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Effects of Starter Cultures on Physicochemical Properties of Fermented Sausages

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Abstract

Fermented sausages prepared by inoculation with different starter cultures were analyzed for their physicochemical characteristics. Three types of fermented sausages were processed separately, without starter culture (control), with a commercial culture mix, and culture mix plus *Lactobacillus plantarum* (LP). On proximate analysis, two inoculated sausages showed an increase in moisture and fat contents ($p<0.05$). The inoculated sausages showed lower hardness and gumminess values ($p<0.05$) than control. The combination of starter culture with LP displayed the lowest chewiness and cohesiveness values and showed a more intensive red color ($p<0.05$). Two inoculated batches showed significantly lower pH values and water activity than control, in accordance with the increase in lactic acid bacteria ($p<0.05$). The inoculated sausages reduced the extent of lipid oxidation ($p<0.05$) and induced an increase in lauric acid, linoleic acid, eicosadienoic acid, and arachidonic acid, as well as they had a higher polyunsaturated fatty acid content and ratio of n-6 and n-3 fatty acids ($p<0.05$). The addition of LP to the starter culture in a suitable combination resulted in a positive effect on the physicochemical and microbiological attributes of fermented sausages.

Key words: fermented sausages, starter culture, *Lactobacillus plantarum*, physico-chemical trait, bacteria counts

Introduction

Fermented sausages are generated by biochemical, microbiological, physical and sensorial changes in meat mixtures during ripening (Aro et al. 2010). When sausages are fermenting and ageing, complex physicochemical reactions take place that result in decreased pH and changes in the microbial populations (Casaburi et al. 2007). Lactic acid bacteria (LAB) in fermented sausages can be present naturally or added by the manufacturer (Metaxopoulos et al. 1981). The main microbial groups of technological interest isolated from fermented sausages are LAB and *Staphylococcus* species (Corbière Morot-Bizot et al. 2006). After the commercial introduction of pure bacterial starter cultures, traditional sausage production shifted to starter culture processes to provide a controlled fermentation, especially for large scale production requirements (Incze, 1998).

Starter cultures are commonly used in manufacturing fermented sausages to ensure hygienic and safety properties by reducing the risk of pathogenic bacteria, as well as to contribute to the development of color and flavor (Casaburi et al. 2007; Casquete et al. 2012; Essid and Hassouna, 2013). Appropriate starter cultures should be selected from microorganisms that are well adapted to meat environments and more competitive because of their specific metabolic capabilities (Leroy et al. 2006). Two of the most important cultures added to starter preparations commonly used in fermentation for longer periods and at lower temperatures are *Staphylococcus carnosus* and *S. xylosus* due to their ability to delay rancidity (Hammes and Knauf, 1994). Lactic acid bacteria are the most important bacteria for producing fermented sausages among the starter culture options because they have a positive effect on the hygienic properties of the product, inhibiting the growth of pathogenic and spoilage flora by acidification (Villani

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et al. 1994). *Lactobacillus sakei, L. curvatus, L. plantarum, Pediococcus pentosaceus*, and *P. acidilactici* are starter cultures commonly used in the production of fermented sausages (Tabanelli et al. 2012).

Lactobacillus plantarum (LP) is commonly found in saliva, many fermented food products, and anaerobic plant matter, known to liquefy gelatin (Kroulik et al. 1955). Although LP may impart characteristic eating quality of fermented foods, use of LP as part of start culture has not been tried in meat products. The present study was conducted to compare the physicochemical and microbial properties of a fermented sausage produced with various starter culture combinations and without starter culture.

Materials and Methods

1. Sausage production

Fermented sausages were manufactured, one without starter culture and the other two with the addition of different starter cultures as follows: (i) control without starter culture, (ii) T1, with *Staphylococcus carnosus* (SC)+*S. xylosus* (SX)+*Debaryomyces hansenii* (DH)+*Pediococcus pentosaceus* (PP)+*Lactobacillus curvatus* (LC), (iii) T2, with *Staphylococcus carnosus*+*S. xylosus*+*Debaryomyces hansenii*+*Pediococcus pentosaceus*+*Lactobacillus curvatus*+*Lactobacillus plantarum* (LP). The 3 types of sausages were manufactured with the same ingredients and formulation. The commercial starter culture was purchased from a company (ALmi2, Germany) and *Lactobacillus plantarum* (Accession No. KCCM11621P, KCCM11622P) collected from blackberry (*Rubus coreanus*) naturally fermented for 6 days at 25 ℃ were used. LP had organic acids and lactolin as antibacterial substances showing antimicrobial activity in *Staphylococcus aureus* (data not shown).

Fermented sausages were made from ground pork, pork backfat, nitrate salt (2.5%; salt: nitrate = 99.5:0.5), pepper (0.2%), sugar (1.5%), sodium erythorbate (0.5%), sodium pyrophosphate (0.3%) and starter culture (0.05%). A commercial starter cultures (ALmi 2, Germany) dissolved in 500 mL of cool water (each 1 ml containing 10^8 CFU) were added to achieve a final concentration of 10^6 CFU/g. Pork and backfat were ground through a 5 mm diameter mincing plate, then vacuum mixed together with the other ingredients and starter cultures (depending on the batch) over 3 min. The mix was then stuffed into natural casings with a diameter of 60 mm and a length of 40 cm. Samples were dried and ripened in a laboratory smoke chamber (BTDS76P, Bradley, USA) under the following conditions of relative humidity (RH) and temperature: day 0 until day 1, 85 to 90% RH and 19±1℃; day 1 until day 2, 81 to 83% RH and 18±2℃; day 2 until day 3, 74 to 76% RH and 11±2℃. Samples were taken at the end of the ripening for subsequent analysis. All the results were expressed as the means of triplicate trials at each sampling time.

2. Proximate composition and physicochemical properties

Before analysis, the fat was manually removed from the sausages using a knife. All determinations were carried out in triplicate on homogenized samples. Moisture, fat, protein and ash were determined on samples using with a slightly modified method of AOAC (2007). The pH values of the samples were measured by blending a 10 g sample with 90 mL distilled water for 60 seconds in a homogenizer (Ultraturrax, T25-S1, IKA, Staufen, Germany). Water activity was measured using a water activity meter (Handheld Instrument HP23-AW-A Instrument, Rotronic AG, Switzerland).

Color measurements were taken with a Minolta Chromometer (Model CR-410, Minolta Co. Ltd., Japan). Commission Internationale de l'Eclairage L^*, a^*, and b^* values were determined by measurements standardized with respect to a white calibration plate ($L^* = 94.4$, $a^* = 0.313$, $b^* = 0.319$) after 30 minutes of blooming at room temperature. Color measurements for each of the three replicates were taken, always while trying to avoid areas with excess fat, and the values recorded. The TBARS values of samples were analyzed as previously described, with slight modification (Witte et al. 1970). Briefly, readings were made on a spectrophotometer (X-MA 3000, Human Ltd., Korea) at 530 nm.

Texture measurements in the form of texture profile analysis (TPA; Bourne, 1978) of the hams were conducted at room temperature with a TA-XT2 Texture Analyzer (Stable Micro Systems, Godalming, UK) and a 2,500 N load cell. The Texture Expert, version 1.20 (Spanish), computer program by Stable Micro Systems was used for data collection and calculations. The samples were 1 cm in height and 1.5 cm in diameter. Each sample was compressed axially in two consecutive cycles of 50% compression with a flat plunger 50 mm in diameter (P50), with 5 s between cycles. The cross-head moved at a constant speed of 1 mm/s. The following texture parameters were measured from force-deformation curves: hardness, springiness, cohesiveness,

gumminess and chewiness.

3. Fatty acids analysis

Total fat for fatty acid analysis was extracted using the method described by Folch et al. (1957). After thawing the samples, the lipids in a 5 g sample were extracted in chloroform/methanol (2:1), with BHT as an antioxidant (Bligh and Dyer, 1959). The methyl esters from fatty acids (FAMES) were formed using a KOH solution in methanol. The fatty acid methyl esters (FAME) were extracted with water and hexane, after which the top hexane layer containing FAME was dehydrated with anhydrous Na_2SO_4 and the extracted and dehydrated hexane was transferred to a vial for analysis. Separation and quantification of the fatty acid methyl esters was conducted using a gas chromatograph (GC, Agilent 7890N, Agilent Technologies, Seoul, Korea) equipped with a flame ionization detector automatic sample injector (HP 7693) and a DB-WAX fused silica capillary column (30 m, 0.25 mm i.d., 0.2 mm film thickness, Agilent Technologies, Seoul, Korea). Helium was applied as the carrier gas at a linear flow of 1 mL/min and the injection volume was 1 μL. The oven temperature was initially held at 180℃ for 1 min, and then increased at 2.5℃ / min to 230℃, where it was held for 12 min. The injector (split mode) and detector temperatures were maintained at 280℃. Linoleic acid (C18:2) was used as an internal standard (Catalogue number H3500, Sigma-Aldrich Inc. USA). The FAME in the total lipids were identified by comparison of the retention times with those of a standard FAME mixture (SuplecoTM 37 Component FAME Mix, Catalogue number 47885-UP, Lot number, LB-85684. Sigma-Aldrich Inc., USA). Fatty acids were expressed as a percentage of total fatty acids identified and grouped as follows: SFA, MUFA and PUFA. PUFA/SFA and n-6/n-3 ratios were calculated.

4. Microbiological analysis

Ten grams of samples from each treatment were also weighed and then homogenized with 90 mL saline solution (0.85 g/100 mL) using a stomacher (STOMACHER 400 CIRCULATOR, Seward Ltd., UK) for 3 min at room temperature. Total aerobic plate counts were analyzed according to the Standards for Processing and Ingredients Specifications of Livestock Products, Animal, Plant and Fisheries Quarantine and Inspection Agency Notification (Quarantine and Inspection Agency, 2014). Homogenized microbial extracts were serially diluted by 10-fold with saline solution. Total aerobic plate counts were enumerated on plate count agar (PCA; Oxoid, Unipath Ltd., UK) after 48 h at 36±1℃. Lactic acid bacteria were cultivated on Man Rogosa Sharpe agar (MRS; Oxoid, Unipath Ltd., UK) after incubation at 30℃ for 72 hours. All analyses were performed in duplicate, and the results were expressed as logarithm colony-forming units per gram of samples (Log CFU/g).

5. Statistical analysis

Statistical analysis was performed using the SAS program (SAS, 2002). ANOVA was used to determine the difference among treatments. Duncan's multiple range test was used to analyze differences among mean values at $p<0.05$. Experimental data in physicochemical traits were reported as mean values with the corresponding standard errors of the mean (SEM) of replicates.

Results and Discussion

1. Physicochemical characteristics

The effects of starter cultures on the proximate composition of fermented sausages are shown in Table 1. Proximate compositions were affected by the use of starter cultures ($p<0.05$). While the moisture and fat contents of sausages were higher in batches inoculated with T1 and T2 starter cultures than in the control group, protein and ash contents were lower in those with T1 and T2 starter cultures ($p<0.05$). In contrast, differences in moisture and fat values were not observed among batches in previous studies (Casaburi *et al*., 2007; Dominguez et al. 2016; Essid and Hassouna, 2013).

The water activity reached values of 0.90 and 0.86 in the samples inoculated with T1 and T2, respectively, while the control samples had a water activity value of 0.93. Our study showed that inoculated sausages had lower pH values (4.98 and 5.06) than the control (5.13) ($p<0.05$), which is in agreement with of the results reported by Yoo et al. (2015), who found pH decreased in sausage fermented with starter cultures in accordance with a dramatic increase in lactic acid bacteria. Baka et al. (2011) also investigated whether the pH reduction was due to lactic acid bacteria rapidly becoming the predominant microorganisms, producing lactic acid and leading to pH reduction. Other authors have reported similar results in dry-fermented sausages (Dominguez et al. 2016; Essid and Hassouna, 2013; Wang et al. 2015). Zhao et al. (2011) found that a combination of *Pediococcus acidilactici*, *Staphylococcus xylosus* and *P. pentosa-*

Table 1. Effect of starter cultures on proximate and physicochemical composition of fermented sausages

	Starter culture[1)]			SEM[2)]
	Control	T1	T2	
Moisture (%)	32.63[b]	34.78[a]	31.26[a]	0.53
Crude protein (%)	32.27[a]	25.92[b]	26.23[b]	1.38
Crude fat (%)	19.94[b]	28.53[a]	31.03[a]	1.26
Crude ash (%)	5.90[a]	3.82[b]	3.73[b]	0.20
Water activity	0.93[a]	0.90[b]	0.86[c]	0.02
pH	5.13[a]	4.98[c]	5.06[b]	0.05

[1)] Control: without starter culture; T1: with a commercial culture mix (*Staphylococcus carnosus+Staphylococcus xylosus+Debaryomyces hansenii+Pediococcus pentosaceus+Lactobacillus curvatus*); T2: with a commercial culture mix+*Lactobacillus plantarum*.
[2)] Standard error of the means (n=10).
[a~c] Figures with different letters within the same row differ significantly ($p<0.05$).

ceus, and a combination of *S. carnosus* and *Lactobacillus pentosus* in fermented sausages reduced the pH and increased the lactic acid content relative to a control. Low pH values confirmed the effectiveness of *L. curvatus* and *L. plantarum* in the starter cultures at inducing strong acidification (Casaburi et al. 2007; Dominguez et al. 2016; Essid and Hassouna, 2013; Zhao et al. 2011).

Texture profiles for the control and starter-inoculated sausages are reported in Table 2. Sausage inoculated with T1 and T2 starter cultures showed significantly lower hardness and gumminess than the control ($p<0.05$). Additionally, chewiness and cohesiveness values were significantly lower in T2 than in other groups ($p<0.05$). Added LP in T2 was effective in changing meat texture due to its activity to degrade collagen. Park et al. (1997) noted that sausages with starter cultures of SC plus LP, SC plus PP, and SX plus PP had higher hardness values than sausages with SC plus LP.

Instrumental color results for the control and the starter-inoculated sausages are presented in Table 2. The lowest L^* values of sausages were in batches inoculated with T2 starter cultures, whereas the highest L^* values were observed for the control group ($p<0.05$). The T2 group had higher a^* values than T1 group ($p<0.05$). The inoculated sausages showed higher b^* values than the control ($p<0.05$). The inoculated sausages had significantly lower TBARS values than the control ($p<0.05$). These results are concordant with those of Bingol et al. (2014), who found that the differences in TBARS values would be the result of decreased pH because of lactic activities of starter cultures. Bozkurt and Erkmen (2002) also observed that Sucuks made without starter culture had higher TBARS values than those

Table 2. Effect of starter cultures on texture, color, and lipid oxidaiton of fermented sausages

	Starter culture[1)]			SEM[2)]
	Control	T1	T2	
Hardness (kg/cm^2)	15.14[a]	12.15[b]	11.77[b]	0.51
Gumminess (kg/cm^2)	4.33[a]	3.18[b]	2.13[b]	0.31
Chewiness (kg/cm^2)	3.68[a]	2.84[a]	1.84[b]	0.24
Cohesiveness (kg/cm^2)	0.28[a]	0.26[a]	0.19[b]	0.02
L^* (Lightness)*	34.22[a]	31.57[b]	28.22[c]	0.70
a^* (Redness)	16.96[b]	17.95[b]	19.85[a]	0.54
b^* (Yellowness)	12.71[b]	17.82[a]	18.53[a]	1.11
TBARS (mg MDA/kg)	0.59[a]	0.43[b]	0.46[b]	0.06

[1)] Control: without starter culture; T1: with a commercial culture mix (*Staphylococcus carnosus+Staphylococcus xylosus+Debaryomyces hansenii+Pediococcus pentosaceus+Lactobacillus curvatus*); T2: with a commercial culture mix+*Lactobacillus plantarum*.
[2)] Standard error of the means (n=10).
[a~c] Figures with different letters within the same row differ significantly ($p<0.05$).

made with starter cultures.

2. Fatty acid compositions

Fatty acids were measured to evaluate the effects of starter cultures of the fermented sausages (Table 3). Regarding individual fatty acids, oleic acid (C18:1) presented the highest values, followed by palmitic (C16:0), stearic (C18:0), and linoleic (C18:2) acids. The sum of these four fatty acids represented between 88 and 92% of total fatty acids. Individual fatty acids were significantly influenced by the use of starter cultures ($p<0.05$). The inoculated batch presented higher values of lauric acid (C12:0), linoleic acid (C18:2), eicosadienoic acid (C20:2), and arachidonic acid (C24:1) than non-inoculated sausages ($p<0.05$). This could have been related to the presence of *S. carnosus*, which has higher lipolytic activity than any other inoculated microorganisms (Dominguez et al. 2016). Two inoculated batches also had significantly higher amounts of polyunsaturated fatty acids (PUFA) and ratios of n-6 and n-3 fatty acids (n-6/n-3) than non-inoculated batches ($p<0.05$). The reduced PUFA content in the control could cause a greasy, oily and rancid off-flavor (Gandermer, 2002). On the other hand, the non-inoculated batch presented higher values of stearic acid (C18:0), oleic acid (C18:1), linolenic acid (C18:3), and monounsaturated fatty acids (MUFA) than inoculated sausages.

The sausage inoculated with T2 starter cultures produced the highest percentage of myristic acid (C14:0), while the control batch produced the lowest. The main fatty acids in all batches were monounsaturated fatty acids (MUFA), followed by saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFA). A similar fatty acid profile was previously described by other authors (Gomez and Lorenzo, 2013; Dominguez et al. 2016), who noted that the MUFA was present in higher proportions

Table 3. Effect of starter cultures on fatty acid composition of fermented sausages

	Starter culture[1)]			
	Control	T1	T2	SEM[2)]
C10:0	0.08	0.07	0.08	0.02
C12:0	0.10^{b}	0.12^{a}	0.13^{a}	0.00
C14:0	1.39^{c}	1.57^{b}	1.61^{a}	0.00
C16:0	24.34	23.79	25.18	0.62
C16:1	2.62^{b}	2.64^{b}	2.89^{a}	0.02
C18:0	12.22^{a}	11.17^{b}	11.03^{b}	0.19
C18:1t	0.17	0.18	0.19	0.03
C18:1	45.90^{a}	40.12^{b}	39.00^{b}	0.58
C18:2	9.13^{b}	12.89^{a}	12.72^{a}	0.09
C18:3	0.83^{a}	0.76^{b}	0.73^{b}	0.05
C20:2	0.38^{b}	0.51^{a}	0.48^{a}	0.04
C20:3	0.09	0.11	0.09	0.01
C20:4	0.53^{a}	0.45^{b}	0.47^{ab}	0.02
C24:1	0.12^{b}	0.15^{a}	0.15^{a}	0.00
SFA[4]	38.13	36.72	38.03	2.64
UFA	59.76	57.81	56.67	3.60
PUFA	10.96^{b}	14.72^{a}	14.48^{a}	0.11
MUFA	48.80^{a}	43.08^{b}	42.19^{b}	0.59
UFA/SFA	1.57	1.57	1.49	0.09
n-6/n-3	10.99^{b}	16.86^{a}	17.35^{a}	0.18

[1)] Control: without starter culture; T1: with a commercial culture mix (*Staphylococcus carnosus*+*Staphylococcus xylosus*+*Debaryomyces hansenii*+*Pediococcus pentosaceus*+*Lactobacillus curvatus*); T2: with a commercial culture mix+*Lactobacillus plantarum*.

[2)] Standard error of the means (n=10).

[a~c] Figures with different letters within the same row differ significantly ($p<0.05$).

SFA: saturated fatty acid, UFA: unsaturated fatty acid, MUFA: monounsaturated fatty acid, PUFA: polyunsaturated fatty acid.

than the PUFA and SFA, indicating that the liberation also originates from the triglycerides that are richer in MUFA.

3. Microbiological analyses

The influence of starter cultures on the bacteria count of fermented sausages is shown in Table 4. The total plate bacteria and lactic acid bacteria counts of sausages were significantly influenced by the addition of starter cultures ($p<0.05$). Lactic acid bacteria counts were about 7.69 Log CFU/g in the control and 9.19 and 9.41 Log CFU/g in those inoculated with T1 and T2, respectively. As expected, the populations of total aerobic bacteria and lactic acid bacteria of the inoculated batch were more abundant than those of the control batch ($p<0.05$). This could be due to a decrease in pH values of the inoculated batch, which caused an increase in the bacteria count. The growth of total bacterial counts in samples inoculated with starter culture combinations appeared to be affected by acidification, probably because of the strong competitive ability of lactic acid bacteria on the rest of the endogenous bacteria (Zhao et al. 2011). These findings are in agreement with those reported by previous authors (Dominguez et al. 2016; Essid and Hassouna, 2013; Yoo et al. 2015), who noted that the populations of lactic acid bacteria were higher in sausages inoculated with starter cultures than in the control batch. The total aerobic counts closely paralleled the lactic acid bacteria, which is in agreement with the results of previous studies (Dominguez et al. 2016; Lim et al. 2008; Yoo et al. 2015). The high population of bacteria is likely because of the addition of the starter culture, in which lactic acid bacteria dominated the microflora during fermentation and ripening (Lim et al. 2008). Consequently, we showed that the addition of starter culture to fermented sausages increased the populations of total aerobic bacteria and lactic acid bacteria.

Conclusions

The addition of starter cultures had a significant effect on the proximate composition, physicochemical, and microbiological characteristics of fermented sausages. Starter cultures had activity to reduce pH of sausages induced by lactic acid production. The addition of starter cultures resulted in lower values for texture profile and reduced TBARS values. Also, the use of starter cultures led to higher PUFA and n-6/n-3 than the control. The combination of starter culture with LP can improve the quality of sausages. In particular, decreased chewiness, cohesiveness, and redness could be caused by the characteristic enzymes of LP. Each lactic acid bacterium has different activities in the quality characteristics of fermented meat products. These findings provide an update on the criteria to consider when choosing new bacterial strains to be used as starter cultures in fermented meat products.

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Table 4. Effect of starter cultures on microbiological characteristics of fermented sausages

	Starter culture[1)]			SEM[2)]
	Control	T1	T2	
Total bacterial counts (Log CFU/g)	7.84[b]	9.59[a]	9.19[a]	0.53
Lactic acid bacteria (Log CFU/g)	7.69[b]	9.19[a]	9.41[a]	0.68

[1)] Control: without starter culture; T1: with a commercial culture mix (*Staphylococcus carnosus*+*Staphylococcus xylosus*+*Debaryomyces hansenii*+*Pediococcus pentosaceus*+*Lactobacillus curvatus*); T2: with a commercial culture mix+*Lactobacillus plantarum*.

[2)] Standard error of the means (n=10).

[a–c] Figures with different letters within the same row differ significantly ($p<0.05$).

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