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ORAL COMMUNICATION

Effect of autochthonous starter cultures in the production of *Paio*, a traditional Portuguese dry-cured sausage

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Additional keywords

Traditional dry-cured sausages.
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SUMMARY

In Mediterranean countries, such as Portugal, traditional dry-cured sausages are highly appreciated. They are often still being manufactured in small processing units, according to traditional procedures. The aims of the present study were to evaluate the effect of different starter cultures and their optimal concentration, to reduce the microbial load and biogenic amines in end-products, with the purpose to improve the sausages' safety, without deteriorating sensory acceptability. pH, a_w, microbiological profile, biogenic amines, colour and texture profile analysis were assessed. The strains and concentrations used were selected based on previous results: *Staphylococcus xylosus*, *Lactobacillus sakei* and an unidentified yeast strain at a concentration of 106 cfu/g meat batter each, added 0.25% dextrose. A control batch without starter cultures was always used. aw values were lower in the inoculated sausages. In general, pH values were slightly higher in the inoculated sausages. The treatment with *L. sakei* alone was the most effective in reducing the contamination level with *L. monocytogenes*, however this effect seems to be lost in the mixed cultures. Inoculation, generally decreased the content of putrescine, cadaverine and tyramine. Yeast inoculation seems to contribute to the darker colour of *Paios*. Regarding texture, control *Paios* showed higher hardness values.

Efecto de los cultivos autóctonos en la producción de *Paio*, un embutido curado tradicional portugués

Palabras clave adicionales

Embutido curado tradicional.
Carne de cerdo ibérico.
Cultivos iniciadores.
Inocuidad de los alimentos.
Aminas biogénicas.

RESUMEN

En los países mediterráneos, como Portugal, los embutidos curados tradicionales son muy apreciados. A menudo se siguen fabricando en pequeñas unidades de procesamiento, de acuerdo con los procedimientos tradicionales. Los objetivos del presente estudio fueron evaluar el efecto de diferentes cultivos iniciadores y su concentración óptima, para reducir la carga microbiana y las aminas biogénicas en productos finales, con el objetivo de mejorar la seguridad de los embutidos sin deteriorar la aceptación sensorial. pH, a_w, perfil microbiológico, aminas biogénicas, análisis del perfil de color y textura fueron evaluados. Las cepas y las concentraciones a utilizar, se seleccionaron en base a resultados previos: *Staphylococcus xylosus*, *Lactobacillus sakei* y una cepa de levadura a una concentración de 106 ufc / g de masa de carne cada una, se añadió 0,25% de dextrosa. Siempre se utilizó un lote control sin cultivos iniciadores. Los valores de aw fueron menores en los embutidos inoculados. En general, los valores de pH fueron ligeramente superiores en los embutidos inoculados. El tratamiento con *L. sakei* solo fue el más efectivo en la reducción del nivel de contaminación con *L. monocytogenes*, sin embargo este efecto parece perderse en cultivos mixtas. La inoculación, disminuyó generalmente el contenido de putrescina, de cadaverina y de tiramina. La inoculación de levadura parece contribuir al color más oscuro de los *Paios*. Con respecto a la textura, el *Paio* control demostró valores más altos de la dureza.

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INTRODUCTION

In the past, people produced sausages with the purpose to preserve meat, a source of animal protein, which was a very important resource to certain rural populations until about 50 years ago. Nowadays, consumers are becoming more demanding and prefer homemade regional products of high sensorial quality. The specificity of these sausages is recognised through *Protected Designation of Origin* (PDO) and *Protected Geographical Indication* (PGI). Increasingly the food industry has attempted to react to the demands and expectations of the consumer concerning food quality and safety, through the improvement of the sausage

making technology at different levels throughout the process. The use of starter cultures, a practice not currently used in the Portuguese traditional sausage making industry, primarily intends to ensure and improve the sanitary, nutritional and sensorial qualities and may result in further advantages to the technological process, as the higher degree of conformity and the increase in the sausages' shelf-life. Furthermore, starter cultures may add new sensory properties to old products, enable products' diversification, as well as to speed up and increase production, with the consequent economic impact, not only for the manufacturer, but also for the region, due to the valorisation of products and the increase in competitiveness in todays' exigent global market. Starter cultures are part of the native microbiota of meat and meat products, but not in the required quantities to ensure their optimal performance. They significantly contribute to the quality and safety of the products mainly through their bioprotector, (inhibition of foodborne pathogens growth) probiotic (production of substances essential to consumers' health) and fermentative action (production of secondary metabolites) on the substrates (Aro Aro et al. 2010; Babić et al. 2011; Essid & Hassouna, 2013; Van Ba et al. 2016).

Nowadays, in the meat processing industry, the microorganisms mainly used as starters belong to four groups: [1)]lactic acid bacteria (LAB) from the genus *Lactobacillus*, *L. sakei*, *L. plantarum* and *L. curvatus*; [2)] coagulasenegative staphylococci (CNS), such as *S. xylosus* and *S. equorum*, *Micrococcaceae* (*Kocuriae*), [3)]moulds of the genus *Penicillium* and [4)]*Debaromyces* spp. yeasts. The first two groups are used for inoculation of meat batters, whereas the last two are mostly used for superficial inoculation of sausages (LatorreMoratalla et al. 2010; Elias et al. 2014; Simion et al. 2014; Cocconcelli & Fontana 2015).

The aims of the present study were to evaluate the effect of different starter cultures and their optimal concentration. Furthermore, we intended to reduce the microbial load and biogenic amines in endproducts, with the purpose to improve the sausages safety.

MATERIAL AND METHODS

Commercial black pig breed meat was used to prepare dryfermented sausages known as *Paios*. Inoculation experiment was designed considering three types of inoculum): (1) *Staphylococcus xylosus*, (2) *Lactobacillus sakei*, (3) mixed culture (*S. xylosus* and *L. sakei*) and (4) mixed culture (*S. xylosus*, *L. sakei* and yeast). With a concentration of 10^6cfug^{-1} meat batter each, added 0.25% dextrose. A control batch without starter cultures was always used. Three independent batches were produced in a local factory. Two replicates per treatment were considered and samples were endproducts (38-40% weight loss). For pH assessment, sausages casings were removed and values measured with a Crison 507 pH-meter (Barcelona, Spain) following the procedures described in ISO 2917 (1999). Water activity was determined with a hygrometer (Hygroskop Rotronic DT, Zurich, Switzerland) equipped with a WA-40 probe at 25 °C. Microbiological analyses were carried out according the analytical protocols described by Laranjo et al. (2015) and Laranjo et al. (2017). Psycrophiles were incubated in Tryptone Glucose Extract (TGE) Agar (Scharlau, Spain) at 10 °C for 7 days.

Biogenic amines quantification was performed according to the experimental protocol described by Roseiro et al. (2006). Colour was measured with a CR-400 colorimeter (Konica Minolta) and the chromatic coordinates L* a* b* of each sausage were determined using the CIELab System. All measurements were performed using the standard illuminant D65.

Texture profile analysis (TPA) experiments were conducted at room temperature (20 ºC ±1 ºC) according the analytical protocol disclosed in Laranjo et al. (2015).

Results were analysed with a factorial ANOVA using StatisticaTM v.8.0, software from Statsoft (StatSoftInc, 1984–2007). Differences between groups were identified based on Tukey's Honest Significant Difference (Tukey's HSD) test ($P < 0.05$). Elimination of outliers in biogenic amines data was carried out according to the Grubbs test ($\alpha = 0.05$).

RESULTS AND DISCUSSION

In **Table I** we see, in general, that a_w values were lower in the inoculated sausages. This contributes to the safety of the sausages. Our results are similar to those of Elias & Carrascosa (2010, 2013) and Simon et al. (2014), and lower than those of Elias et al. (2014). In general, pH values were slightly higher in the inoculated sausages, with the exception of *L. sakei* treatment, which presented the lowest mean value. Bozkurt & Erkman (2002) refer that high quality ripened sausages should have pH values between 4.7 and 5.2. In this work, results are within this range and were lower than those of Elias & Carrascosa (2010, 2013), Elias et al. (2014) and Simon et al. (2014). Thus, pH values are low and contribute to safety. Results along the cure are not shown, but normally a pH increase was observed throughout the curing process. This may be due to the action mainly of bacteria and, eventually, moulds capable of triggering proteolysis (Gonzalez Fernandez et al. 2003). With the action of endogenous proteases,

Table I. pH and a_w for three independent batches of end-product *Paio* inoculated with starter cultures (pH y a_w en tres lotes independientes de producto final *Paio* inoculado con cultivos iniciadores).

	Treatments				
Parameters	Control	*S. xylosus*	*L. sakei*	*S. xylosus***L. sakei*	*S. xylosus***L. sakei**yeast
pH	4.97^{bc}±0.14	5.05^{ab}±0.14	4.94^{c}±0.07	5.10^{a}±0.01	5.10^{a}±0.10
a_w	0.845^{a}±0.024	0.826^{b}±0.031	0.852^{a}±0.002	0.823^{b}±0.030	0.824^{b}±0.014

Data are expressed as means ± SD. In the same row, different letters indicate significantly different values (P<0.05).

Table II. Microbiological parameters for three independent batches of end-product *Paio* inoculated with starter cultures (Parámetros microbiológicos en tres lotes independientes de producto final *Paio* inoculado con cultivos iniciadores).

	Treatments				
Parameters	Control	*S. xylosus*	*L. sakei*	*S. xylosus***L. sakei*	*S. xylosus***L. sakei***yeast*
mesophiles	7.38±0.60	7.65±0.54	8.39±0.97	8.03±1.06	8.48±1.19
psychrophiles	5.66±0.29	5.69±0.26	6.20±0.18	5.89±0.44	6.48±0.52
LAB	8.06±0.77	7.96±0.67	8.49±1.15	8.15±1.09	8.56±1.10
staphylococci	8.68±1.03	8.34±0.49	8.49±0.71	8.38±2.14	10.31±1.29
enterobacteria	2.75±0.36	2.69±0.50	2.24±0.39	2.48±0.40	2.51±0.55
moulds	0.58±1.20	n.d.	0.33±0.82	n.d.	n.d.
yeasts	4.61±0.35	4.70±0.47	4.85±0.42	4.96±0.74	4.74±0.69
L. monocytogenes	5.83±12.01	7.50±11.72	1.67±2.58	0.83±2.24	7.50±13.69
Salmonella spp.	Absent in 25 g	Absent in 25 g	Absent in 25 g	Absent in 25 g	Absent in 25 g

n.d.: none detected. Data are expressed as means ± SD. *Listeria monocytogenes* is reported in cfu/g. The remaining countable parameters are presented in log cfu/g.

resulting in formation of peptides, free amino acids, amines, ammonia, among others, thereby increasing the concentration of buffer substances (Elias & Carrascosa, 2010). Flores et al. (1997) report, at this stage, that yeasts may also be responsible for the rise in pH.

Table II shows a tendency for inoculated sausages to have a higher concentration of mesophiles. The same can be observed for psychrophiles and LAB. Interestingly, for staphylococci, the treatment that showed higher counts was the one inoculated with *S. xylosus***L. sakei**yeast. Inoculation with the yeast strain seems to have a positive effect on the multiplication of staphylococci. Moulds were detected only in the control and in the inoculation with *L. sakei*. Regarding yeasts, there were no significant differences between treatments in any case. Enterobacteria showed no significant differences between treatments. When *L. sakei* was inoculated alone or combined with *S. xylosus*, elimination of *L. monocytogenes* was effective; however this effect seems to be lost in the co-inoculation with yeast. *Salmonella* spp. was not detected in any of the treatments.

Table III general indicate that biogenic amines contents of inoculated treatments were lower than of control. Tryptamine was statistically lower in the treatments with *S. xylosus* and *S. xylosus***L. sakei* when compared to the control. The treatment with yeast increased the concentration of tryptamine and was the only one that was not statistically lower than the control for **β**phenylethylamine. For putrescine, the treatment with *S. xylosus* and *L. sakei* were statistically lower than the control. The treatment with yeast and *S. xylosus***L. sakei* were statistically lower than the control for cadaverine. The last mentioned treatment, for histamine, were the only statistically lower than the control. *L. sakei* and *S. xylosus***L. sakei* were significantly reduced the content in tyramine.

No significant differences between treatments were observed for spermine and spermidine. Inoculation generally decreased the contents of putrescine, cadaverine and tyramine by, approximately, 20%, when compared to control. For histamine the reduction was, approximately; 70%, for treatment *S. xylosus***L. sakei,* when compared to the control. In the present work, cadaverine and putrescine were the most abundant biogenic amines, followed by tyramine. Latorre-Moratalla et al. (2010) and Singh et al. (2012) reported that, in general, tyramine, putrescine, cadaverine and histamine are the amines most frequently detected in fermented meat sausages. On the other hand, VidalCarou et al. (2015) reported that tyramine is the most abundant biogenic amine in fermented sausages.

Table III. Biogenic amines for three independent batches of end-product *Paio* inoculated with starter cultures (Aminas biogénicas en tres lotes independientes de producto final *Paio* inoculado con cultivos iniciadores).

	Treatments				
Biogenic amines (mg/kg)	Control	*S. xylosus*	*L. sakei*	*S. xylosus***L. sakei*	*S. xylosus***L.sakei**yeast
Triptamine	26.21^{b}±5.59	14.73^{c}±4.61	19.28^{bc}±5.20	15.70^{c}±6.08	35.60^{a}±16.43
β - phenylethylamine	4.80^{a} ±0.83	3.85^{b}±0.28	3.98^{b}±0.70	3.69^{b}±0.51	5.16^{a}±0.57
Putrescine	329.11^{a}±50.82	255.70^{b}±90.69	270.25^{b}±50.34	278.92^{ab}±65.91	283.86^{ab}±77.31
Cadaverine	439.42^{a}±98.35	407.69^{ab}±47.15	403.29^{ab}±71.73	353.27^{b}±49.91	360.81^{b}±89.05
Histamine	10.58^{ab}±8.01	10.13^{ab} ± 5,03	8.20^{b} ± 6.96	3.17^{c}±2.20	12.96^{a}±3.92
Tyramine	113.99^{a}±32.99	108.80^{ab}±40.96	89.72^{b} ± 16.95	88.44^{b}±21.49	94.40^{ab}±19.52
Spermidine	11.02±1.38	10.86±1.34	10.78±1.12	11.37±0.94	10.82±0.78
Spermine	37.88±10.92	35.97±10.21	34.40±9.31	38.16±5.95	35.29±6.24

Data are expressed as means ± SD. In the same row, different letters indicate significantly different values (P<0.05).

Table IV. Colour parameters for three independent batches of end-product *Paio* inoculated with starter cultures (Parámetros de color en tres lotes independientes de producto final Paio inoculado con cultivos iniciadores).

	Treatments				
Parameters	Control	*S. xylosus*	*L. sakei*	*S. xylosus*L. sakei*	*S. xylosus*L. sakei**yeast
L*	42.32^{a}±4.63	43.41^{a}±5.03	41.32^{ab}±4.26	42.00^{a}±4.66	38.14^{b}±5.24
a*	18.58±2.86	19.43±3.66	19.15±3.80	19.13±2.97	18.37±2.61
b*	15.64±5.00	15.72±5.26	15.87±5.53	16.26±4.40	15.02±4.79
C*	24.44±6.74	25.14±5.81	25.00±6.17	25.21±4.79	23.90±4.62
H°	39.16±6.74	38.13±5.91	38.69±6.14	39.80±5.31	38.55±6.42

Data are expressed as means ± SD. In the same row, different letters indicate significantly different values (P<0.05).

Table V. Texture profile analysis for three independent batches of end-product Paio inoculated with starter cultures (Análisis del perfil de textura en tres lotes independientes de producto final Paio inoculado con cultivos iniciadores).

	Treatments				
Parameters	Control	*S. xylosus*	*L. sakei*	*S. xylosus*L. sakei*	*S. xylosus*L. sakei**yeast
Hardness (N)	63.169^{a}±15.151	49.606^{c}±10.171	58.404^{ab}±14.308	52.785^{bc}±9.826	51.220^{bc}±11.199
Adhesiveness (N·s^{-1})	-3.398±1.741	-2.778±1.529	-2.837±1.852	-3.003± 1.827	-2.629±1.553
Cohesiveness	0.594^{ab}±0.035	0.600^{ab}±0.053	0.622^{a}±0.058	0.581^{b}±0.044	0.609^{ab}±0.046
Springiness	0.881±0.094	0.913±0.097	0.901±0.173	0.889±0.070	0.966±0.256
Resilience (N·s)	0.133^{ab}±0.014	0.134^{ab}±0.029	0.144^{a}±0.022	0.128^{b}±0.025	0.136^{ab}±0.016
Chewiness (N)	33.325^{a}±10.504	27.036^{b}±6.168	32.158^{ab}±8.002	27.192^{b}±5.355	29.777^{ab}±8.926

Data are expressed as means ± SD. In the same row, different letters indicate significantly different values (P<0.05).

For colour **(Table IV)**, only L* showed statistically significant differences.The treatment with yeast was the darkest, and the control, *S. xylosus* and *S. xylosus* L.sakei* were the lightest. Talon et al. (2007) and Ravyts et al. (2012) reported that starter cultures contribute to the improvement of colour. However, Essid & Hassouna (2013) and Whang et al. (2015) in their studies report that colour values of fermented sausages were only affected by ripening time and not by inoculation with starter cultures.

Table V indicates that hardness values was tendentiously higher in the control treatment. For cohesiveness and resilience, the treatment with *L. sakei* showed the highest values, although with no statistical significance, denoting a tendency of the meat batters to bind better. For chewiness, higher values were obtained in the control treatment, which indicates that the inoculated *Paios* were easier to chew.

To conclude, it is important to point out that when *L. sakei* was inoculated alone or combined with *S. xylosus*, elimination of *L. monocytogenes* was effective. Furthermore, it is noteworthy that inoculation generally decreased the contents of putrescine, cadaverine and tyramine.

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