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EFEITO DA ADIÇÃO DE CULTURAS DE ARRANQUE NAS PROPRIEDADES FÍSICO-QUÍMICAS E SENSORIAIS DE "ALHEIRA DE VITELA"

EFFECT OF ADDITION OF A STARTER CULTURES ON PHYSICOCHEMICAL AND SENSORY PROPERTIES OF "ALHEIRA", A SMOKED SAUSAGE-LIKE PRODUCT

EFECTO DE LA ADICIÓN DE CULTIVOS INICIADORES SOBRE LAS PROPIEDADES FISICOQUÍMICAS Y SENSORIALES DE "ALHEIRA", UN EMBUTIDO AHUMADO

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RESUMO

Introdução: A alheira é um produto fumado tradicional português que pode ser produzido com diferentes tipos de carne sendo o pão um ingrediente fundamental. A bioconservação de alimentos pela adição de bactérias do ácido láctico (BAL) e das suas bacteriocinas tem ganho especial destaque como forma de controlar naturalmente o crescimento de microrganismos patogénicos e de degradação presentes nos alimentos.
Objectivos: Investigar o efeito de uma cultura de arranque de *Lactobacillus plantarum* ST153Ch fresca e liofilizada nas características físico-químicas e sensoriais de alheira de vitela.
Métodos: As amostras foram produzidas a nível industrial. As amostragens para todos os procedimentos analíticos foram efetuadas aos dias 0, 15, 30, 45, 60, 75, 90 de armazenamento. As características físico-químicas (humidade, pH, atividade de água (a_w), dureza, adesividade, cor (CIE L*, a*, b*) e a análise sensorial realizada à aparência visual, odor, textura e sabor (11 atributos), por um painel de 9 provadores, foram avaliadas ao longo do armazenamento em atmosfera modificada a 4 °C.
Resultados: Foram observadas diferenças significativas (p<0,05) entre todas as amostras no teor de humidade, pH, a_w, dureza, adesividade e cor ao longo do armazenamento.
Conclusões: O painel sensorial não detetou diferenças significativas nas amostras de alheira inoculadas em relação ao controlo até aos 75 dias de armazenamento o que possibilita a aplicação da cultura de *L. plantarum* ST153Ch pelo menos durante esse período.

Palavras-chave: Alheira; Enchidos fumados e curados; Bactérias ácido lácticas; Caraterísticas físico-químicas; Análise sensorial

ABSTRACT

Introduction: "Alheira" is a traditional Portuguese smoked sausage-like product that can be manufactured with different types of meat and bread. Biopreservation of foods has gained special prominence due to the possibility of naturally controlling the growth of pathogenic and spoilage microorganisms, with a growing interest in the use of lactic acid bacteria (LAB) and their bacteriocins.
Objectives: To ascertain the effect of adding fresh and dried starter LAB cultures (*Lactobacillus plantarum* ST153Ch) on the physicochemical and sensory characteristics of "Alheira".
Methods: All samples were produced at the industrial level. Sampling for analytical procedures was performed at 0, 15, 30, 45, 60, 75 and 90 days of storage at 4 °C. Physicochemical characteristics (moisture content, pH, water activity (a_w), instrumental hardness and adhesiveness, instrumental colour (L*, a*, b*)) and sensory evaluation, performed by 9 panellists regarding visual appearance, odour, texture and taste (11 attributes), were evaluated along the storage at 4 °C.
Results: Significant differences (p<0.05) were observed in all samples regarding moisture content, pH, a_w, instrumental hardness, adhesiveness and in colour parameters, during the storage period.
Conclusions: No significant differences in both inoculated "Alheira" samples compared to the control assay were found in sensory analysis until day 90, meaning that *L. plantarum* ST153Ch starter culture, fresh or dried, could be applied successfully until at least until day 75.

Keywords: "Alheira"; Cured smoked sausages; Lactic acid bacteria; Physicochemical characteristics; Sensory evaluation

RESUMEN

Introducción: "Alheira" es un embutido ahumado tradicional portugués que se puede producir con distintos tipos de carne siendo el pan un ingrediente clave. La biopreservación de los alimentos por la de la adicción de bacterias del acido láctico (BAL) y de sus bacteriocinas ha ganado especial destaque como forma de controlar naturalmente el crecimiento de microorganismos patógenos y de deterioro presentes en los alimentos.
Objetivos: Determinar el efecto de la adición de cultivos iniciadores de LAB (*Lactobacillus plantarum* ST153Ch) frescos y liofilizados en las características fisicoquímicas y sensoriales de "Alheira".
Métodos: Todas las muestras fueron producidas a nivel industrial. Los muestreos para todos los procedimientos analíticos fueron realizados en los días 0, 15, 30, 45, 60, 75, 90 de almacenamiento. Las características fisicoquímicas (humedad, pH, actividad del agua (a_w), dureza y adhesividad, color (CIE L*, a*, b*) y el análisis sensorial realizado a la apariencia visual, olor, textura y sabor (11 atributos), por un panel de 9 probadores, fueron evaluados a lo largo del almacenamiento en atmósfera modificada a 4 ° C.
Resultados: Se observaron diferencias significativas (p<0,05) entre todas las muestras en el contenido de humedad, pH, a_w, dureza, adhesividad y color a lo largo del almacenamiento.
Conclusiones: El panel sensorial no detectó diferencias significativas en la muestras de "Alheira" inoculada en relación al control hasta los 75 días de almacenamiento lo que posibilita la aplicación del cultivo *L. plantarum* ST153Ch al menos durante ese período.

Palabras Clave: "Alheira"; Embutidos ahumados/curados; Bacterias de ácido láctico; Características fisicoquímicas; Evaluación sensorial

INTRODUCTION

Consumer demand for foods without chemical additives is increasing forcing industry to meet consumers' interests, but food should remain safe and sensory changes should be minimized. In the last decade a great number of food products, named "natural", "free of additives", "biological", "probiotics", have been launched in the market (Zegler, 2017).
Application of bioprotective bacterial strains in foods has been shown to help reduce the amount of chemical preservatives as well as the intensity of the heat treatment, greatly improving the organoleptic and nutritional properties of food in addition to having control over many undesirable microorganisms, managing to increase the lifetime of food and increase the safety of the food itself (Vasquez, Suarez, & Zapata, 2009).
LAB is the dominant microbiota in refrigerated and vacuum-packed or AM-CO_2 meat products, and the use of these protective cultures has been studied as alternatives to the use of chemical additives to ensure food quality and safety (Holzapfel, Geisen, & Schillinger, 1995; Stiles, 1996; Mataragas, Drosinos, & Metaxopoulos, 2003; Vasquez et al., 2009). Some bacteriocins produced by LABs selectively inhibit certain high-risk bacteria in foods such as *L. monocytogenes*, without affecting the harmless microbiota. The efficacy of bacteriocins is often dictated by environmental factors, such as pH, temperature, food composition and structure, as well as the microbiota naturally present in the food (O'Sullivan, Ross, & Hill, 2002; Galvez, Abriouel, Lopez, & Ben Omar, 2007).
Today, a wide variety of sausages are produced, depending on their final and quality characteristics of a large number of variables related to raw materials, microbial population and processing conditions (Flores & Toldrá, 2011). The production of meat products is a long-standing tradition in the rural areas of Portugal, and these traditional meat products, with unique technological and sensorial characteristics, are very appellative for both, rural and urban consumers (Ferreira et al., 2007; Patarata, Judas, Silva, Esteves & Martins, 2008) and should be framed according to the current requirements of hygiene / health, from a perspective of consumer protection. This, not only values the organoleptic and nutritional properties of the products, but also increases the importance of food safety (Vieira da Silva, Teixeira, Hogg, & Couto, 2003).
The use of selected LAB strains as starter cultures strongly inhibited the bacterial growth of decomposition and left intact the organoleptic properties of fermented meat products, and thus LAB strains can be efficiently used to preserve meat products for quality purposes. Meat starter cultures are defined as "preparations that include live or inactive microorganisms that accumulate the desired metabolic activity in the meat" (Singh, Pathak & Verma, 2012). The starter cultures used in meat are strains of lactic acid bacteria (LAB), such as *Lactobacillus pentosus, L. casei, L. curvatus, L. plantarum, L. sakei, Pediococcus acidilactici, P. pentosaceus* (Singh et al., 2012).
Lactobacillus plantarum ST153Ch, a lactic acid bacteria (LAB), originally identified as *L. sakei* ST153Ch but later re-identified as *L. plantarum*, is an autochthonous strain isolated from a traditional Portuguese salami product - "Salpicão" (Todorov, Vaz Velho, Franco, & Holzapfel, 2013). The use of this strain combined with AM enriched in CO_2 reduced the number of cells of *Listeria* spp. in "Alheira" (Vaz-Velho et al., 2013), in sliced "Chouriço" (Jácome et al., 2014) and in smoked "Lombo" (Vaz Velho et al., 2015). Todorov et al., (2013), characterized the bacteriocin produced by *L. plantarum* (bacteriocin ST153Ch) as heat resistant, stable between pH 2.0 and 10.0 and showing higher levels during the stationary fermentation phase in the presence of 2% (w/v) D-Glucose. In addition, they have also shown that it is a safe strain that could contribute to the safety of fermented food products (Todorov, Franco & Wild, 2014).
Thus, this work aims to validate the use of a biocontrol process, using the autochthonous *L. plantarum* ST153ch, in the production, at a pilot scale, of "Alheira de Vitela", a traditional Portuguese smoked sausage.

1. MATERIALS AND METHODS

1.1 Sample preparation

"Alheira de Vitela" samples were manufactured in a meat plant according to traditional recipes and techniques. The ingredients used were as follows: Veal (35%), wheat bread (wheat flour, yeast and salt), pork, rooster meat, the cooking juice of meat, spices, onion, olive oil and salt. The sausage mixture, before stuffing, was divided into three batches: one batch was inoculated with *L. plantarum* ST153Ch fresh (2AlF), another batch with *L. plantarum* ST153Ch dried (2AlP) and the other one was non-inoculated (2AlC), to act as a control experiment. *L. plantarum* ST153Ch, fresh or dried, was added before stuffing, in order to reach ~10^6 CFU/mL in the final product. Dried culture was dissolved in 1.5 L of water and sucrose (6% w/v) and then added to the mixture. Fresh culture in 1.5 L of water was directly added to the mixture. Samples were prepared in triplicate. The samples of ''Alheira de Vitela" were cold-smoked by natural convection, in an artisanal smoker, by slow burning of *Quercus* spp. wood. After smoking and cooling, samples were packed under modified atmosphere (20 % CO_2 and 80 % N_2) and stored for 90 days at 4 °C. Sampling for analytical procedures was performed at 0, 15, 30, 45, 60, 75 and 90 days of storage.

1.2 Analytical procedures

Moisture content was determined by oven drying according to 1441 (ISO, 1997). Five g of each sample were heated at 103±2 °C until they reached constant weight (Vacucell MMM VU55, Germany). The results were expressed as % moisture content. Water activity (a_w) was measured in a portable a_w Pawkit meter (Decagon, EUA). The pH was measured directly using a pH meter (model CRISON pH 25+, Spain).

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Texture evaluation was carried out using a texture analyser TA-XTplus (Stable Micro Systems, UK). Textural parameters were measured using a Cylinder Probe P/10 (10 mm diameter) of surface contact. Sausage pieces of 1×1×2.5 cm (height × width × length) were compressed at a crosshead speed of 60 mm/min. Compression measurements were recorded with regard to hardness (expressed in N) and the adhesiveness (expressed in N/sec) force using the computer software Texture Exponent 32 (Stable Micro Systems, UK).
A portable colorimeter Minolta CR-300 (Konica Minolta, USA) was used to measure meat colour in the CIELAB space: lightness, L*; redness, a*; yellowness, b*.
A semi-trained 9-taster panel was used to evaluate sausage samples regarding to eleven sensory parameters: characteristic colour, brightness, meat binding, characteristic odour, strange odour, hardness, adhesiveness in the mouth, characteristic flavour, bitter taste, spicy flavour and acid taste. A scale of intensity of 1 to 13 (1-low intensity; 13-high intensity) was used and a score of 7 was previously established for the standard sample. The panellists were asked to classify the differences detected from this central point, except for the strange odour parameter, where the reference point of the sample was set as value 1. A general attribute of conformity with standard "Alheira", was set using a 5-point hedonic scale that allowed perceiving potential defects that were not expressed in the attributes. The score 3 was established as the limit of conformity, meaning that values lower than 3 correspond to detectable defective changes and values equal or higher than 3 correspond to a conforming product.

1.3 Statistical Analysis

All data were analysed statistically using an ANOVA procedure (IBM SPSS Statistics 25). The Tukey HSD test was used to investigate significant differences in physicochemical and sensory parameters (level of significance: $p<0.05$). A canonical variates analysis (CVA) was applied to sensory results to reduce the number of variables, selecting the few that best characterized the product in the sensory evaluation and to evaluate the separation of the groups analysed. Two variables were selected based on factor loadings modulus higher than 0.70: "characteristic colour" and "meat binding".

2. RESULTS AND DISCUSSION

The results of moisture, pH and water activity determinations throughout the 90 days of storage are summarized in Table 1. Significant differences in moisture values were found at 0, 15, 30 and 45 sampling days, with moisture being higher up to 30 days compared to the control sample. Inoculated samples presented lower pH values compared to the control, meaning that the inoculation of the starter cultures resulted in stronger acidification during the storage period.
In all samples the pH decrease during storage, pH being lower in the tested samples, as expected, due to LAB addition. The decrease of pH is related to an accumulation of organic acids, mainly lactic, because of carbohydrate breakdown during the fermentation (Casaburi et al., 2007; Essid & Hassouna, 2013; Lorenzo, Gómez, & Fonseca, 2014). This pH reduction, noticeable in all samples, is essential as it contributes to the inhibition of undesirable microorganisms, accelerates the red colour development of fermented sausages, affects taste and reduces the water binding capacity of proteins, ensuring the drying (Casaburi et al., 2007; Essid & Hassouna, 2013; Lorenzo et al., 2014).
Significant differences in water activity values were found at 15, 45, 60 and 90 days, the non-inoculated samples presenting lower aw values than both inoculated samples, but it must be pointed that moisture content of inoculated samples was also higher at day 0. Heterogeneity of samples is expected when dealing with a food matrix, such as "Alheira", where the diversity in its structural organization is noticeable at macroscopic level.

Table 1. Effect of inoculation starter cultures on moisture content, pH and water activity throughout the storage of "Alheira" at 4 °C.

		Storage (days)						
	Samples	0	15	30	45	60	75	90
Moisture content (%)	2AlC	56,68±0,10^{a}	56,65±0,38^{a}	56,67±0,20^{a}	56,06±0,17^{a}	56,84±0,42^{a}	56,21±0,35^{a}	56,64±0,1^{a}
	2AlF	57,47±0,58^{a,b}	57,75±0,48^{b}	57,71±0,36^{b}	57,49±0,26^{b}	55,75±1,99^{a}	57,09±0,42^{a}	57,33±0,1^{a}
	2AlP	57,84±0,30^{b}	58,02±0,34^{b}	58,53±0,21^{c}	57,83±0,28^{b}	57,92±0,21^{a}	57,64±0,99^{a}	57,46±0,6^{a}
pH	2AlC	5,92±0,07^{a}	4,90±0,04^{a}	4,52±0,01^{a}	4,34±0,04^{a}	4,62±0,04^{a}	4,44±0,11^{a}	4,79±0,17^{a}
	2AlF	5,69±0,02^{b}	4,88±0,04^{a}	4,46±0,01^{b}	4,25±0,02^{a}	4,43±0,07^{b}	4,53±0,06^{a}	4,55±0,02^{b}
	2AlP	5,84±0,02^{a}	4,85±0,01^{a}	4,53±0,01^{a}	4,27±0,04^{a}	4,40±0,02^{b}	4,38±0,06^{a}	4,47±0,02^{b}
Water activity	2AlC	0,87±0,04^{a}	0,96±0,01^{a}	0,96±0,01^{a}	0,95±0,01^{a}	0,94±0,03^{a}	0,94±0,01^{a}	0,90±0,01^{a}
	2AlF	0,93±0,02^{a}	0,97±0,01^{a}	0,98±0,01^{a}	0,99±0,01^{b}	0,98±0,02^{a}	0,98±0,02^{a}	0,97±0,05^{b}
	2AlP	0,92±0,01^{a}	0,98±0,01^{a}	0,98±0,00^{a}	0,99±0,01^{b}	0,99±0,01^{b}	0,96±0,02^{a}	0,98±0,02^{b}

a,b,c: Mean values in the same column (corresponding to the same days of storage) not followed by a common letter differ significantly ($p<0.05$).
Samples: 2AlC: control without starter culture; 2AlF: with *L. plantarum* ST153Ch fresh; 2AlP: with *L. plantarum* ST153Ch dried.

The results of the instrumental analysis of the texture and adhesiveness are shown in table 2. The texture is a predominant element of food quality and acceptability and is perceived from the sensory sensations of the physical properties of a material (Hussein, Razavi, & Emam-Djomeh, 2017). A decrease in hardness was found in all samples up to 60 days of storage when compared to the control (p<0.05). As reported by Benito et al., (2007) in a study with traditional Iberian sausages, starter cultures decreased hardness, probably due to its effect on protein hydrolysis. Regarding the adhesiveness, significant differences were found on 0, 75 and 90 days of storage (p<0.05). The sample inoculated with dried culture showed the highest values.

Table 2. Effect of the inoculated starter cultures on texture parameter throughout the storage of "Alheira" (means and standard deviations of ten replicates).

		Storage (days)						
	Samples	0	15	30	45	60	75	90
Hardness (N)	2AlC	5,73±0,64^{a}	4,45±0,81^{a}	5,03±0,55^{a}	3,93±0,66^{a}	2,38±0,23^{a}	4,23±0,79^{a}	4,90±1,10^{a}
	2AlF	4,49±0,56^{b}	3,74±0,80^{a}	4,66±0,76^{a}	3,57±0,49^{a,b}	2,77±0,42^{b}	4,36±0,60^{a}	3,50±0,67^{b}
	2AlP	5,44±0,72^{a}	4,06±0,59^{a}	3,25±0,97^{b}	3,05±0,58^{b}	2,46±0,16^{a}	4,59±1,03^{a}	3,78±0,45^{b}
Adhesiveness (N/sec)	2AlC	0,25±0,12^{a,b}	0,06±0,02^{a}	0,04±0,02^{a}	0,04±0,02^{a}	0,05±0,02^{a}	0,04±0,03^{a}	0,06±0,03^{a}
	2AlF	0,18±0,07^{a}	0,04±0,02^{a}	0,05±0,03^{a}	0,04±0,02^{a}	0,04±0,02^{a}	0,11±0,04^{b}	0,04±0,02^{a}
	2AlP	0,32±0,14^{b}	0,05±0,03^{a}	0,06±0,03^{a}	0,03±0,02^{a}	0,05±0,02^{a}	0,09±0,04^{b}	0,10±0,04^{b}

a,b,c: Mean values in the same column (corresponding to the same days of storage) not followed by a common letter differ significantly (p<0.05).
Samples: 2AlC: control without starter culture; 2AlF: with *L. plantarum* ST153Ch fresh; 2AlP: with *L. plantarum* ST153Ch dried.

The results of instrumental colour analysis are presented in Table 3.

Table 3. Effect of the inoculated starter cultures on colour parameter throughout the storage of "Alheira" (means and standard deviations of ten replicates).

		Storage (days)						
	Samples	0	15	30	45	60	75	90
L*	2AlC	49,81±1,38^{a}	43,49±2,60^{a}	51,86±4,22^{a}	57,53±2,93^{a}	52,76±2,76^{a}	54,14±4,37^{a}	60,16±2,77^{a}
	2AlF	49,58±2,74^{a}	41,51±4,73^{a}	50,73±4,50^{a}	54,26±5,73^{a}	52,56±3,58^{a}	56,12±3,05^{a}	57,61±1,89^{b}
	2AlP	49,14±2,09^{a}	41,90±3,60^{a}	50,87±4,78^{a}	52,88±4,03^{a}	52,12±5,00^{a}	54,49±2,87^{a}	57,91±2,43^{a,b}
a*	2AlC	17,09±2,63^{a}	16,05±3,16^{a}	12,63±2,86^{a}	13,92±2,19^{a}	11,89±1,51^{a,b}	13,41±1,09^{a}	12,46±0,86^{a}
	2AlF	15,57±1,19^{a}	16,32±2,80^{a}	13,58±2,71^{a}	12,45±1,41^{a}	13,00±1,32^{a}	14,48±2,03^{a}	13,69±1,12^{b}
	2AlP	15,53±1,49^{a}	15,37±1,48^{a}	11,80±2,22^{a}	12,98±1,64^{a}	11,26±1,06^{b}	13,79±1,25^{a}	13,45±1,01^{b}
b*	2AlC	16,38±2,57^{a}	15,98±2,15^{a}	19,68±5,11^{a}	22,69±1,77^{a}	25,22±1,44^{a}	28,07±1,97^{a}	27,13±2,84^{a}
	2AlF	15,86±1,74^{a}	15,83±2,53^{a}	21,21±2,92^{a}	21,67±2,36^{a}	26,87±1,56^{a}	29,91±2,46^{a}	29,02±2,62^{a}
	2AlP	15,76±1,49^{a}	15,81±2,85^{a}	19,60±5,37^{a}	20,95±1,92^{a}	25,69±2,77^{a}	28,36±2,24^{a}	27,39±0,93^{a}

a,b,c: Mean values in the same column (corresponding to the same days of storage) not followed by a common letter differ significantly (p<0.05).
Samples: 2AlC: control without starter culture; 2AlF: with *L. plantarum* ST153Ch fresh; 2AlP: with *L. plantarum* ST153Ch dried.

Colour is one of the most important quality attributes of sausages, since it affects overall quality (Essid & Hassouna, 2013). The addition of *L. plantarum* ST153Ch initially did not affect the colour parameters L* (brightness), a* (red) and b* (yellow), but significant differences were found from 75 days of storage.

Results of sensory analysis with respect to the 11 attributes showed that most of the attributes for both inoculated samples were similar to the control in the seven sampling periods. This is in accordance with what Jácome et al. (2014) observed, which is that the application methodology of LAB in this type of product did not affect the sensory properties. Only significant differences (p<0.05) were found in the "acid taste" parameters at 45 days (Suplementary Table S1).

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The "Alheira" sensory profile is also represented in figure 1 and figure 2, on a scale of intensity of the main descriptors. Regarding the attributes "brightness", "hardness" and "characteristic flavour" in the both inoculated samples showed lower values when compared to the control and only sample inoculated with fresh *L. plantarum* showed lower "characteristic odour". For the "adhesiveness", "spicy, acid and bitter taste" the result showed some increased along storage period in both LAB conditions but only "acid taste" presented significant differences (p<0.05), as already mentioned.

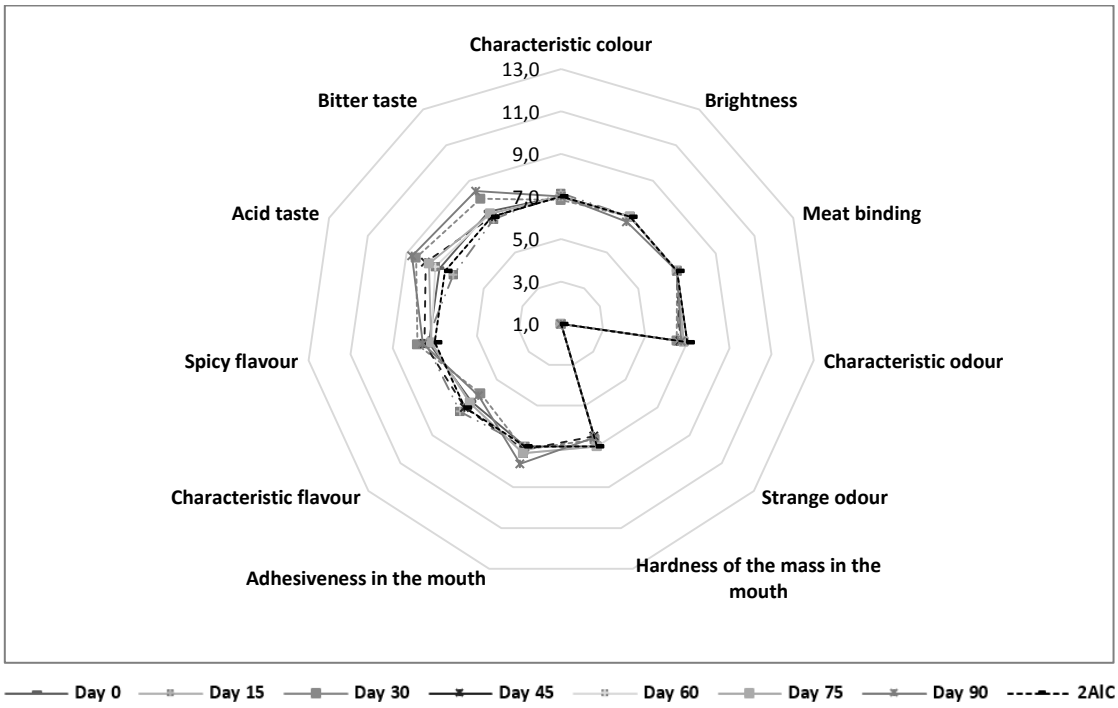


Figure 1. Sensory profile of "Alheira" during storage at 4 °C in an intensity scale of the main descriptors considered in a QDA for 2AlF with *L. plantarum* ST153Ch fresh. 2AlC: control without starter culture.

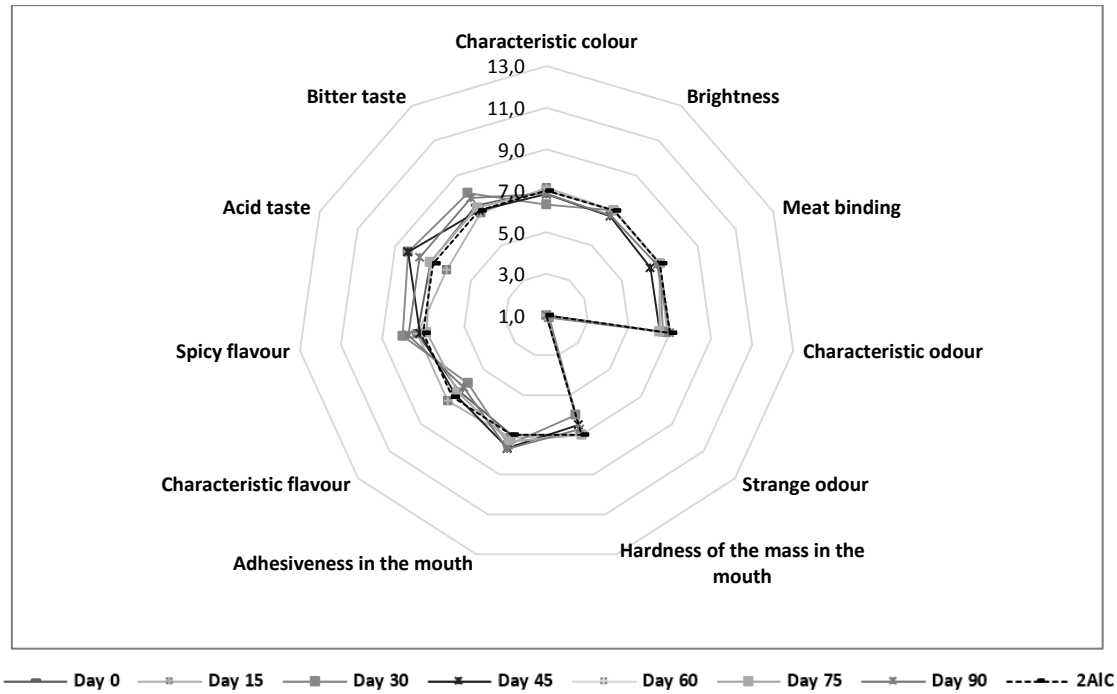
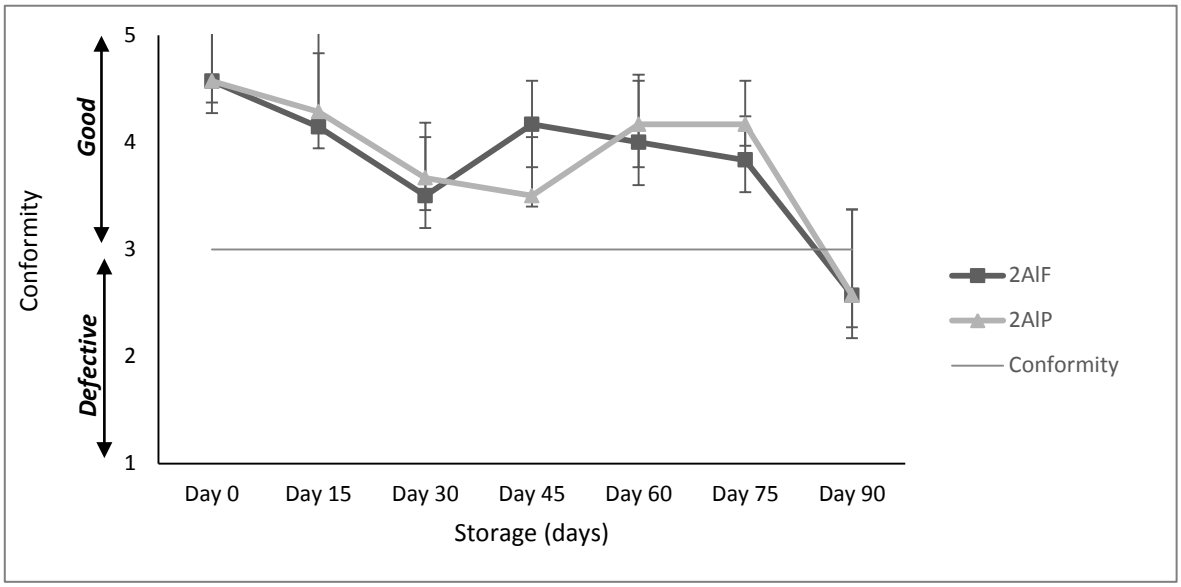


Figure 2. Sensory profile of "Alheira" during storage at 4 °C in an intensity scale of the main descriptors considered in a QDA for 2AlP with dried *L. plantarum* ST153Ch. 2AlC: control without starter culture.

Regarding the conformity parameter (Figure 3), the panellists considered that at day 90 the product is no longer conform as it obtained a score lower than 3. However, the commercial shelf-life of "Alheira" is 60 days and, in the present study, at day 75, the product was considered conform, this meaning a 15 days extension of its shelf-life.
The sensory characteristics of the final product are the result of a complex interaction of physicochemical, biochemical and microbiological processes with role in the formation and equilibrium of chemical compounds and in the modification of molecules responsible for texture and appearance (Ciuciu Simion, Vizireanu, Alexe, Franco, & Carballo, 2014). The present sensory analysis aimed to compare the effect of the addition of a fresh and lyophilized LAB culture on the sensory properties of "Alheira de Vitela".



(b)

Figure 3. Conformity variations over the storage period of "Alheira" samples (2AlF: with *L. plantarum* ST153Ch fresh; 2AlP: with *L. plantarum* ST153Ch dried) (mean ± standard deviation).

Figure 4 displays CVA applied at the sensory analysis data of the two inoculated samples of "Alheira" studied at 0, 15, 30, 45, 60, 75 and 90 days. As it can be seen dispersion of points representing any "Alheira"/time is very high, due to the lack of agreement among judges panellist that might be related with the natural sample heterogeneity. Loadings of each variable to each CV after factor structure matrix from two canonical roots were performed using the 11 original variables in order to determine which aspects were of greatest importance in "Alheira" characterization. Two variables were selected based on factor loadings modulus higher than 0.70: "characteristic colour" and "meat binding". The Eigen value of the second canonical variate (CV2) is inferior to 1 and therefore differences observed between the top and the bottom of all figures are not significant.

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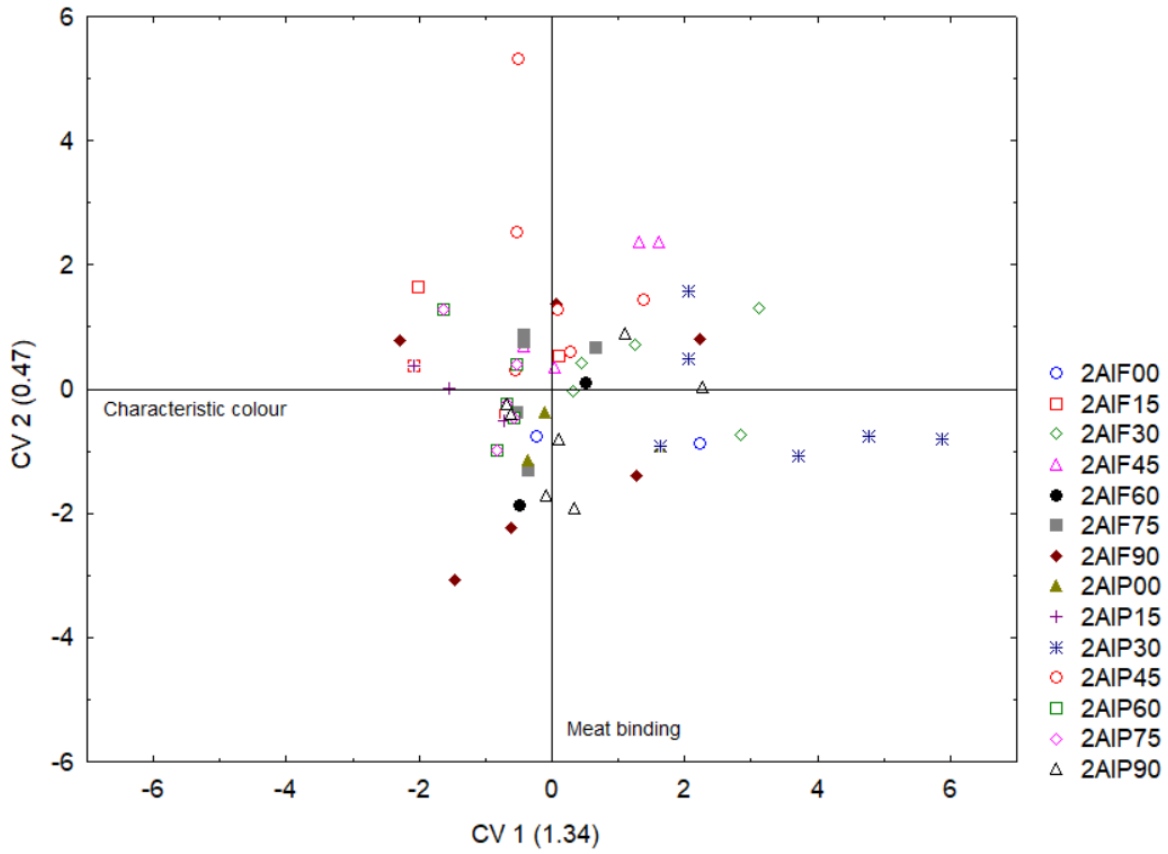

Figure 4. Canonical variance analysis projection applied to the dimensions obtained from the 2 variables selected sensorial analysis data of "Alheira" with case projection (storage time) (2AIF: with *L. plantarum* ST153Ch fresh; 2AIP: with *L. plantarum* ST153Ch dried).

Studies on the effect of starter cultures and packaging methods on amino acid profile and eating quality characteristics of pork ham, showed that the use the starter cultures brought about desirable changes regarding to all the traits studied (Gogoi, Borpuzari, Borpuzari Hazarika, & Bora, 2015), Pork ham is a more homogeneous food matrix compared to "Alheira" and the dispersion of the LAB cultures might be more distributed throughout the samples.
As for the role and use of LAB in the quality of dry-fermented meat products, LAB take part in the coagulation of muscle proteins by acidifying the batters, which results in increased slice stability, firmness, and cohesiveness of the final product. Besides, they contribute to the flavour of the final product through the formation of noticeable acidic and vinegary (acetic acid) tastes. Moreover, the existing acidic conditions may increase the activity of cathepsin D, which is responsible for muscle proteolysis (El Adab, Essid, & Hassouna, 2014; Laranjo, Elias, & Fraqueza, 2017).

CONCLUSIONS

Overall, no significant differences were found between application methodologies (fresh or dried LABs), hence industry might be able to choose the most appropriate technique according to their manufacture process. On the other hand, it was also verified that at 90 days the panellists report acid taste and spicy flavour and samples were scored below the conformity level, therefore this technique has changed organoleptic characteristics. The extension of 15 days of shelf-life was achieved but further assays must be performed to optimize the sensory properties and to assure an extension of shelf life up to 90 days.

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