Evaluation of Technological and Sensory Properties of Dry-Fermented Sausages with No Added Sodium Chloride and Enriched with Probiotics, Phytosterols, Flavonoids and Fructooligosaccharides

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Abstract—**The aim of this work was to study the effect of including phytosterols (Phy), flavonoids (Fla), fructooligosaccharides (FOS) and the probiotic strain *L. plantarum* 299V (Pro) on physicochemical and sensory properties of sliced fermented sausages with no added sodium chloride (100% NaCl molar substitution by KCl and K-Lactate). Six batches containing the probiotic strain (Pro), inoculated at ca. 10^7 cfu/g at the time of manufacturing (Pro, Pro+Phy, Pro+Fla, Pro+FOS, Pro+Phy+Fla, Pro+Phy+Fla+FOS) plus one control with no culture added were elaborated. Slices were dried by Quick-Dry-Slices (QDS®) process at 32% of weight loss. No significant differences in pH and a_w between batches were observed. The F_0 (hardness) and lightness (L*) were higher in all probiotic and prebiotic batches when compared with the control. The addition of Phy resulted in an increase in redness (a*) while the addition of FOS and Fla resulted in lower a* values when compared with the control batch. Hardness increased in all treatments compared to control, especially in sausages with higher concentration of FOS. The addition of FOS resulted in a decrease of crumbliness. No significant differences between batches were observed regarding the overall liking. Thus, the addition of probiotics, phytosterols and flavonoids to dry-fermented sausages with no added NaCl had no an important effect on quality and could be useful to produce nutritional enhanced fermented sausages.**

Keywords— **Dry-cured fermented sausages; NaCl reduction; probiotic and prebiotic addition; sensory quality; QDS®.**

I. INTRODUCTION

Functional meat derivatives could be an excellent opportunity to diversify and take up positions in an important emerging market [1]. Their positive effects are due to either addition of active ingredients (e.g. probiotics and prebiotics) or removal or replacement of substances such as NaCl. Na^+ content in meat products can be lowered by NaCl reduction or substitution others salts such as KCl, potassium lactate and/or by altering the processing techniques. KCl has similar properties to NaCl, but its addition to meat products is mainly limited by its bitter taste [2]. Even though, reduction of Na^+ in meat products is possible from a technological and sensorial point of view [3, 4], commercial application of probiotic in fermented sausages is not common yet. In addition, no information is available on the effect of including prebiotic on physicochemical and sensory properties of dry-fermented sausages with no added NaCl. Therefore, the aim of this work was to study the effect of including a probiotic strain *L. plantarum* 299V, phytosterols, flavonoids, and fructooligosaccharides on physicochemical and sensory properties of sliced fermented sausages with no added NaCl (100% NaCl molar substitution by KCl and K-Lactate) dried with QDS process.

II. MATERIALS AND METHODS

A. Preparation of fermented sausages

Seven independent batches of sausages were elaborated. A mixture of lean (71.4 %) and fat (11.0 %) meat was minced (model PM114, Castellvall S.A., Castellar del Vallès, Barcelona. Spain) at 12 mm and mixed under vacuum condition with KCl, K-lactate Vol. 78 %, nitrite, nitrate, GDL, lactose, dextrose, isolate soy protein, spices, antioxidant (E-316), carmine (E-124), starter (*Staphylococcus spp*.) at 0 ºC for 2 min in a mixer (model AVT50, Castellvall S.A., Castellar del Vallès, Barcelona. Spain). After that, probiotics and/or prebiotic were added and mix for an additional minute. Samples were stuffed into 70-mm diameter collagen casings (1.5 kg of weight), fermented at 21–23 ºC and a relative humidity of 90–95% until the pH (Crison Instruments, S.A., Alella,

Spain) decreased to 5.3. Sausages were then vacuum packed (model EV-25, Tecnotrip S.A., Terrassa, Spain) in PA/PE plastic bags (Sacoliva S.L., Castellar del Vallés, Spain) and frozen at -10 ºC for at least one week.

B. Dry cured process

Frozen sausages were sliced at 1.6 mm-thick for QDS process® [5]. Each batch for drying contained 80 slices from the central part of 4 different sausages per batch. Drying time was 34 ±2 min (to reach a weight loss of 32 %). The initial temperature of the slices was around -0.5 ±1ºC while at the end of drying it was 15 ±2 ºC. After drying 10 slices was overlapped, vaccum packed in PA/PE plastic bags and stored in darkness at 3 ±2ºC for 15 days until the analysis were carried out.

C. Colour analysis

Instrumental colour measurements were recorded on the upper and lower surfaces of 4 packs for L* (lightness), a* (redness), and b* (yellowness) using a Minolta Chromameter (CR-400, Minolta, Co., Ltd., Japan) in the CIE Lab space (Commission Internationale de l'Eclairage, 1976). Two readings was taken per pack, D65 illuminant was used.

D. Instrumental texture test

One specimen from each pack was carved with scalpel into parallelepipeds of 20 mm × 20 mm × 15 mm (length × width × height). The specimens were then stored for 2 h at 3 ºC before the stress relaxation (SR) tests were carried out. The SR test was performed using a texture analyser TA.TX2 (Stable Micro system Ltd., Surrey, England) equipped with a 5 kg load cell and 50 mm diameter compression plate. The specimens were compressed to 25% of their original height and a crosshead speed of 1 mm/s was applied. The force versus time after the compression was recorded at a speed of 50 point per second for 90 s (relaxation time). The relaxation curves obtained for each specimen were normalized, i.e., the force decay Y(t) calculated as follow: $Y(t) = F_0 - F(t) / F_0$, where F_0 (kg) is the time initial force and F (t) is the force recorded after t seconds of relaxation. In order to better understand the SR test parameters it can be assumed that low F_0 and high Y_2 and Y_{90} indicate a high softness [6].

E. Physicochemical analysis

pH (Crison GLP 21, Crison Instruments, S.A., Alella, Spain) and water activity (a_w) at 25 ºC (AquaLab CX-2 instrument; Decagon Devices, Inc., Washington) of minced samples (specimens included) per duplicate were determined in each batch.

F. Sensory analysis

Six selected and trained assessors [7, 8] performed the sensory analysis on the slices of dry-fermented sausages. The generations of the descriptors was carried out by open discussion in 3 previous sessions. The descriptors retained were: acid flavour (acid flavour sensation elicited by lactic acid), bitterness (basic taste elicited by L-tryptophan), hardness (force required to bite through the sample), crumbliness (texture property characterized by ease with each a sample can be separated into smaller particles during chewing), fibrousness (the perception of long and parallel particles during chewing) and overall liking (hedonic liking for panelists). A non-structured scoring scale [9] was used, where 0 meant absence of the descriptor and 10 meant high intensity of the descriptor. Sensory evaluation was carried out in 6 sessions and an incomplete block design was used [10], where each taster assessed control and 4 different treatments in each session. Samples were coded with three random numbers and were presented to the assessors balancing the first order and the carry over effects [11].

G. Statistical analysis

Results were analyzed using the GLM procedure of SAS version 9.1 [12]. For colour and texture data, batch was included in the model as a main factor while moisture content was used as covariant. The sample was the experimental unit. Data from the Quantitative Descriptive Analysis was performed over the mean

score (5 assessors) for each sausage. The model included the batch and the taste session as fixed effects. Means were compared using Tukey test ($P \leq 0.05$).

III. RESULTS AND DISCUSSION

1. *Physicochemical parameters:* Fermentation period resulted in a decrease in pH from 5.85 ±0.02 to 5.32 ±0.03 but differences between batches were not significant ($P > 0.05$). Contrarily to a traditional drying process, the decrease of the pH was limited to the fermentation of whole pieces and it did not change with the QDS process during drying of the slices [13]. No other significant effect of probiotic and prebiotics on a_w was observed.

2. *Instrumental colour*: The probiotic (Pro) did not significantly affect instrumental colour (Table 1). L* values fluctuated between 45.9 and 48.7 with the higher values in Pro+Phy when compared with the control batch. The addition of Phy also resulted in an increase in redness (a*) while the addition of FOS and Fla resulted in lower a* values when compared with the control. Huebner et al., [14] reported that only heat treatment (85 ºC for 2 h) at low pH (pH = 4) can promote a reduction in prebiotic activity and higher brown colour formation when FOS is added due to Maillard reactions. The lower a* values observed in Fla (cacao extract) were probably due to its higher brown colour formation. On the other hand, phytosterols increased redness.

3. *Instrumental texture:* F_0, Y_2 and Y_{90} were significantly higher ($P \leq 0.05$) in all probiotic and prebiotic batches when compared with the control samples (Table 2). The inclusion of prebiotics in some dairy products can have different effects on texture depending on the structure (liquid, semi-solid or solid) and composition (fat content, concentration of other carbohydrates) of each product [15-16]. These effects could be also strongly influenced by the interaction of prebiotics with other ingredients present in the product. However, in our study, these reasons are not applicable because the composition, ingredient and drying process were similar for all batches.

Table 1. Instrumental colour

Treatments	L*	a*	b*
Control	45.9^{b}	23.8^{bc}	7.05^{b}
Pro	46.9^{ab}	24.5^{ab}	6.69^{bc}
Pro+Phy	48.7^{a}	25.1^{a}	7.51^{a}
Pro+Fla	47.1^{ab}	21.5^{e}	5.71^{d}
Pro+FOS	47.1^{ab}	23.9^{bc}	6.26^{c}
Pro+Phy+Fla	46.4^{b}	23.0^{cd}	6.59^{c}
Pro+Phy+Fla+FOS	47.1^{ab}	22.5^{de}	6.44^{c}
Root MSE	1.221	0.667	0.282
P value	0.0018	<.0001	<.0001

Table 2. Instrumental texture

Treatments	F_0	Y_2	Y_{90}
Control	1.38^{c}	0.73^{c}	1.02^{c}
Pro	1.63^{bc}	0.97^{bc}	1.26^{bc}
Pro+Phy	1.87^{ab}	1.21^{ab}	1.49^{ab}
Pro+Fla	2.17^{a}	1.51^{a}	1.80^{a}
Pro+FOS	2.16^{a}	1.49^{a}	1.79^{a}
Pro+Phy+Fla	1.99^{ab}	1.31^{ab}	1.60^{ab}
Pro+Phy+Fla+FOS	1.99^{ab}	1.31^{ab}	1.60^{ab}
Root MSE	0.172	0.171	0.172
P value	<.0001	<.0001	<.0001

4. Sensory characterization: Acid flavour was higher in all probiotic batches compare with the control probably due to higher number of lactic acid bacteria. The intensity of bitterness was very low in all batches. Sensory hardness increased in all treatments when compared to the control ones, especially in sausages with FOS added. Pro+FOS batch also resulted in a lower crumbliness when compared with the control batch. On another hand, control slices recorded lower values of fibrousness compared with the rest of batches. No significant differences regarding the overall liking were observed between the tested batches. These results are useful as a starting point for studies where KCl and K-lactate are added to reduce the Na^{+} content of dry-cured products and probiotic, prebiotics phytosterols and flavonoids are added to improved nutrition, microbial balance, and immune enhancement of the intestinal tract, as well as lower cholesterol [17].

IV. CONCLUSIONS

Addition of probiotics, prebiotics phytosterols and flavonoids to dry-fermented sausages with on added NaCl showed no important technological and sensory parameters effects and could be useful to produce nutritionally enhanced fermented sausages.

V. ACKNOWLEDGMENT

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Table 3. Sensory qualities

Treatments	Control	Pro	Pro+Phy	Pro+Fla	Pro+FOS	Pro+Phy+Fla	Pro+Phy+Fla+FOS	*Root MSE*	*P value*
Acid flavour	2.1^{b}	2.5^{ab}	2.8^{a}	2.8^{a}	3.0^{a}	2.8^{a}	2.7^{a}	0.3189	0.0092
Bitterness	1.3	1.2	1.4	1.5	1.4	1.4	1.2	0.4208	0.9259
Hardness	2.2^{c}	2.8^{b}	2.9^{b}	3.1^{b}	3.7^{a}	2.9^{b}	3.2^{ab}	0.2387	< 0.0001
Crumbliness	6.2^{a}	5.8^{ab}	5.5^{ab}	5.2^{ab}	5.0^{b}	5.9^{ab}	5.6^{ab}	0.5023	0.0466
Fibrousness	1.6^{b}	2.6^{ab}	2.6^{ab}	2.8^{a}	3.3^{a}	3.2^{a}	3.3^{a}	0.4954	0.0005
Overall liking	4.2	4.3	4.1	4.3	4.5	4.6	4.4	0.3760	0.4005