *alheira* were weighed into 85 ml test tubes and extracted with 20 ml of trichloroacetic acid (5%) (Merck). Extracts were derivatized with o-

phthalaldehyde (OPA) (Sigma Diagnostics, St. Louis. MO, USA) and biogenic amines were determined by High-Performance Liquid Chromatography (HPLC) using a method based on that described by [Komprda et al. \(2004\)](#).

2.5 Organic acids quantification by High-Performance Liquid Chromatography (HPLC)

Five grams of each sample were added to 50 ml water and the preparation was vigorously shaken and blended with a vortex. Samples were centrifuged at 3000 x g for 15 min and the supernatant was filtered initially through filter paper and finally through 0.45 μm HPLC certified disposable syringe filters (Chromafil Pet-45/25; Macherey-Nagel GmbH & Co) before injecting into HPLC. The chromatographic system consisted of a Beckman model 126 pump, a Beckman automatic injector (model 508), a Beckman DAD detector (model 168), and an Aminex HPX-87H cation exchange column at 40 °C. Quantification of each sample was done at 210 nm, after the quantification of several standard compounds at different concentrations. The mobile phase was composed by 2.5 mM of H_2SO_4 with 0.6 ml/min flow. Eleven standard solutions were analyzed at different concentrations: between 0.1 and 2 g/L of acetic, citric, lactic and malic acids and between 0.01 and 0.2 g/L of succinic, butyric, isobutyric and isovaleric acids (all from Merck).

2.6 Microbiological analyses

Twenty-five grams of each composite sample (in duplicate) were added to 225 ml of sterile buffered peptone water (Biokar Diagnostics, Beauvais, France), and homogenized in a stomacher (Interscience, Saint Nom la Brèteche, France) for 2 min. Appropriate decimal dilutions were prepared in Ringer's solution (Biokar Diagnostics) for microbial enumeration: colony counts at 30 °C on Plate Count Agar according to

ISO 4833-1:2013; lactic acid bacteria (LAB) counts at 30 °C on De Man-Rogosa Sharpe (MRS) medium according to ISO 15214:1998; *Enterobacteriaceae* on RAPID[®] *Enterobacteriaceae* medium (Bio-Rad, Hercules, CA, USA; ISO 16140:2016; ISO 21528-2:2017); *Staphylococcus* coagulase positive according to ISO 6888-1:1999; *E. coli* according to ISO 16649-2:2001; enterococci on Bile Esculin Azide Agar (Biokar Diagnostics) incubated at 30 °C for 72 h (Ferreira et al., 2007b); *L. monocytogenes* according to ISO 11290-2:2017 and yeasts and moulds according to ISO 21527-1:2008. Detection of *L. monocytogenes* (ISO 11290-1:2017), *Salmonella* spp. (ISO 6579-1:2017) and sulphite-reducing *Clostridium* spores (NP 2262:1986) were also performed. After appropriate incubation, colonies were counted and/or confirmatory tests performed and the colony forming units (cfu)/g calculated.

2.7 Statistical analysis

An analysis of variance was carried out for microbiological analysis to test any significant difference between each lot and formulation of *alheira*. Multiple comparisons were evaluated by Tukey's post-hoc test and all analyses were performed using IBM SPSS Statistics, 24 (IBM Corporation, USA). The mean difference was considered significant at the 0.05 level.

3. Results and discussion

3.1. Chemical analysis

The results of the physicochemical analyses are shown in Tables 1 and 2. No differences were observed between traditional and “innovative” *alheiras* with pH values ranging 4.2 to 6.0 and 4.0 to 6.0, respectively (Table 1). Also, evident differences between water activity values (Table 1) were not observed for traditional (0.959 –

0.988) and “innovative” *alheiras* (0.961 – 0.991). Pathogenic bacteria development is partially inhibited during various sausage production steps, as well as reduction of pH and a_w during this process (Ferreira et al., 2007a). In this kind of products, pH below 4.0 and water activity lower than 0.85 are considered sufficiently to ensure microbiological safety (Marcos, Viegas, de Almeida, & Guerra, 2016); in this study, none of the samples showed values of pH and/or water activity below those mentioned, which means that contaminants may be able to grow, specially before product filling or if the product is exposed to temperature abuse (Ferreira et al., 2007a).

In food analysis, pH and titratable acidity are two interconnected concepts when dealing with acidity. While pH is important to determine if a microorganism is able to grow in a specific food, titratable acidity can indicate the impact organic acids will have on food flavor (Prieto, Capita, Llorente-marigo, & Alonso-calleja, 2006). Overall, *alheiras* made with traditional ingredients, such as meat and fat, demonstrated higher titratable acidity values, from 0.25 to 1.16 (Table 1).

3.2 Nitrite and nitrate concentrations

In all analyzed samples (Table 2), nitrite and nitrate concentrations were lower than the legal standards, i.e. 150 mg/kg (European Parliament and Council, 2011). Even though in fermented meat products, nitrites and/or nitrates are well-known for their antimicrobial effects against pathogenic bacteria, such as *Clostridium*, *L. monocytogenes* and *Salmonella* spp. (Cammack et al., 1999; Christieans, Picgirard, Parafita, Lebert, & Gregori., 2018; Hammes, 2012; Hospital, Hierro, & Fernández., 2014, 2012). As far as we know (since this information is not specified on all labels), unlike many similar products, *alheiras* are not traditionally prepared using nitrites or

nitrates and for that reason it was expected to find very low concentrations of these compounds.

3.3 Biogenic amines determination

Considerable variability was observed in the content of biogenic amines in *alheiras* from different producers (Table 2). Histamine (0.0 – 18.9 mg/kg), tyramine (0.0 – 32.1 mg/kg), methylamine (0.0 – 59.0 mg/kg) and ethylamine (0.0 – 14.3 mg/kg) were the biogenic amines more often detected in the samples analyzed. Histamine and tyramine were detected in 21 samples, while methylamine and ethylamine were found in 15 and 14 analyzed products, respectively. Additionally, the highest concentrations of putrescine were detected in samples A2 (39.7 mg/kg; vegetarian) and K20 (27.2 mg/kg; traditional) and cadaverine in samples A2 (29.7 mg/kg) and C7 (40.4 mg/kg; traditional). No correlation between type of *alheira* and/or producer was found. Biogenic amines contents found in this study were similar to those previously reported for fermented sausages ([Ekici & Omer, 2018](#); [Gong, Qi, Wang, Lin, & Li, 2014](#); [Hernández-Jover, Izquierdo-Pulido, Veciana-Nogués, Mariné-Font, & Vidal-Carou, 1997](#); [Vidal-Carou, Veciana-Nogués, Latorre-Moratalla, & Bover-Cid, 2014](#); [Singh, Pathak, & Verma, 2012](#)). Histamine, tyramine, putrescine and cadaverine are the most commonly found biogenic amines in dairy products, fish and fish products, meat and meat products, fermented vegetables and soy products being derived by decarboxylation from amino acids ([Alvarez & Moreno-Arribas, 2014](#); [Landete, De Las Rivas, Marcobal, & Muñoz, 2011](#)).

3.4 Organic acids

In Table 3 is presented the quantification of organic acids for all samples analyzed. As expected, lactic acid was found in all samples analyzed (0.50 – 7.02 mg/g) which corroborates the fact that LAB are the predominant bacteria found in *alheiras* microbiota (Franciosa, Alessandria, Dolci, Rantsiou, & Cocolin, 2018; Macori & Cotter, 2018). One vegan and two tofu *alheira*-like products showed low lactic acid values 0.50, 0.94 and 0.96 mg/g respectively, which corroborate the low values of LAB counts found.

Malic acid, for example, largely found in fruits and vegetables like apple and carrots, was mainly found in “innovative” *alheiras*: sample A2 (vegetarian) with 2.50 mg/g, followed by sample E11 (Apple) with 0.62 mg/g. Succinic acid, another by-product, seems to be predominant in soy, tofu, vegetables and mushrooms “innovative” *alheiras*, except for sample B6 which was the only *alheira* that presents similar values to the same producer of “innovative” *alheira*, 0.58 and 0.62 mg/g, respectively. Acetic (producer C) and citric acid (producer A and C) are often added as food preservatives, however no relation was found since higher values were obtained in samples with no preservatives declared. Butyric acid found in sample B6 (18.11 mg/g) may indicate that the fat present in this *alheira* could be rancid, however no sensorial analysis was performed to support this (Mortera, Zuljan, Magni, Bortolato, & Alarcón, 2018).

Organic acids quantification is important to monitor bacterial growth and activity and their presence has been analyzed in several fermented foods and dairy products since acidic conditions minimize pathogen growth and spoilage development (Bandić et al., 2015; Califano & Bevilacqua, 2000; González de Llano, Rodríguez, & Cuesta, 2008; Mullin & Emmons, 1997; Sirén, Sirén, & Sirén, 2015; Tormo & Izco, 2004).

Furthermore, they also contribute to the flavor and aroma of fermented products.

3.5 Microbiological analyses

Regarding the microbiological characterization, in general, variability was observed between producers and even between lots from the same producer (Table 4), but no significant differences were observed ($p > 0.05$). Ingredients quality and specific conditions during processing of meat fermented sausages, determines the microbiota developed during fermentation. Some differences found might be related not only with small variations during production, different raw materials used, producer's different geographic locations but also due to the "age" of the sample, since all analyses were performed before the due date, but some may have been packed longer than others. Significant differences ($p < 0.05$) were observed between traditional and "innovative" *alheiras* and it is possible to observe that any of the biological hazards investigated were found in "innovative" *alheiras* (table 4). A more detailed analysis revealed that total aerobic microorganisms at 30 °C in traditional *alheiras* showed higher values, mainly above 6.1 log cfu/g, when compared to "innovative", except for producer C which showed similar values possibly due to the use of natural casing in both types of *alheiras* (Djordjevic, Pecanac, Todorovic, & Dokmanovic, 2015). Curiously, tofu and vegan *alheiras*-like products showed no microbiological contamination and this may be due to not being traditionally fermented or because a sterilization step might be applied during production/packaging (table 1).

Numerous authors have described the importance of the natural microbiota throughout the fermentation process, namely LAB, responsible for inhibition of unwanted microbial growth and development of particular color and flavor (Ferreira et al., 2009). Since traditional *alheiras* are fermented products, it is quite common to find high numbers of LAB and these types of bacteria have been shown to play an important role in the production of numerous fermented sausages (Correia Santos, Fraqueza, Elias,

Salvador Barreto, & Semedo-Lemsaddek, 2017; Moretti et al., 2004; Papamanoliet, Kotzekidou, Tzanetakis, & Litopoulou-Tzanetaki, 2002; Papamanoliet, Tzanetakis, Litopoulou-Tzanetaki, & Kotzekidou, 2003). For this reason, and for most of the *alheiras* analyzed, except tofu and vegan *alheiras*-like products as also described, LAB was the dominant microbiota, in some cases higher than 6.0 log cfu/g. These LAB, in particular *Lactobacillus* and *Enterococcus* can be found in relatively high numbers (Albano et al 2009; Albano, Henriques, Correia, Hogg, & Teixeira, 2008; Barbosa, Ferreira & Teixeira, 2009; Correia Santos, Fraqueza, Elias, Barreto & Semedo-Lemsaddek, 2017; Hugas, Garriga, & Aymerich, 2003; Rantsiou & Cocolin, 2006). Enterococci, found in most of the products from this study in numbers higher than 4.0 log cfu/g, are commonly found in fermented sausages, as they contribute to their aromatization through glycolytic, proteolytic and lipolytic actions (Sarantinopoulos et al., 2001). Nevertheless presence of enterococci could be a concern as many strains possess virulence factors (Barbosa, Gibbs, & Teixeira, 2010; Franz, Stiles, Schleifer, & Holzapfel, 2003).

In addition to LAB, yeasts and molds play an important role in the development of organoleptic characteristics in fermented sausages. In this study, they were commonly found in most of the samples of both types of *alheiras*. *Alheiras* made with apple (E11), vegetables and mushrooms (B5, D9) presented more yeasts than those mainly constituted by meat, varying from 2.8 to 5.6 log cfu/g. An exception was found in traditional sample J18, which presented yeasts values higher than the similar samples, possibly due to the fact that sampling has been performed closer to the expiration date of the product.

Enterobacteriaceae were found in less than half of the samples (37%), presenting values higher than 3.1 log cfu/g, though they were mainly found in traditional *alheiras*

in which meat is the main ingredient. An exception was found in producer A - codfish *alheira*-like product - and one possible explanation is the fact that it contains 1% of cow natural casing (Djordjevic et al., 2015).

Escherichia coli is usually transmitted to humans through food, environmental contact or directly from person to person and some outbreaks have been associated with fermented sausage consumption, but also with vegetables and fruits (Sartz et al., 2008). In this study, *E. coli* was found in six products from five different producers, all of them in traditional *alheiras* samples, presenting values higher than 0.8 log cfu/g. This may be indicative of poor sanitary conditions during manipulation/production or even cross contamination.

Listeria monocytogenes was present in products from three different producers, in levels higher than 2.3 log cfu/g. This was previously described in other studies and may indicate poor facility conditions (Felício, Hogg, Gibbs, Teixeira, & Wiedmann, 2007; Ferreira et al., 2011; Ferreira et al., 2009; Ferreira et al., 2007a, 2007b; Ferreira et al., 2006; Ramalheira, Silva, Hogg, & Teixeira, 2007; Talon & Leroy, 2011; Xavier, Gonzales-Barron, Paula, Estevinho, & Cadavez, 2014). Despite being present in traditional *alheiras*, contamination by this pathogen was not found in “innovative” products which may indicate that contaminations, including cross-contamination, did not occur during common processes. Also, these innovative products are produced in lower numbers which might indicate that better control exists during innovative *alheiras* manufacturing, when compared to traditional *alheiras* production.

Staphylococcus aureus, *Salmonella* spp. and sulphite reducing *Clostridium* spores were found neither in the traditional nor in the “innovative” samples.

Alheiras, traditional or “innovative”, are generally cooked before consumption, except for vegan *alheira* which is supposed to be ready to eat, either baked in the oven, fried or

grilled. However previous studies suggested that internal temperatures may often not be sufficient to kill all of the pathogens initially present (Felício et al., 2011). Therefore, and in the absence of official microbiological criteria, guidelines for the interpretation of results of microbiological testing of ready-to-eat (RTE) foods placed on the market were followed (Food Safety Authority of Ireland, 2019; revision 3). According to these criteria, all vegetarian *alheira* samples but also vegan and tofu ones presented satisfactory results related to hygiene indicators like *Enterobacteriaceae* and *E. coli* (*Enterobacteriaceae* were found below 10^2 cfu/g and no *E. coli* was found). However, some borderline results were found for *Enterobacteriaceae* in traditional samples like A4, B5 and E21 (results between 10^2 and 10^4 cfu/g) and in samples C7 and E21 for *E. coli* ($\geq 20 - 10^2$ cfu/g). Traditional samples like A1, A3 and J18 were considered unsatisfactory for both *Enterobacteriaceae* and *E. coli* presenting results higher than 10^4 cfu/g and 10^2 cfu/g, respectively. *Alheiras* B6, C7, J18 and K20 were also considered unsatisfactory, but only for *Enterobacteriaceae*. Looking at these values, unsatisfactory results represent unacceptable levels of microbial contamination and this should be investigated in order to detect the cause of the elevated levels. Measures as part of Hazard Analysis and Critical Control Point (HACCP) based procedures and Good Manufacturing Practices (GMP) should be taken into consideration in order to ensure levels in subsequent batches of food are satisfactory. For borderline results, the action carried out should be proportional to the levels detected.

Regarding the presence of pathogens like coagulase-positive staphylococci and *Salmonella* spp., values under 20 cfu/g and absence in 25g, respectively, are requested in order to consider a product as satisfactory. None of the analyzed samples presented *Salmonella* nor coagulase-positive staphylococci. Concerning *L. monocytogenes*, the allowed limit is either '100 cfu/g' or 'absence in 25g' depending on the category (Food

Safety Authority of Ireland, 2019; revision 3). Samples A3, C7 and J18 were considered unsatisfactory, due to the presence of values above the legal limit (100 cfu/g). *Listeria monocytogenes* can be found on a wide range of foods and can persist in food production facilities and contaminate food after processing. Consuming food containing *L. monocytogenes* at levels > 100 cfu/g is considered a major food safety risk. It is important to emphasize that *alheiras* are generally cooked before consumption and, if properly cooked, these products may in fact no longer be considered unsatisfactory. According to the study of Felício et al. (2011), in which internal temperature profiles of *alheiras* were monitored during frying, electric grilling and roasting (in both gas and wood-fired oven), the authors reported that, with exception of roasting, the remaining evaluated cooking methods might not be sufficient to inactivate the foodborne pathogen *L. monocytogenes*. The authors highlighted the fact that some of the internal temperature profiles of *alheiras* were quite low during frying or grilling, which suggest insufficient thawing of frozen *alheiras* (Felício et al., 2011). Freezing *alheiras* is a common practice so, in the absence of cooking treatments guidelines, the absence of pathogens in these products becomes important. Comparatively, the differences between “innovative” and traditional *alheiras* indicate that even being manufactured by the same producer, samples analyzed do not present the same biological hazards.

4. CONCLUSIONS

In this study, even though pH and water activity levels are insufficient to assure microbiological safety, nitrites, nitrates, biogenic amines and organic acids were found to be within accepted limits for this kind of product. No biological threat was detected in “innovative” *alheiras*, but some of the traditional products analyzed have shown

concerning microbiological status in terms of food safety. Even though no *S. aureus*, *Salmonella* spp. nor sulphite reducing *Clostridium* spores were detected, *E. coli* and *L. monocytogenes* were found in traditional *alheiras* available in the market.

In conclusion, unlike traditional *alheiras* which often contain pathogenic agents, no harmful organisms nor chemical hazards were found in these new products, even though produced by the same companies. An important aspect to take into consideration in future confirmation studies, is to understand why traditional are more contaminated than “innovative” *alheiras*. Although being produced in the same facilities and eventually under the same conditions, it is undisputed that some ingredients used in their manufacture are different and meats used in traditional *alheiras* might be more contaminated. However, the production of traditional *alheiras* in higher amounts could be one of the answers to diverse types of contamination found and, consequently, to different associated hazards.

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Journal Pre-proof

Food safety of innovative vs. traditional fermented sausages

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None of the authors have conflict of interest to declare.

Journal Pre-proof

Non meat-based alheiras– a safer novel trend?

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Credit Author Statement:

Inês Azevedo: investigation, validation, writing – original draft

Joana Barbosa: conceptualization, validation, formal analysis, writing – review and editing, supervision

Helena Albano: conceptualization, validation, writing – review and editing, supervision

Paula Teixeira: conceptualization, validation, writing – review and editing, supervision

Highlights

- Microbiological and chemical characterization of “innovative” *alheiras* was performed
- No pathogenic bacteria were found
- Nitrites, nitrates and biogenic amines were within acceptable levels
- pH and a_w were not always sufficient to assure microbiological safety

Journal Pre-proof

Table 1 – Information and main characteristics of analyzed *alheiras*

Producer / Sample	Denomination	Information and Main Characteristics								
		Main Ingredient	Casing type	Food Preservatives	Packaging type	Consumption Indications	a_w	pH	TA ^a	
A	1	Traditional White Label	Chicken meat; Bread; Bisaro pork meat; Water; Olive oil; Spices	Natural cow gut	E281; E300; E321	Protective atmosphere	No indication	0.981 ± 0.001	6.0 ± 0.02	0.70 ± 0.04
	2	Vegetarian	Bread; Green and black olives; Olive oil; Carrot; Green and red pepper; Spices	100% synthetic	E281; E330	Protective atmosphere	Do not bake in the oven / 75°C Max.	0.991 ± 0.001	4.2 ± 0.01	0.12 ± 0.01
	3	Traditional	Chicken meat; Bread; Bisaro pork meat; Water; Olive oil; Spices	Natural cow gut	E281; E321; E330	Protective atmosphere	Cook until 75°C	0.988 ± 0.001	5.1 ± 0.02	0.34 ± 0.03
	4	Codfish	Bread; Codfish; Green and black olives; Olive oil; Spices	Natural cow gut	E281; E321; E330	Protective atmosphere	Cook until 75°C	0.991 ± 0.002	4.7 ± 0.04	0.17 ± 0.01
B	5	Traditional with Leek & Mushrooms	Chicken meat; Mushrooms; Bread; Pork loin; Leek; Water; Olive oil; Oregano; Sweet chili	Natural casing	No indication	Protective atmosphere	Cook over 65°C	0.986 ± 0.000	5.3 ± 0.00	0.61 ± 0.03
	6	Traditional	Poultry meat (Chicken, turkey, duck); Bread; Spices	No indication	No indication	Protective atmosphere	Grilled until complete cooking	0.982 ± 0.001	5.7 ± 0.05	0.25 ± 0.01
C	7	Traditional	Pork meat; Wheat bread; Chicken meat; Pork fat; Water; Olive oil; Spices	No indication	E200; E215; E260; E270; E330; E334	Protective atmosphere	Consume after cooking	0.983 ± 0.002	5.5 ± 0.01	0.67 ± 0.02
	8	Soy	Soy; Wheat bread; Olive oil; Spices; Starter cultures	Natural casing	E260; E270; E330; E334; E407; E508; E516	Protective atmosphere	Consume after cooking	0.982 ± 0.003	4.9 ± 0.03	0.33 ± 0.02
D	9	Vegetables & mushrooms	Shiitake mushrooms; Water; Wheat bread; Vegetables; Olive oil; Spices	Inedible cellulose gut	No indication	No indication	Consume after cooking	0.987 ± 0.001	5.5 ± 0.01	0.34 ± 0.01
E	21	Traditional	Bread; Pork meat and fat; Poultry meat; Spices	Natural pork gut	E250; E252; E262; E301; E331; E450; E451; E452;	Protective atmosphere	Consume after cooking	0.972 ± 0.001	5.6 ± 0.04	0.41 ± 0.01
	10	Traditional with Shiitake mushrooms	Bread; Pork meat and fat; Poultry meat; Mushrooms; Spices	Natural pork gut	E262; E331; E452	Protective atmosphere	Consume after cooking	0.976 ± 0.001	4.6 ± 0.02	1.00 ± 0.02
	11	Traditional with Apple	Bread; Pork meat and fat; Apple; Poultry meat; Spices	Natural pork gut	E250; E252; E262; E301; E331; E450; E451; E452;	Protective atmosphere	Consume after cooking	0.970 ± 0.001	4.6 ± 0.05	1.03 ± 0.03
	12	Traditional with Cheese	Bread; Pork meat and fat; Cheese; Poultry meat; Spices	Natural pork gut	E250; E252; E262; E301; E331; E450; E451; E452	Protective atmosphere	Consume after cooking	0.977 ± 0.001	4.8 ± 0.05	1.16 ± 0.01
F	13	Vegan	Water; Bread; Sunflower oil; Olive oil; Spices; Vinegar	Inedible synthetic gut	No indication	Sterilization heat treatment	Ready to eat	0.979 ± 0.001	6.0 ± 0.08	0.13 ± 0.01
G	22	Traditional	Bisaro pork meat; Chicken broth; Wheat bread; Olive oil; Spices	Cow gut	No indication	No indication	Heat treatment before consumption	0.981 ± 0.001	4.9 ± 0.01	0.46 ± 0.01
	14	Wild boar with thyme & marjoram	Wild boar; Pork fat; Water; Wheat bread; Olive oil; Spices natural extracts	Cow gut	E262; E331	No indication	Heat treatment before consumption	0.980 ± 0.001	4.3 ± 0.02	n.d.
H	15	Tofu	Tofu; Whole grain bread; Seitan; Soy sauce; Parsley, Coriander, Cumin, Fennel, Ginger	Inedible cellulose film	No indication	No indication	Grilled, fried or in oven	0.973 ± 0.000	5.2 ± 0.03	0.35 ± 0.00
I	16	Tofu	Tofu; Whole grain bread; Seitan; Soy sauce; Parsley, Coriander, Cumin, Fennel, Ginger	Inedible cellulose film	No indication	No indication	Grilled, fried or in oven	0.983 ± 0.000	5.1 ± 0.03	0.35 ± 0.00
J	17	Vegetarian	Mushrooms; Wheat bread; Olive oil; Spices	Natural pork gut	No indication	No indication	Grilled, fried or in oven	0.973 ± 0.002	4.2 ± 0.01	0.25 ± 0.01
	18	Traditional	Rooster meat; Wheat bread; Pork meat; Spices	Natural pork gut	No indication	No indication	Grilled, fried or in oven	0.965 ± 0.002	5.1 ± 0.02	0.80 ± 0.03
K	19	Vegetarian	Mushrooms; Wheat bread; Green asparagus; Olive oil; Spices	Edible gut	No indication	Vacuum packed	Cook until 72°C	0.961 ± 0.001	4.0 ± 0.01	0.18 ± 0.01
	20	Traditional	Chicken meat; Wheat bread; Bisaro pork meat; Olive oil; Spices	Edible gut	No indication	Vacuum packed	Cook until 72°C	0.959 ± 0.001	4.2 ± 0.04	0.32 ± 0.01

^a – Titratable Acidity (TA) (lactic acid, %); a_w , pH and TA are reported as means ± standard deviations; n.d. – non determined.

Table 2 – Nitrite (mg NaNO₂/kg), nitrate (mg NaNO₃/kg) and biogenic amine (mg/kg) content found in analyzed *alheiras*

Producer / Sample	Denomination	Nitrite	Nitrate	Histamine	Methylamine	Ethylamine	Tyramine	Phenylethylamine	Isoamylamine	Putrescine	Cadaverine	
A	1	Traditional White Label	6.4	12.7	0.7	59.0	1.2	2.1	1.8	3.1	0.0	0.0
	2	Vegetarian	0.6	37.6	16.7	0.4	2.2	32.1	3.3	0.0	39.7	29.7
	3	Traditional	2.2	4.5	0.5	0.0	1.2	1.0	0.0	0.0	0.0	0.0
	4	Codfish	1.7	13.6	2.2	0.0	2.4	0.9	0.0	0.0	0.0	0.0
B	5	Traditional with Leek & Mushrooms	2.3	58.1	9.8	0.8	10.0	1.5	0.0	3.1	0.5	0.0
	6	Traditional	11.9	26.0	7.3	1.0	14.3	1.8	0.0	0.0	0.0	0.0
C	7	Traditional	3.5	18.3	18.9	1.0	3.5	7.4	0.0	0.0	5.2	40.4
	8	Soy	2.5	40.9	18.8	1.1	6.0	2.1	0.0	0.0	3.0	0.0
D	9	Vegetables & mushrooms	6.3	31.0	1.3	0.5	0.0	0.0	0.4	0.7	1.0	0.0
E	21	Traditional	1.0	21.0	8.7	0.4	2.7	1.6	2.4	0.0	0.0	0.0
	10	Traditional with Shiitake mushrooms	0.5	3.1	1.5	0.0	0.0	20.7	0.0	0.0	0.0	0.0
	11	Traditional with Apple	1.1	2.9	4.2	42.8	1.5	10.7	1.8	0.0	0.0	0.0
	12	Traditional with Cheese	0.9	34.7	2.7	0.0	0.0	6.2	0.3	0.4	0.0	0.0
F	13	Vegan	0.0	16.2	1.4	10.2	1.1	0.9	0.6	1.1	0.5	0.0
G	22	Traditional	0.5	9.2	0.3	58.2	1.1	1.0	0.0	0.0	0.0	0.0
	14	Wild boar with thyme & marjoram	0.0	13.0	0.0	7.9	1.1	18.7	1.7	1.6	2.2	0.0
H	15	Tofu	0.0	87.6	1.4	0.0	0.0	0.7	0.6	0.0	0.0	0.0
I	16	Tofu	0.1	87.0	4.6	0.0	0.0	0.9	0.0	0.0	0.0	0.0
J	17	Vegetarian	1.0	53.0	2.3	0.3	0.0	1.0	0.0	0.0	0.0	0.0
	18	Traditional	4.8	73.0	5.8	1.4	0.0	1.8	1.5	0.0	1.5	3.5
K	19	Vegetarian	1.7	27.0	4.4	0.0	1.3	1.9	0.0	0.0	0.0	0.0
	20	Traditional	1.7	1.3	12.4	0.5	0.0	4.8	0.0	0.0	27.2	0.0

Table 3 – Quantification of organic acids (mg/g) in analyzed *alheiras* (mg/g)

Producer / Sample	Denomination	Organic Acids (mg/g)							
		Acetic	Citric	Lactic	Malic	Succinic	Butyric	Isobutyric	Isovaleric
A	1 Traditional White Label	0.74	< Q.L	4.69	N.D.	N.D.	N.D.	0.70	N.D.
	2 Vegetarian	N.D.	0.16	1.20	2.50	< Q.L	N.D.	0.31	N.D.
	3 Traditional	< Q.L	< Q.L	5.13	N.D.	< Q.L	N.D.	0.81	N.D.
	4 Codfish	N.D.	0.12	1.04	< Q.L	< Q.L	N.D.	0.18	N.D.
B	5 Traditional with Leek & Mushrooms	N.D.	< Q.L	3.47	N.D.	0.62	N.D.	1.56	N.D.
	6 Traditional	N.D.	< Q.L	1.33	< Q.L	0.58	18.11	N.D.	0.36
C	7 Traditional	< Q.L	N.D.	5.28	N.D.	N.D.	N.D.	0.94	N.D.
	8 Soy	N.D.	1.24	1.60	0.13	0.14	N.D.	< Q.L	N.D.
D	9 Vegetables & mushrooms	N.D.	0.24	3.18	N.D.	1.68	N.D.	0.41	N.D.
	10 Traditional with Shiitake mushrooms	1.22	0.35	6.55	< Q.L	0.66	N.D.	1.63	N.D.
E	11 Traditional with Apple	0.55	< Q.L	4.74	0.62	N.D.	N.D.	1.24	N.D.
	12 Traditional with Cheese	0.52	< Q.L	6.69	0.12	N.D.	N.D.	1.61	N.D.
F	21 Traditional	N.D.	0.50	1.67	N.D.	< Q.L	N.D.	0.23	N.D.
	13 Vegan	N.D.	0.20	0.50	< Q.L	< Q.L	N.D.	< Q.L	N.D.
G	14 Wild boar with thyme & marjoram	---	---	---	---	---	---	---	---
	22 Traditional	1.84	0.10	4.10	N.D.	N.D.	N.D.	0.54	N.D.
H	15 Tofu	N.D.	0.28	0.96	< Q.L	1.04	N.D.	0.15	N.D.
I	16 Tofu	< Q.L	0.28	0.94	< Q.L	1.34	N.D.	0.20	N.D.
	17 Vegetarian	N.D.	N.D.	3.23	0.34	0.69	N.D.	0.32	N.D.
J	18 Traditional	1.01	N.D.	7.02	N.D.	N.D.	N.D.	0.73	0.35
	19 Vegetarian	N.D.	N.D.	1.70	< Q.L	< Q.L	N.D.	0.28	N.D.
K	20 Traditional	N.D.	< Q.L	2.53	N.D.	N.D.	N.D.	0.28	1.97

N.D. – Non-detected; < Q.L – Below Quantification Limit (0.05 g/L for acetic, lactic and succinic acids; 0.01 g/L for citric, malic, butyric, isobutyric and isovaleric acids).

Table 4 – Microbiological characterization of *alheiras* (log cfu/g): important microbial parameters in fermented products

Producer /Sample	Type	Lot	Moulds	Yeasts	<i>Enterococcus</i>	LAB	Total Counts*	<i>Enterobacteriaceae</i>	<i>E. coli</i>	<i>L. monocytogenes</i>	
A	1	Traditional white label	A	0.7 ± 0.0 ^A	1.5 ± 0.0 ^A	5.8 ± 0.1 ^{ADFGI}	8.5 ± 0.4 ^{ACN}	8.7 ± 0.0 ^{AC}	6.7 ± 0.2 ^{AGM}	3.3 ± 0.1 ^{AB}	<1 ± 0.0 ^A
			B	<1 ± 0.0 ^A	<1 ± 0.0 ^A	5.2 ± 0.0 ^{ADFGI}	6.1 ± 0.0 ^{ACN}	6.1 ± 0.0 ^{AC}	<1 ± 0.0 ^{AGM}	<1 ± 0.0 ^{AB}	<1 ± 0.0 ^A
	2	Vegetarian	A	<1 ± 0.0 ^A	1.3 ± 0.3 ^A	7.1 ± 0.1 ^{AC}	7.5 ± 0.3 ^{ADN}	7.7 ± 0.1 ^{AC}	<1 ± 0.0 ^{BD}	<1 ± 0.0 ^A	<1 ± 0.0 ^A
			B	<1 ± 0.0 ^A	<1 ± 0.0 ^A	5.2 ± 0.0 ^{AC}	5.8 ± 0.0 ^{ADN}	6.0 ± 0.0 ^{AC}	<1 ± 0.0 ^{BD}	<1 ± 0.0 ^A	<1 ± 0.0 ^A
	3	Traditional	A	<1 ± 0.0 ^A	<1 ± 0.0 ^A	7.5 ± 0.0 ^{ADE}	7.9 ± 0.0 ^{BCEGK}	8.2 ± 0.1 ^{AC}	7.2 ± 0.1 ^{CE}	0.9 ± 0.2 ^B	<1 ± 0.0 ^{AD}
			B	<1 ± 0.0 ^A	<1 ± 0.0 ^A	6.0 ± 0.0 ^{ADE}	8.7 ± 0.1 ^{BCEGK}	8.8 ± 0.0 ^{AC}	7.5 ± 0.0 ^{CE}	3.3 ± 0.0 ^B	2.3 ± 0.0 ^{AD}
	4	Codfish	A	1.0 ± 0.0 ^A	<1 ± 0.0 ^A	5.2 ± 0.2 ^{ADFGI}	5.8 ± 0.0 ^{AN}	6.0 ± 0.0 ^C	4.2 ± 0.0 ^{AF}	<1 ± 0.0 ^A	<1 ± 0.0 ^A
			B	<1 ± 0.0 ^A	<1 ± 0.0 ^A	4.3 ± 0.0 ^{ADFGI}	6.6 ± 0.0 ^{AN}	6.7 ± 0.0 ^C	4.2 ± 0.0 ^{AF}	<1 ± 0.0 ^A	<1 ± 0.0 ^A
5	Traditional with Leek & Mushrooms	A	2.4 ± 0.0 ^{BC}	5.6 ± 0.3 ^B	5.4 ± 0.2 ^{ADFGI}	7.3 ± 0.3 ^{AHN}	7.5 ± 0.0 ^C	3.9 ± 0.0 ^{AGHJL}	<1 ± 0.0 ^A	<1 ± 0.0 ^A	
		B	2.3 ± 0.0 ^{BC}	5.2 ± 0.0 ^B	4.5 ± 0.0 ^{ADFGI}	5.5 ± 0.0 ^{AHN}	5.8 ± 0.1 ^C	3.8 ± 0.1 ^{AGHJL}	<1 ± 0.0 ^A	<1 ± 0.0 ^A	
6	Traditional	A	3.3 ± 0.2 ^B	4.5 ± 0.4 ^{BC}	5.8 ± 0.2 ^{ADFGH}	6.0 ± 0.1 ^{AINR}	6.4 ± 0.1 ^{AC}	5.2 ± 0.1 ^{AEG}	<1 ± 0.0 ^{AB}	<1 ± 0.0 ^{AE}	
		B	2.5 ± 0.0 ^B	4.5 ± 0.0 ^{BC}	6.1 ± 0.1 ^{ADFGH}	7.2 ± 0.0 ^{AINR}	7.3 ± 0.0 ^{AC}	5.3 ± 0.0 ^{AEG}	0.8 ± 0.2 ^{AB}	<1 ± 0.0 ^{AE}	
7	Traditional	A	1.8 ± 0.2 ^{ACD}	0.7 ± 0.0 ^A	8.1 ± 0.1 ^{BCEHK}	8.6 ± 0.1 ^{BCFKQ}	8.8 ± 0.0 ^{AC}	6.3 ± 0.0 ^{CEF}	1.5 ± 0.0 ^{AB}	<1 ± 0.0 ^A	
		B	<1 ± 0.0 ^{ACD}	<1 ± 0.0 ^A	7.3 ± 0.0 ^{BCEHK}	8.7 ± 0.0 ^{BCFKQ}	8.7 ± 0.0 ^{AC}	7.1 ± 0.0 ^{CEF}	<1 ± 0.0 ^{AB}	2.5 ± 0.0 ^A	
8	Soy	A	0.8 ± 0.2 ^A	0.7 ± 0.0 ^A	8.2 ± 0.0 ^{BCEJM}	8.2 ± 0.0 ^{BCFKP}	8.6 ± 0.0 ^{AC}	<1 ± 0.0 ^{AC}	<1 ± 0.0 ^A	<1 ± 0.0 ^A	
		B	<1 ± 0.0 ^A	<1 ± 0.0 ^A	8.2 ± 0.1 ^{BCEJM}	8.4 ± 0.0 ^{BCFKP}	8.6 ± 0.0 ^{AC}	<1 ± 0.0 ^{BI}	<1 ± 0.0 ^A	<1 ± 0.0 ^A	
9	Vegetables & Mushrooms	A	1.3 ± 0.0 ^A	3.5 ± 0.1 ^{CDE}	<1 ± 0.0 ^L	7.4 ± 0.0 ^{AINR}	7.6 ± 0.0 ^{AC}	<1 ± 0.0 ^{BI}	<1 ± 0.0 ^A	<1 ± 0.0 ^A	
		B	<1 ± 0.0 ^A	3.0 ± 0.0 ^{CDE}	1.5 ± 0.1 ^L	5.9 ± 0.0 ^{AINR}	5.9 ± 0.0 ^{AC}	<1 ± 0.0 ^{BI}	<1 ± 0.0 ^A	<1 ± 0.0 ^A	
10	Traditional with Shiitake Mushrooms	A	2.4 ± 0.0 ^{BDE}	<1 ± 0.0 ^A	5.6 ± 0.0 ^{ADFGIK}	7.4 ± 0.0 ^{AEIJPQ}	7.5 ± 0.0 ^{AC}	<1 ± 0.0 ^{BI}	<1 ± 0.0 ^A	<1 ± 0.0 ^A	
		B	2.0 ± 0.1 ^{BDE}	<1 ± 0.0 ^A	6.1 ± 0.0 ^{ADFGIK}	8.2 ± 0.0 ^{AEIJPQ}	8.4 ± 0.1 ^{AC}	<1 ± 0.0 ^{BI}	<1 ± 0.0 ^A	<1 ± 0.0 ^A	
11	Traditional with Apple	A	0.7 ± 0.0 ^A	3.1 ± 0.1 ^{CFG}	5.8 ± 0.0 ^{ADFGI}	8.1 ± 0.0 ^{BCDFHJR}	8.1 ± 0.0 ^{AC}	<1 ± 0.0 ^{BN}	<1 ± 0.0 ^A	<1 ± 0.0 ^A	
		B	<1 ± 0.0 ^A	2.8 ± 0.0 ^{CFG}	5.1 ± 0.0 ^{ADFGI}	7.9 ± 0.0 ^{BCDFHJR}	7.9 ± 0.0 ^{AC}	<1 ± 0.0 ^{BN}	<1 ± 0.0 ^A	<1 ± 0.0 ^A	
12	Traditional with Cheese	A	1.2 ± 0.0 ^A	1.0 ± 0.0 ^A	4.0 ± 0.0 ^{BL}	8.8 ± 0.0 ^{BCFJ}	8.8 ± 0.0 ^{AC}	<1 ± 0.0 ^{BN}	<1 ± 0.0 ^A	<1 ± 0.0 ^A	
		B	<1 ± 0.0 ^A	<1 ± 0.0 ^A	3.6 ± 0.0 ^{BL}	8.8 ± 0.0 ^{BCFJ}	8.8 ± 0.0 ^{AC}	<1 ± 0.0 ^{BN}	<1 ± 0.0 ^A	<1 ± 0.0 ^A	
21	Traditional	A	0.7 ± 0.0 ^A	3.3 ± 0.0 ^{ADG}	4.7 ± 0.0 ^{ADFGI}	7.7 ± 0.0 ^{BCJL}	7.8 ± 0.0 ^{AC}	<1 ± 0.0 ^{DIJKMN}	<1 ± 0.0 ^{AB}	<1 ± 0.0 ^A	
		B	<1 ± 0.0 ^A	<1 ± 0.0 ^{ADG}	6.7 ± 0.0 ^{ADFGI}	9.2 ± 0.0 ^{BCJL}	9.4 ± 0.0 ^{AC}	3.1 ± 0.0 ^{DIJKMN}	1.4 ± 0.3 ^{AB}	<1 ± 0.0 ^A	
13	Vegan	A	<1 ± 0.0 ^A	<1 ± 0.0 ^A	<1 ± 0.0 ^{LN}	<1 ± 0.0 ^O	<1 ± 0.0 ^B	<1 ± 0.0 ^{BN}	<1 ± 0.0 ^A	<1 ± 0.0 ^A	
		B	<1 ± 0.0 ^A	<1 ± 0.0 ^A	<1 ± 0.0 ^{LN}	<1 ± 0.0 ^O	<1 ± 0.0 ^B	<1 ± 0.0 ^{BN}	<1 ± 0.0 ^A	<1 ± 0.0 ^A	
14	Wild boar with thyme & marjoram	A	<1 ± 0.0 ^A	<1 ± 0.0 ^A	<1 ± 0.0 ^{LN}	7.7 ± 0.0 ^{AGJNPQ}	7.7 ± 0.0 ^{AC}	<1 ± 0.0 ^{BN}	<1 ± 0.0 ^{AB}	<1 ± 0.0 ^{AC}	
		B	N.D.	N.D.	N.D.	N.D.	N.D.	N.D. ^{BN}	N.D.	N.D.	
22	Traditional	A	<1 ± 0.0 ^A	<1 ± 0.0 ^A	3.2 ± 0.0 ^I	7.1 ± 0.0 ^{BCDHI}	7.7 ± 0.0 ^{AC}	<1 ± 0.0 ^{BN}	<1 ± 0.0 ^A	<1 ± 0.0 ^A	
		B	<1 ± 0.0 ^A	1.6 ± 0.0 ^A	3.7 ± 0.0 ^I	7.6 ± 0.0 ^{BCDHI}	7.9 ± 0.0 ^{AC}	<1 ± 0.0 ^{BN}	<1 ± 0.0 ^A	<1 ± 0.0 ^A	
15	Tofu	A	<1 ± 0.0 ^A	<1 ± 0.0 ^A	<1 ± 0.0 ^{LN}	<1 ± 0.0 ^O	<1 ± 0.0 ^B	<1 ± 0.0 ^{BN}	<1 ± 0.0 ^A	<1 ± 0.0 ^A	
		B	<1 ± 0.0 ^A	<1 ± 0.0 ^A	<1 ± 0.0 ^{LN}	<1 ± 0.0 ^O	<1 ± 0.0 ^B	<1 ± 0.0 ^{BN}	<1 ± 0.0 ^A	<1 ± 0.0 ^A	
16	Tofu	A	<1 ± 0.0 ^A	<1 ± 0.0 ^A	<1 ± 0.0 ^{LN}	<1 ± 0.0 ^O	<1 ± 0.0 ^B	<1 ± 0.0 ^{BN}	<1 ± 0.0 ^A	<1 ± 0.0 ^A	
		B	<1 ± 0.0 ^A	<1 ± 0.0 ^A	<1 ± 0.0 ^{LN}	<1 ± 0.0 ^O	<1 ± 0.0 ^B	<1 ± 0.0 ^{BN}	<1 ± 0.0 ^A	<1 ± 0.0 ^A	
17	Vegetarian	A	<1 ± 0.0 ^A	<1 ± 0.0 ^A	2.8 ± 0.0 ^{BLN}	7.5 ± 0.0 ^{AGIKLN}	7.6 ± 0.0 ^{AC}	<1 ± 0.0 ^{BN}	<1 ± 0.0 ^A	<1 ± 0.0 ^A	
		B	<1 ± 0.0 ^A	<1 ± 0.0 ^A	2.1 ± 0.1 ^{BLN}	6.8 ± 0.0 ^{AGIKLN}	6.9 ± 0.0 ^{AC}	<1 ± 0.0 ^{BN}	<1 ± 0.0 ^A	<1 ± 0.0 ^A	
18	Traditional	A	2.4 ± 0.1 ^{ACE}	2.9 ± 0.0 ^{BEF}	5.3 ± 0.1 ^{ADFGIKM}	8.8 ± 0.0 ^{BFMQ}	8.8 ± 0.0 ^A	6.4 ± 0.0 ^{AEGH}	<1 ± 0.0 ^{AB}	3.8 ± 0.0 ^{BCDE}	
		B	<1 ± 0.0 ^{ACE}	5.3 ± 0.0 ^{BEF}	7.4 ± 0.0 ^{ADFGIKM}	9.9 ± 0.0 ^{BFMQ}	9.9 ± 0.0 ^A	4.4 ± 0.0 ^{AEGH}	3.4 ± 0.0 ^{AB}	<1 ± 0.0 ^{BCDE}	
19	Vegetarian	A	<1 ± 0.0 ^A	1.6 ± 0.6 ^A	5.2 ± 0.0 ^F	5.4 ± 0.0 ^N	5.7 ± 0.0 ^C	<1 ± 0.0 ^{BK}	<1 ± 0.0 ^A	<1 ± 0.0 ^A	
		B	<1 ± 0.0 ^A	<1 ± 0.0 ^A	3.3 ± 0.0 ^F	6.8 ± 0.0 ^N	6.8 ± 0.0 ^C	<1 ± 0.0 ^{BK}	<1 ± 0.0 ^A	<1 ± 0.0 ^A	
20	Traditional	A	2.3 ± 0.0 ^{ACE}	2.0 ± 0.4 ^A	7.4 ± 0.1 ^{ADFGIKM}	7.8 ± 0.1 ^{AGIJKMQ}	8.0 ± 0.0 ^{AC}	6.5 ± 0.1 ^{AEGHL}	<1 ± 0.0 ^A	<1 ± 0.0 ^A	
		B	<1 ± 0.0 ^{ACE}	<1 ± 0.0 ^A	5.2 ± 0.1 ^{ADFGIKM}	7.7 ± 0.1 ^{AGIJKMQ}	7.7 ± 0.0 ^{AC}	5.2 ± 0.0 ^{AEGHL}	<1 ± 0.0 ^A	<1 ± 0.0 ^A	

N.D. – Non-determined; * - Total counts at 30 °C; Equivalent capital letters, by column, mean no significant differences between each *alheira* (p>0.05).