Original article
Shelf-life extension and quality improvement of a Portuguese traditional ready-to-eat meat product with vinegar

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Summary   *Cabeça de xara* is a traditional ready-to-eat meat product (RTEMP) from the Portuguese region of Alentejo. It is a moulded galantine made of low value pork pieces. The aim of this work was to test the addition of vinegar in reducing the spoilage microbiota, as well as controlling *Listeria monocytogenes*, in order to increase the shelf-life of *cabeça de xara*. Physicochemical (fatty acids and biogenic amines profiles), microbiological (mesophiles, psychrotrophic bacteria, enterobacteria, yeasts and *L. monocytogenes*) and sensory analyses were performed throughout the storage period. RTEMP vinegar samples always showed lower microbiological counts than control samples. Moreover, RTEMP vinegar samples showed significantly lower contents in vasoactive amines throughout the storage period, which might be explained by their significantly lower enterobacteria counts. Concerning sensory analysis, RTEMP vinegar samples generally scored higher in overall appreciation. Our results showed that shelf-life of *cabeça de xara* may be extended from 1 to 3 months.

Keywords   Biogenic amines, fatty acids, galantine, *Listeria monocytogenes*, ready-to-eat food, sensory quality, vinegar.

Introduction

Cabeça de xara is a ready-to-eat meat product (RTEMP), whose production is very characteristic in a particular region of Portugal, Alentejo. It is a galantine usually moulded into semicylindrical shape made with various pork meat pieces, namely deboned pork heads, tongue and cartilaginous tissue to which a number of condiments like salt, parsley, wine and pepper are added. The usual shelf-life period of *cabeça de xara* is 1 month.

The use of food additives in the food industry has promoted important developments to ensure the food safety, stability and quality. However, notwithstanding their clear benefit, consumers are increasingly concerned about the use of chemical additives.

Alternatively, naturally occurring compounds have been investigated as replacements for conventional additives. These are compounds derived from natural sources such as spices, fruits, vegetables and oil, which are 'Generally Recognised As Safe' (GRAS) where oregano (*Origanum vulgare* L.), rosemary (*Rosmarinus officinalis* L.), clove (*Syzygium aromaticum* L.), garlic, grape seed extract and marjoram (*Origanum majorana* L.) are just a few examples (Tajkarimi *et al.*, 2010; Xi *et al.*, 2011a, 2012; García-Díez *et al.*, 2016, 2017). Very encouraging results have arisen with the use of organic additives as regards the containment of various pathogens including *Escherichia coli*, *Salmonella* Typhimurium, *Listeria monocytogenes* (Over *et al.*, 2009; Shan *et al.*, 2009; Cui *et al.*, 2010).

Following this line of research into the use of natural antimicrobial ingredients, vinegar has been standing out

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for its antimicrobial effect. Vinegar is an inexpensive natural additive with no side effects, easy to obtain and manipulate. It is a fermented product with several active compounds. Those with antimicrobial effects are organic acids, polyphenols and melanoidins (Chen et al., 2016). This is an ingredient whose use dates back to ancient times for its preservative properties, as well as for its aroma. The fact that acetic acid is considered as a GRAS substance (Chang & Fang, 2007) has also contributed to the increasing interest in studies of its efficacy in reducing the number of foodborne pathogens. Acetic acid, in its protonated form, is able to pass through the membrane and diffuse into the bacterial cells (Budak et al., 2014). Its effect consists in destroying the outer membrane of bacteria, inhibiting macromolecular synthesis, consuming energy, increasing intracellular osmotic pressure and promoting the generation of antibacterial peptides in host cells (Chen et al., 2016).

Regarding meat products, one of the most common applications of acetic acid has been to eliminate or prevent the development of *L. monocytogenes*, a challenging foodborne bacterium causing severe foodborne diseases (Barbuddhe et al., 2012; Mateus et al., 2014). It is resistant to low pH, as well as extreme temperatures (between -0.4 °C and 45 °C; Xi et al., 2011b).

Positive results have been obtained in the reduction of pathogens by acetic acid alone or in combination with other natural antimicrobial agents. For example, a reduction in *L. monocytogenes* counts was observed by Xi et al. (2012) in frankfurter sausages naturally cured by mixing powdered vinegar, lime and cherry.

This aim of the present work was to use vinegar to improve food quality through a better control of spoilage, safety and sensory acceptability, of a traditional RTEMP. More detailed objectives included the nutritional characterisation and shelf-life extension of *cabeça de xara*, by reducing spoilage microorganisms and controlling the pathogenic *L. monocytogenes*.

Materials and methods

Cabeça de xara manufacture

Cabeça de xara, a Portuguese traditional meat product characteristic of the Alentejo region, was manufactured from the head muscles, tongue and cartilaginous tissue of hybrid Iberian x Duroc pigs. First, the heads were thoroughly washed and covered with a layer of a mixture consisting of salt, sugar and nitrite and allowed to stand, for seasoning purposes, for 5 days at a temperature of 2 °C. After this period, the heads were cooked at 105 °C for 10 min in water previously seasoned with white wine, salt, parsley, black and white pepper, marjoram and cloves. The heads were then deboned and coarsely cut and again heated at

80 °C in the cooking water for 10 min, placed in metallic semicylindrical moulds (with 40 cm length and 14 cm height) and kept under refrigeration (0–5 °C) for 48 h. Afterwards, *cabeça de xara* was demoulded. Before packaging, *cabeça de xara* was sliced and subjected to two treatments: control samples were submerged in water while vinegar samples were submerged in a 50% (v/v) aqueous solution of white wine vinegar containing 6% acetic acid (Paladin, Mendes Gonçalves, Portugal), at room temperature, for 5 min. At last, all samples were vacuum packed in a 700 STE-XL equipment (Turbovac, The Netherlands) using polyamide/polyethylene co-extruded film packages (Alempack, Portugal) and stored at low temperature (between 0 and 5 °C) for 3 months.

Three independent batches were prepared for each treatment. Two samples per treatment from each batch were collected throughout the storage period.

Physicochemical analyses

The proximate composition of *cabeça de xara* samples was analysed as described before (Laranjo et al., 2016b) following the standard methodologies for moisture content ISO 1442 (1997), total ash ISO 936 (1998), crude protein ISO 937 (1978) and total fat content ISO 1443 (1973). Furthermore, the 'Atwater factors' were the energy conversion factors used for proteins, lipids and carbohydrates, for calorie count, respectively 4, 9 and 4 kcal g^{-1} (Kim & Choi, 2015). These are considered the EU reference values to determine energy values, as recommended in the in the 1169/2011 Commission Regulation (EC 2011).

For pH assessment, procedures described in the standard method ISO 2917 were followed (1999). Measurements were accomplished with a Crison 507 (Barcelona, Spain) pH-meter. Water activity (a_W) was determined with a hygrometer (Hygroskop Rotronic DT, Zurich, Switzerland) equipped with a WA-40 probe at 25 °C. Total basic volatile nitrogen (TBV-N) was determined as described before (Fraqueza et al., 2008) and peroxide values were determined according to ISO 3960 (2017a).

Microbiological analysis

The following microbiological parameters were analysed as described before (Laranjo et al., 2015) and according to the corresponding international standard methods: total mesophilic bacteria ISO 4833-1 (2013), psychrotrophic bacteria ISO 17410 (2001), enterobacteria ISO 21528-2 (2017b), yeasts and moulds ISO 21527-1 (2008) and *L. monocytogenes* ISO 11290-2 (2014). Anaerobic psychrotrophic bacteria were counted in Tryptone Glucose Extract (TGE; Scharlau) kept under anaerobic conditions, in an anaerobic jar (Merck) using

Anaerocult® A (Merck) and incubated at 10 °C for 7 days.

Biogenic amines analysis

Biogenic amines extraction and quantification was performed following the analytical protocol described by Laranjo *et al.* (2016a).

Fatty acids profile

Samples were mechanically homogenised, lyophilised and stored at 4 °C in glass flasks until further analysis. Fatty acids were extracted as described by Laranjo *et al.* (2015). Identification of Fatty Acids Methyl Esthers (FAMEs) was based on comparison of retention times of known composition standards mixtures (37-component FAME mix, Supelco).

Sensory evaluation

A trained panel of 10 qualified assessors (five women and five men) were selected according to ISO 8586-1 (1993). Products sensory evaluation took place in a room especially prepared for that purpose following the methodology previously described (Costell & Durán, 1981a,b,c,d). Thirty minutes prior each session, *cabeça de xara* samples were sliced (5 mm thick) and three slices were randomly disposed in white dishes. Each dish was identified with a three digit number. Moreover, neutral water and crackers were also provided so tasters could rinse their mouths between evaluations. Each sample was rated in triplicate, during different sessions. Tasters were asked to rate samples colour intensity, off colour, aroma intensity, off aroma, hardness, succulence, flavour intensity, off flavour, salt perception and overall acceptability based on a 0 ('minimum perception') to 100 ('maximum perception') scale. Salt perception was the exception, where 50% corresponding to the optimum value. In this case, lower rates would indicate a low salt perception while higher rates would correspond to high salt contents.

Statistical analysis

Results were analysed according to ANOVA using Statistica™ v.7.0, software from Statsoft (StatSoft Inc, Palo Alto, CA, USA, 1984–2007). Significant differences ($P < 0.05$) were identified based on Tukey Honest Significant Difference (HSD) test.

Results and discussion

In the present study, the quality of *cabeça de xara* was assessed according to its pH and a_W, main factors responsible for product stability and influencing spoilage and potential pathogenic bacteria multiplication during storage as well its sensory acceptability. Furthermore, this RTEMP was evaluated regarding nutritional components through the determination of its proximate composition and the analysis of fatty acids profile.

Nutritional characterisation of cabeça de xara

Table 1 shows the proximate composition of *cabeça de xara*, evidencing no significant differences between control and vinegar samples. This is a high moisture meat product (57.9–59.9%), whose major fraction is lipids, followed by proteins.

Oleic acid (C18:1) accounts for the major proportion of the lipid fraction (Table 2), followed by palmitic (C16:0), stearic (C18:0) and linoleic (C18:2). All other fatty acids are present only in small quantities (<10%). This is in accordance with the fatty acid profile reported for other pork meat products (Elias, 2004). Unsaturated fatty acids (MUFA and PUFA) are predominant over saturated fatty acids, with MUFA accounting for over 50% of the total lipid fraction. The PUFA/SFA (P/S) ratio should be above 0.4, while the normal P/S ratio of meat is approximately 0.1 (Wood *et al.*, 2003), which means that our results although below the recommended values are all above the usual ratio. Regarding the MUFA+PUFA/SFA (U/S) ratio, although we found no reference values, our results are around 2.0, which may be considered a favourable ratio. Correspondingly, Elias (2004) found U/S ratios between 1.6 and 1.7 for dry-cured pork meat sausages.

Regarding fatty acids composition, no significant differences were observed between treatments or throughout storage (Table 2). No differences were expected with the addition of vinegar, but degradation of fatty acids could occur with time, although it was not probable, because the samples were vacuum packaged. The effect of fatty acids on shelf-life may be explained by the susceptibility of unsaturated fatty

Table 1 One-way ANOVA for proximate composition (g/100 g)

	Treatment		
	Control	**Vinegar**	**Significance**
Moisture	59.9 ± 3.6	57.9 ± 4.1	ns
Fat	19.2 ± 5.0	22.8 ± 5.7	ns
Protein	16.75 ± 2.55	16.42 ± 1.86	ns
Carbohydrates	1.7 ± 1.9	0.6 ± 0.5	ns
Ash	2.35 ± 0.38	2.34 ± 0.33	ns
Energy (kcal/100 g)	247 ± 40	274 ± 45	ns
Energy (kJ/100 g)	1032 ± 166	1143 ± 190	ns

Data are shown as means ± standard deviation.
ns, not significant at $P < 0.05$.

Table 2 Fatty acids composition (% of total fatty acids)

Treatment Storage time (months)	Control				Vinegar			
	0	1	2	3	0	1	2	3
C12:0	0.24 ± 0.11	0.18 ± 0.10	0.21 ± 0.13	0.22 ± 0.11	0.26 ± 0.20	0.16 ± 0.09	0.27 ± 0.15	0.25 ± 0.11
C14:0	1.93 ± 0.54	2.17 ± 0.62	2.45 ± 1.15	1.89 ± 2.06	2.18 ± 1.20	2.13 ± 0.62	2.07 ± 2.06	1.64 ± 0.97
C16:0	18.94 ± 1.19	19.43 ± 1.60	19.99 ± 2.97	19.35 ± 0.67	18.41 ± 0.91	20.34 ± 2.77	19.04 ± 0.61	19.13 ± 1.37
C16:1	3.23 ± 0.61	3.17 ± 0.60	3.67 ± 0.70	2.92 ± 0.66	3.16 ± 0.67	3.12 ± 0.45	3.14 ± 0.58	3.27 ± 0.62
C17:0	0.28 ± 0.05	0.28 ± 0.04	0.25 ± 0.08	0.24 ± 0.10	0.25 ± 0.03	0.28 ± 0.05	0.28 ± 0.05	0.26 ± 0.05
C17:1	0.38 ± 0.05	0.39 ± 0.03	0.37 ± 0.05	0.30 ± 0.12	0.36 ± 0.05	0.39 ± 0.04	0.38 ± 0.04	0.36 ± 0.05
C18:0	8.15 ± 1.55	8.29 ± 1.98	8.35 ± 2.29	8.72 ± 1.33	7.64 ± 1.49	5.95 ± 2.43	8.13 ± 1.22	7.82 ± 1.81
C18:1	47.40 ± 0.73	49.36 ± 2.70	47.47 ± 3.28	46.72 ± 1.15	48.82 ± 0.90	48.68 ± 6.57	47.54 ± 2.40	48.91 ± 1.42
C18:2	5.40 ± 1.12	5.66 ± 0.76	5.31 ± 1.86	4.99 ± 1.01	5.46 ± 1.35	6.55 ± 2.99	5.16 ± 0.94	5.58 ± 1.22
C18:3	1.75 ± 0.35	1.68 ± 0.21	1.80 ± 0.33	1.64 ± 0.33	1.73 ± 0.25	1.77 ± 0.23	1.52 ± 0.11	1.64 ± 0.28
C20:1	0.73 ± 0.25	0.65 ± 0.08	0.62 ± 0.10	0.63 ± 0.36	0.64 ± 0.07	0.67 ± 0.22	0.62 ± 0.09	0.61 ± 0.08
C21:1	0.82 ± 0.37	0.53 ± 0.17	0.74 ± 0.38	0.93 ± 0.41	0.62 ± 0.22	0.58 ± 0.32	0.80 ± 0.33	0.56 ± 0.09
MUFA	52.56 ± 2.01	54.10 ± 3.58	52.87 ± 4.51	51.50 ± 2.70	53.60 ± 1.91	53.44 ± 7.60	52.48 ± 3.44	53.71 ± 2.26
PUFA	7.15 ± 1.47	7.34 ± 0.97	7.11 ± 2.19	6.63 ± 1.34	7.19 ± 1.60	8.32 ± 3.22	6.68 ± 1.05	7.22 ± 1.50
SFA	29.54 ± 3.44	30.35 ± 4.34	31.25 ± 6.62	30.42 ± 4.27	28.74 ± 3.83	28.86 ± 5.96	29.79 ± 4.09	29.10 ± 4.31
P/S	0.24 ± 0.43	0.24 ± 0.22	0.23 ± 0.33	0.22 ± 0.31	0.25 ± 0.42	0.29 ± 0.54	0.22 ± 0.26	0.25 ± 0.35
U/S	2.02 ± 1.01	2.02 ± 1.05	1.92 ± 1.01	1.91 ± 0.95	2.12 ± 0.92	2.14 ± 1.82	1.99 ± 1.10	2.09 ± 0.87

MUFA, Monounsaturated Fatty Acids; PUFA, Polyunsaturated Fatty Acids; SFA, Saturated Fatty Acids. P/S, PUFA/SFA ratio; U/S, Unsaturated (MUFA + PUFA)/SFA ratio.

Data are expressed as mean % ± standard deviation.

acids (UFA) to oxidation, leading to the development of rancidity with time (Wood *et al.*, 2003).

Spoilage and safety of cabeça de xara

The spoilage and safety of *cabeça de xara* during storage at low temperature was evaluated though microbiological analyses, TBV-N and peroxide content and the profile and content in biogenic amines.

The control of the product's stability throughout storage was accessed through the determination of pH and a_W values.

pH significantly differs with treatment ($P < 0.001$), with lower values in the samples containing vinegar (Table 3), and throughout storage ($P < 0.05$). Regarding a_w, the values were from 0.979 to 0.987, with no significant differences between samples (Table 3). These results suggest that *cabeça de xara* may be a more stable product than other galantines (containing fish and pork), which had higher pH values and similar a_W values (Herrero *et al.*, 2008).

The content in total basic volatile nitrogen (TBV-N) shows significant differences between treatments ($P < 0.001$), with the vinegar treated samples showing lower values. Furthermore, the TBV-N values significantly increase throughout storage ($P < 0.001$) (Table 3), which is more consistent in control samples. This increase in TBV-N values essentially corresponds to an increase in nitrogen compounds resulting from the deterioration caused by bacteria. No significant differences were found for peroxide values, although an increasing trend was observed with storage time, which is more pronounced from the second to the third month.

Microbiological results

Significant differences were observed between *cabeça de xara* treatments for mesophiles ($P < 0.01$), psychrotrophic ($P < 0.001$) and anaerobic psychrotrophic ($P < 0.001$) bacteria, enterobacteria ($P < 0.001$) and *L. monocytogenes* ($P < 0.05$; Table 4). Control samples always showed higher microbial counts than RTEMP vinegar samples.

Regarding storage time, there is an increase in log cfu g^{-1} for all microbial groups until 2 months of RTEMP storage, followed by a slight decrease from the second to the third month, mainly in enterobacteria ($P < 0.001$; Table 4). Comparing RTEMP control and vinegar samples, the increase from 0 to 3 months was about one log cfu g^{-1} less in RTEMP vinegar samples, with the maximum difference (3 log cfu g^{-1}) in enterobacteria.

The rapid increase in the first month suggests that there was a post contamination after thermal treatment, probably due to cross-contamination from operators or industrial surfaces during product moulding and demoulding. On the other hand, the decrease from the second to the third month could be explained through both the decrease in pH by the production of organic acids, such as lactic acid, and the increasing

Table 3 Two-way ANOVA for physicochemical analyses throughout storage time

Treatment	Control				Vinegar				Significance		
Storage time (months)	0	1	2	3	0	1	2	3	T	t	T × t
pH	6.23^{c} ± 0.02	5.97^{bc} ± 0.04	5.80^{abc} ± 0.08	5.93^{bc} ± 0.04	5.64^{ab} ± 0.18	5.44^{a} ± 0.11	5.57^{ab} ± 0.06	5.39^{a} ± 0.08	***	*	ns
a$_w$	0.982 ± 0.006	0.986 ± 0.004	0.981 ± 0.004	0.979 ± 0.002	0.981 ± 0.006	0.987 ± 0.004	0.987 ± 0.002	0.984 ± 0.004	ns	ns	ns
TBV-N	11.70^{a} ± 0.62	13.50^{ab} ± 0.83	17.48^{bc} ± 1.12	19.70^{c} ± 2.16	10.43^{a} ± 0.33	9.72^{a} ± 0.93	14.72^{abc} ± 1.12	14.95^{abc} ± 1.25	***	***	ns
Peroxide value	8.12 ± 2.77	8.40 ± 1.92	10.33 ± 3.62	15.98 ± 5.99	9.05 ± 3.76	8.12 ± 1.05	10.95 ± 3.02	17.55 ± 6.04	ns	ns	ns

Data are shown as means ± standard deviation. Different letters indicate statistically different means (Tukey's HSD).
TBV-N: total basic volatile nitrogen (expressed in mg NH$_3$ 100 g^{-1}); peroxide value (expressed in meq O2 kg^{-1}).
T, treatment; t, storage time; T × t, interaction between the two factors.
ns, not significant, *P < 0.05, ***P < 0.001.

Table 4 Two-way ANOVA for microbiological analyses

Treatment	Control				Vinegar				Significance		
Storage time (months)	0	1	2	3	0	1	2	3	T	t	T × t
Mesophiles	2.73^{a} ± 0.67	7.88^{bc} ± 0.12	8.88^{c} ± 0.38	7.67^{bc} ± 0.24	2.51^{a} ± 0.28	6.13^{b} ± 0.74	7.18^{bc} ± 0.60	6.41^{a} ± 0.55	**	***	ns
Psychrotrophic bacteria	3.02^{ab} ± 0.37	7.67^{c} ± 0.24	8.54^{c} ± 0.36	7.04^{c} ± 0.37	2.18^{a} ± 0.48	4.40^{b} ± 0.45	7.21^{c} ± 0.61	4.44^{b} ± 0.16	***	***	*
Anaerobic psychrotrophic bacteria	2.67^{ab} ± 0.51	7.90^{d} ± 0.12	8.50^{d} ± 0.36	7.54^{d} ± 0.21	1.89^{a} ± 0.47	4.06^{bc} ± 0.42	5.29^{c} ± 0.74	4.99^{c} ± 0.49	***	***	*
Enterobacteria	1.77^{a} ± 0.79	7.17^{d} ± 0.31	8.39^{d} ± 0.30	6.33^{cd} ± 0.40	0.87^{a} ± 0.55	2.47^{ab} ± 0.57	4.21^{bc} ± 0.43	2.23^{ab} ± 0.73	***	***	**
Yeasts	2.57^{ab} ± 0.53	4.52^{bc} ± 0.60	5.64^{c} ± 1.36	4.06^{abc} ± 0.46	1.45^{a} ± 0.33	4.11^{abc} ± 0.24	4.96^{bc} ± 0.79	4.16^{abc} ± 0.14	ns	***	ns
Listeria monocytogenes	0.00 ± 0.00	1.75 ± 2.24	1.98 ± 3.07	2.03 ± 3.15	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.35 ± 0.87	*	ns	ns

Data are expressed as log cfu g^{-1}. Data are shown as means ± standard deviation. Different letters in the same line indicate statistically different means (Tukey's HSD).
T, treatment; t, storage time; T × t, interaction between the two factors.
ns, not significant, *P < 0.05, **P < 0.01, ***P < 0.001.

trend in peroxides. However, when peroxides accumulate, they inhibit the microbiota, mainly enterobacteria.

The slight decrease in pH throughout storage may be due to the TBV-N buffer effect, which limits pH variations. The increase in the microbiota with storage time may also have contributed to this decrease in pH through the production of organic acids, such as lactic acid among others. Since these acids are mainly in the undissociated form, their effect is hardly noticed in the pH value. However, these organic acids may have an antimicrobial effect over several microbial groups, mostly in enterobacteria.

The growth of psychrotrophic and anaerobic psychrotrophic bacteria was also less pronounced in RTEMP vinegar samples than in control samples. This is in agreement with Stelzleni *et al.* (2013), which concluded that the addition of vinegar to ground beef patties was effective in controlling the growth of psychrotrophic organisms, such as *L. monocytogenes*.

Listeria monocytogenes was present from the first month on but only in control batch 1. However, it was inhibited by the addition of vinegar until the third month of storage, where *L. monocytogenes* was present but below the limit established in the 1441/2007 Commission Regulation (EC 2007). The other two batches were free from contamination with *L. monocytogenes* throughout the assayed storage period (Table 4). *L. monocytogenes* is a pathogen that tolerates cold temperatures and poses a potential health risk for ready-to-eat meat based products that are typically stored under refrigeration (Zhang *et al.*, 2009; Henriques *et al.*, 2017).

Organic acids, such as acetic acid (vinegar) are more effective against psychrotrophic bacteria, such as *L. monocytogenes*, than mesophiles (Smulders & Greer, 1998), which may explain the higher reduction (2.5 log cfu g^{-1}) observed in psychrotrophic and psychrotrophic anaerobic bacteria than in mesophiles (Table 4). The deleterious effect of vinegar on *L. monocytogenes*, a psychrotrophic bacterium, can also explain why it could not be detected until the third month of storage, while in control samples it was already detected after 1 month of storage.

The major microbiological concerns associated with these products focus on two types of microorganisms, psychrotrophic and mesophilic, which may grow during extended refrigerated storage or temperature abuse, and cause food spoilage. European or Portuguese legislation that applies to these kind of meat products considers only the safety indicator *Listeria monocytogenes* since was previously submitted to a thermic treatment. Therefore, we refer to the Irish guidelines (FSAI 2016), which include hygiene criteria. According to the 'Guidelines for the Interpretation of Results of Microbiological Testing of Ready-to-Eat

Table 5 Two-way ANOVA for biogenic amines content expressed on a dry matter basis in mg kg^{-1}

Treatment Storage time (months)	Control				Vinegar				Significance		
	0	1	2	3	0	1	2	3	T	t	T × t
Tryptamine	8.25^b ± 10.40	4.07^{ab} ± 4.58	3.27^a ± 3.36	3.40^a ± 3.64	2.65^a ± 3.88	2.19^a ± 3.17	3.51^a ± 3.69	1.73^a ± 2.66	**	*	*
β-phenylethylamine	0.48^{ab} ± 0.09	0.00^a ± 0.00	0.00^a ± 0.00	0.00^a ± 0.00	0.68^b ± 1.55	0.00^a ± 0.00	0.00^a ± 0.00	0.00^a ± 0.00	ns	***	ns
Putrescine	187.52^a ± 27.10	406.19^b ± 171.00	606.15^c ± 151.28	741.29^d ± 186.75	175.15^a ± 23.16	195.19^a ± 28.65	209.35^a ± 39.36	206.52^a ± 28.80	***	***	***
Cadaverine	0.76^a ± 1.73	42.63^b ± 27.93	50.08^{bc} ± 29.96	58.49^c ± 26.11	0.66^a ± 1.13	2.69^a ± 3.37	5.61^a ± 6.16	1.80^a ± 4.12	***	***	***
Histamine	2.64^{ab} ± 1.04	3.28^{ac} ± 1.10	3.42^{ac} ± 1.60	3.95^c ± 1.18	2.64^{ab} ± 0.78	2.30^b ± .072	2.73^{ab} ± 0.96	2.45^{ab} ± 1.32	***	ns	*
Tyramine	0.70^a ± 1.02	6.64^{bc} ± 7.48	11.46^c ± 10.10	20.91^d ± 10.41	1.12^{ab} ± 0.85	2.53^{ab} ± 3.71	4.37^{ab} ± 5.55	5.58^{ab} ± 6.80	***	***	***
Spermidine	13.11^{ab} ± 3.73	14.59^b ± 4.36	13.41^{ab} ± 3.02	12.96^{ab} ± 2.97	13.60^{ab} ± 4.69	11.45^a ± 2.52	13.93^{ab} ± 2.82	13.63^{ab} ± 3.81	ns	ns	*
Spermine	31.06 ± 9.56	33.68 ± 11.84	32.62 ± 9.28	31.14 ± 7.66	30.85 ± 10.46	26.00 ± 7.10	32.98 ± 7.27	30.80 ± 9.58	ns	ns	ns
Vasoactive amines	12.06^{ab} ± 10.58	14.00^{ab} ± 10.51	18.15^b ± 9.77	28.26^c ± 9.37	7.08^a ± 5.55	7.02^a ± 4.85	10.61^a ± 4.47	9.76^a ± 9.54	***	***	***
Total content	244.51^a ± 38.29	511.09^b ± 199.09	720.42^c ± 190.23	872.14^d ± 223.12	227.35^a ± 26.61	242.36^a ± 43.27	272.47^a ± 51.18	262.50^a ± 37.52	***	***	***

Data are expressed as mg kg^{-1}. Data are shown as means ± standard deviation. Different letters indicate statistically different means (Tukey's HSD).

T, treatment; t, storage time; T × t, interaction between the two factors.

ns, not significant, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Foods Placed on the Market' (FSAI 2016), an 'extended shelf-life food products requiring refrigeration' may be classified as unsatisfactory when aerobic colony counts are ≥ 8 log cfu g^{-1}. After 3 months of storage, our results remained between 6 and 8 log cfu g^{-1} allowing us to consider them as borderline (FSAI 2016).

Other authors, studying pork sausage patties have reported a decrease in total plate counts with vinegar; however, their values after 18 days are higher than our after 3 months in both control and vinegar samples (Bradley *et al.*, 2011).

Biogenic amines profile
The results regarding the content in biogenic amines are summarised in Table 5. Putrescine is by far the most abundant biogenic amine, with contents between 175.15 and 741.29 mg kg^{-1}. On the contrary, β-phenylethylamine is the less abundant amine, only detected at time zero (T0), with mean values of 0.48 mg kg^{-1} in control samples and 0.68 mg kg^{-1} in vinegar samples.

Significant differences were observed between treatments for most individual biogenic amines, with the exception of β-phenylethylamine, spermine and spermidine. Furthermore, spermine and spermidine contents remained more or less constant throughout the storage period.

Tryptamine values generally decreased with time for both control and vinegar samples ($P < 0.05$). On the other hand, putrescine, cadaverine and tiramine contents increased with time for both treatments ($P < 0.001$). Histamine values remained mostly unchanged with time.

Tyramine and histamine are biogenic amines with toxic effects like headaches, hypertension and perspiration in levels of 50 mg per person per meal (EFSA 2011). On the other hand, putrescine is not toxic, but it potentiates the toxic effects of other biogenic amines, such as histamine (Chinnici *et al.*, 2016). In our study, the levels of these amines always remained below this threshold, although their contents are lower in vinegar samples, which is very important for consumer health issues.

According to Nuñez *et al.* (2016), food products that contain more than 500 mg kg^{-1} histamine and 1000 mg kg^{-1} tyramine are considered toxic or dangerous to human health. However, for susceptible individuals or individuals whose health is compromised, these are far too high levels (Stadnik & Dolatowski, 2010). In fact, according to Karovičová & Kohajdová (2005), levels as low as 5–10 mg kg^{-1} of histamine may be harmful for susceptible individuals. On the other hand, for β-phenylethylamine, there are evidences that a content of 30 mg kg^{-1} in food products may be toxic (Gardini *et al.*, 2001).

Vinegar samples showed significantly lower contents in vasoactive amines throughout the storage period ($P < 0.001$). The reduced content in biogenic amines of vinegar treated samples might be explained by the significantly lower enterobacterial counts in vinegar samples. Furthermore, these results seem to indicate that the microbiota present in these samples might be mostly decarboxylase-negative. Nevertheless, these are high values for biogenic amines, considering that it is a cooked RTEMP and not a fermented meat product (Ruiz-Capillas & Jimenez-Colmenero, 2004).

Sensory evaluation

The attributes evaluated by the panel in the different sensory sessions are presented in Table 6. Samples contaminated with *L. monocytogenes* were not subjected to a full sensory evaluation by the panellists. In

Table 6 Sensory analysis data

Treatment	Control				Vinegar			
Storage time (months)	0	1	2	3	0	1	2	3
Colour intensity	62.26 ± 15.25	63.02 ± 13.39	57.29 ± 13.95	55.92 ± 14.19	60.46 ± 17.51	58.44 ± 16.85	55.17 ± 16.54	56.83 ± 17.82
Off-colours	2.48 ± 8.59	0.83 ± 2.98	3.08 ± 7.95	3.75 ± 8.96	2.24 ± 8.01	1.83 ± 8.76	2.38 ± 4.78	2.67 ± 6.51
Marbled	56.02 ± 18.26	61.50 ± 17.58	52.25 ± 23.03	54.00 ± 16.06	58.37 ± 18.27	60.81 ± 19.02	55.08 ± 17.06	52.75 ± 19.23
Aroma intensity	60.93 ± 16.10	61.96 ± 14.31	59.83 ± 16.24	53.58 ± 13.07	63.72 ± 15.17	60.40 ± 18.22	58.17 ± 16.03	62.25 ± 11.63
Off-aromas	4.24 ± 13.18	2.65 ± 8.00	7.92 ± 14.70	11.36 ± 9.60	0.74 ± 2.06	0.79 ± 1.68	2.58 ± 5.59	8.75 ± 11.66
Hardness	49.74 ± 11.23	50.72 ± 7.61	50.83 ± 2.04	*	46.83 ± 10.17	45.54 ± 8.84	46.58 ± 11.70	*
Fibrousness	22.52 ± 20.77	29.38 ± 20.81	13.17 ± 10.87	*	21.02 ± 19.28	25.44 ± 20.45	22.71 ± 19.51	*
Succulence	59.83 ± 15.81	59.09 ± 14.20	61.00 ± 17.79	*	62.87 ± 17.05	66.63 ± 12.38	66.08 ± 14.20	*
Flavour intensity	63.15 ± 12.43	63.22 ± 10.85	55.67 ± 10.33	*	67.15 ± 10.62	65.91 ± 10.31	64.79 ± 10.97	*
Off-flavours	5.07 ± 15.78	1.26 ± 2.21	4.67 ± 8.16	*	2.41 ± 8.04	3.55 ± 11.51	0.58 ± 1.28	*
Salt perception	52.13 ± 9.75	51.53 ± 7.65	52.83 ± 5.60	*	50.80 ± 6.96	52.25 ± 7.25	52.46 ± 8.44	*
Overall appreciation	62.78 ± 15.16	61.50 ± 13.65	62.50 ± 18.32	*	67.59 ± 13.12	64.13 ± 12.31	63.88 ± 12.61	*

Data are expressed as means ± standard deviation.
*Samples were not tasted due to the presence of *Listeria monocytogenes*.

these samples, the evaluation only concerned the analysis of colour and aroma, and not the analysis of flavour and texture. No significant differences were observed between treatments or throughout storage. However, regarding succulence, flavour intensity and overall appreciation tasters preferred vinegar samples (Table 6). Regarding overall appreciation, the differences between treatments are not significant and become less pronounced throughout storage time. Off-aromas and off-flavours are less detected in vinegar samples, suggesting that vinegar can mask some defects (Table 6). Furthermore, it is important to notice that no vinegar off-aromas and off-flavours were recorded by tasters. When preservatives like organic acids, such as vinegar, are used, it is necessary to ensure that they have no negative impact on the product regarding organoleptic quality, as has been described before (Smulders & Greer, 1998). However, this was not the case in our products, since overall appreciation values were higher for vinegar samples.

Conclusions

The use of vinegar in the manufacture of a traditional RTEMP significantly improved food quality and hazard control, based on microbiological analyses and biogenic amines content. Furthermore, vinegar did not depreciate sensory acceptability. In fact, although no significant differences were observed, vinegar samples were generally scored with higher values regarding overall appreciation.

Based on these results, and considering that there were no significant differences in the microbial counts between 1 and 3 months storage, we propose the extension of the shelf-life period from 1 to 3 months.

Additionally, this kind of RTEMP appears to be contaminated from the handling by food operators in the after cooking processing and before packaging. Therefore, we suggest a post-packaging pasteurisation of the product before commercialisation. This would ensure food safety and could extend the shelf-life even further.

To our knowledge, there are very few studies on this kind of sliced RTEMP, which is a sort of galantine, but more studies are needed in the future to valorise them. In fact, these traditional RTEMP are manufactured with low value meat pieces, such as pork heads, but may be highly valorised by consumers due to their particularly appreciated sensory characteristics that regard them as *delicatessens* and are willing to pay for them.

Acknowledgments

Conflict of interest

The authors declare that there are no conflicts of interest.

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