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Development of novel *fermented goat* sausage inoculated with *Lactobacillus plantarum* probiotic bacteria

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ABSTRACT. The current study was undertaken to evaluate the performance of *Lactobacillus plantarum* CT28 strain as probiotic culture compared to thecommercial starter cultureon the quality of fermented goat sausage samples during 28 days of ripening period. Microbial populations, parameters such aspH, water activity (aw), moisture, lipid and protein contents, color (L, a*, b*) and texture properties were analyzed, as well as the sensory characteristics.Lactic acid bacteria counts were maintained high population number (>10^8 $CFUg^{-1}$) during ripening in the sausage samples compared to the control (⩾10^7 CFU g^{-1}). The inoculation of *L.plantarum* CT28 preserved the hygienic quality of sausage sampleswith *Enterobacteriaceae*number lower <1 log $CFUg^{-1}$ throughout ripening period. The control and probioticgoat sausage samples exhibitedsimilar physicochemical parameters and composition.Also, goat sausage samples recorded a good textural attributes and appreciated sensory features.Overall, the probiotic culture was found to possess desirable technological characteristics, indicating that probiotic-fermented goat sausage sample could be a novel functional food.

Keywords: Goat meat; fermented sausage; probiotic; functional food.

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Introduction

Consumers are very interested in healthy diet in terms of the quality of food products. Especially, functional foods which could provide health-promoting and nutritional functions (Pereira et al.,2024). As a result, the demand for functional foods was be more and more improved.

Recently, high importance has been paid to develop meat products with physiological functions to promote health effects and prevent the risk of diseases. Particularly, probiotic fermented sausage samples are considered as a type of functional foods which confer benefits to human health (Lafarga & Hayes, 2017). Fermented sausage is a mixture of meat, sodium chloride, sodium nitrate, sodium nitrite, spices, antioxidants and sugar, which are homogeneously mixed and then stuffed into casings (Franciosa et al.,2018). The fermentation/maturation process occurs under controlled temperature and humidity conditions and providedehydration which contribute to the firmness, cohesiveness and safety of fermented sausage samples (Casaburi et al., 2007; Drosinos et at., 2007; Ravyts et at.,2012). Fermented sausage samples are important sources for protein, fat, essential amino acids, minerals, vitamins and other nutrients (Cruxen et al., 2019). The quality of the final product is depended to the ripening. This process is characterized by chemical reactions and physical modifications associated with the microbiological growth of the natural flora.The results of this interaction are decrease in pH, changes in the initial microflora, solubilisation and gelification of myofibrillar and sarcoplasmic proteins, proteolytic, lipolytic and oxidative phenomena and dehydratation (Casaburi et al., 2007).

Incorporation of probiotic lactic bacteriarepresents a new option to add furthervalue to meat products, as reported in various studieson their adequacies as a food matrix and their health benefits (Ayyash et al., 2019). Probiotics, mostly lactic acid bacteria (LAB), have been well applied in the food industry. The use of probiotic LAB starter cultures underscores their essential role in modern food production, contributing not only to the sensory attributes and nutritional value of fermented foods but also to their safety, stability and marketability (Nami et at.,2024).*Lactobacillus plantarum* belonging to LAB, as a probiotic, has been used in fermented sausage samplessuch as traditional chineese sausage as the starter culture for a long time (Shao et at., 2024).

In meat fermentation, its function is to generate rapid acidification of batter leading to the lower pH values with improving the microbial stability of products by inhibiting the activity of pathogens. *L. plantarum* also brings about the physicochemical and biochemical changes to attain the unique sensory features of ripe products during the maturation and fermentation (Leroy et at., 2013).

Due to its, goat meat has been established as lean meat with favorable nutritional quality and it is consumedin many countries in the world. Goat meat hasa protein content about 20 per 100 g, lean and less caloric compared to other meats. Besides, fat content is 2.6 per 100g with 0.79per 100 g of saturated fatty acids. Goat meat contains a low cholesterol level (35 mg per 100 g) which has an advantage for people concerned about low calorie and hypocholesterolemic diets (Food and Agriculture Organization [FAO], 2007).

This study was conducted to determine whether a new probiotic strain could be added to the formulation of goat sausage samples in order to improve the quality and safety of meat products with the least side effects. Therefore, the current study was designed to investigate the technological properties of fermented goat sausage samples, to detect the usability of probiotic strain compared to the commercial starter cultureand to verify the microbiological, technological, texture and sensory properties of sausage samples during 28 days ofripening period.

Material and methods

Starter culture condition

The traditional starter culture (SC) for sausage fermentation (*Lactobacillus Sakei* and *Staphylococcus carnosus)* (CHR Hansen, Nienburg, Germany) was used. The probiotic strain *L.plantarum* CT28 was isolated and selected by Mahmoudiet al. (2019).

Fermented sausage preparation

Goat meats and fats were purchased fresh from a local market in Tunisia. The sausage formulation was prepared according to Essid and Hassouna (2013) with minor modifications. Briefly, the sausage formulation included 6.750 kg of goat meat (75%) (w/w) and 2.250 kg of goat fat (25%). Then, they were minced and mixed in a rotating bowl meat mixer (Rowenta, Universo, Germany) with 400 g of salt, 20 g of black pepper, 20 g of paprika, 100 g of glucose and 1 g of potassium nitrate. After mixing them, the mixture was divided into two equal batches as follows:1) first batch was inoculated with a commercial starter culture(20 g200 kg^{-1}; 7 log $CFUg^{-1}$)(SC) and 2) second one was inoculated with *L. plantarum* (CT28) (7 log $CFUg^{-1}$) (SLP). The mixtures were manually stuffed into a natural casings (25 cm of length and about 4 cm of diameter) at approximately 330 g each one and placed in a fermentation chamber (BCR, CF 1B,1420 x 1820 cell dimension, France). The sausages were fermented for five days at 24ºC and 80% relative humidity (RH). After 5 days of processing, the temperature was decreased to 14ºC for 23 days and the RH value was 80%. For. For sampling, three sausage samples of each batch at 0, 7, 14, 21 and 28 days of ripening were taken for microbiological, physicochemical, composition and textural analysis.

Microbiological analysis

LAB bacteria were enumerated on MRS agar (Biokar Diagnostics, France) after incubation at 37ºC for 48h. Also, total viable counts were enumerated on Plat Count Agar (PCA) (Biokar Diagnostics, France) after incubation at 30ºC for 48h. The number of staphylococci was determined on mannitol salt agar (Biokar Diagnostics, France) after incubation at 30ºC for 72h. *Enterobacteriaceae* were enumerated on Violet Red Bile Glucose agar (Biolife, Italy) and incubated for 24h at 37ºC (El Adab et al., 2020).

Physical attributes

Measurement of pH and water activity

The pH of fermented sausage samples was determined using the pH-meter (Microprocessor pH meter BT-500, Boeco, Hamburg, Germany).Water activity (a_w) was measured with a water activity meter (HygroLab 3, Rotronic, Croissy-Beaubourg, France)(Association ofOfficial Analysis Chemists International [AOAC], 2007).

Color determination

Color parameters of the goatsausage sampleswere determined witha Minolta CR-300 colorimeter (Minolta Chromameter Co., Ltd., Osaka, Japan).Each sausage was cut and the CIE L (lightness), a* (redness) and b*

(yellowness) parameters were measured on the inner surface and the center of the slices.

Compositional experiments analysis

Goat sausage samples were subjected to analysis of moisture and protein, according to the methodology proposed by AOAC (2007). The lipid content was determined by the methodology of cold extraction as described by Folch et al.(1957).

Texture profile analysis

Texture Profile parameters of each goat sausage was determined with TVT-6700 texture analyzer (Tex Cal, Perten Instruments, Sweden) equipped with a cylindrical probe of 50 mm in diametercompressed twice to 50% of their original thickness. Force-time curves were recorded at a crosshead speed of 1 mms^{-1} (Sousa et al.,2017). The sausages were cut in a cylinder 1 cm thick and 3 cm in diameter and the measured parameter settings were Hardness,Chewiness, Gumminess,Cohesiveness and Flexibility.

Sensory evaluation

Goat sausage samples were subjected to sensory evaluation of acceptance or preference with 30 panellists, composed from professors. Panellists were given a white plate containing sausagesamples which cut in pieces (15 g) coded with three numbers. Acceptance testing for overall acceptance, odor, red color, hardness and acidity was performed with a 10-point hedonic scale (1- I unliked extremely; 10- I liked extremely) (El Adab et al., 2020).

Statistical analysis

SPSS statistics 20.0 software was used to perform statistical analysis of the results (one-ANOVA). Tukey test ($p < 0.05$) significance level was performed to determine significant differences between the means. Data are presented as mean ± standard deviation. The experiments were carried out in triplicate.

Results and discussion

Microbiological analysis

The microbial counts of LAB, Staphylococci and Total bacterial of the goat sausage samplessamples are presented in Figure 1.

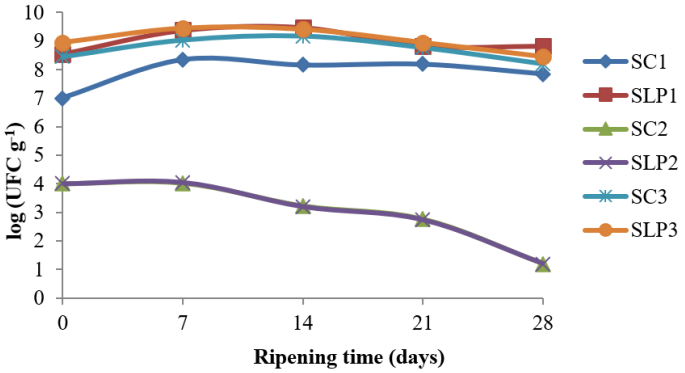

Figure 1. Evolution of microbial population during the ripening of sausage samples.SC1: Lactic acid bacteria count in sausage inoculated with starter culture (control); SLP1: Lactic acid bacteria in sausage inoculated with *L.plantarum*; SC2: *Staphylococci* count in sausage inoculated with starter culture (control); SLP2: *Staphylococci* count in sausage inoculated with *L.plantarum*; SC3: Total viable count in sausage inoculated with starter culture (control); SLP3: Total viable count in sausage inoculated with *L.plantarum*.

The total viable and LAB counts increased ($p < 0.05$) roughly 2 log cycles starting with a population of 7.01±0.77 and 8.54±0.71 log $CFUg^{-1}$ for the SC and SLP goat sausage samples samples, respectively, during the ripening period as except the final day of ripening. At day 28, LAB counts were significantly higher in the inoculated sample SLP than the control one.This indicated that the goat meat is a favorable medium for the growth of probiotics. At the same, Ayyash et al.(2019) have reported that camel meat is favorable for *L. plantarum* growth. LAB were characterized as the dominant population group and the total bacterial counts population followed the dynamics of LAB during the ripening period. The slight decrease of LAB at the end of

ripening is probably due to the decrease of fermentable carbohydrates (Lorenzo & Franco, 2012). These results were in accordance with those observed in other studies of beef and camel sausage samples(Ben Slima et al., 2018; Ayyash et al., 2019). LAB are the main agents, improving the microbiological, sensory and physicochemical quality of the final meat product (Pavliet al.,2019). The population of Staphylococci was similar in probiotic cases at the end of the ripening period compared to the control case. Specifically, the counts of staphylococci in goat fermented sausage samples were ranged from 4to 1.2 log $CFUg^{-1}$ throughout the ripening period. *Enterobacteriaceae*were below the detection limit of the enumeration method (<1 log $CFUg^{-1}$) throughout the ripening period. These results confirm the inhibitory effect of the probiotic culture due to their acidification behaviour (Mahmoudi et al., 2018) against *Enterobacteriaceae* growth, which is crucial to obtain high quality hygienic sausage samples.

Ph and water activity

The evolution of pH and water activity is presented in Table 1. The initial pH was around 6 in samples. These pH values declined significantly ($p < 0.05$) in all tested samples to reach 4.8±0.18for SLP sampleat the end of ripening. The drop in pH values suggests that the probiotic *L. plantarum* CT28 had good fermentation properties as homofermentative LAB and accelerated the fermentation process by producing more organic acids especially lactic acid as a result of carbohydrate breakdown during fermentation have promising technological characteristics in fermented meat. Moreover, the pH decrease contributes to the reduction of spoilage microorganisms, accelerates the reduction of nitrite to nitric oxide, affects the flavor of the product and improves meat binding capacity, firmness and sliceability, thus contributing to the product safety (Essid & Hassouna, 2013). The range of pH values is in accordance with those reported by Ayyash et al. (2019) and Kargozari et al. (2014).

As shown in Table 1, the water activities of goat sausage samples decreased significantly ($p < 0.05$) from 0.979±0.09 to 0.82±0.03 in SC sample, during the ripening period. These results are similar to those found by Ben Slima et al. (2018). The decrease in a_w in fermented goat sausage samples during ripening may be attributed to the loss in moisture contents and the presence of organic acids, peptides (Hughes et al., 2002) and salts. At this moment, myofibrillar proteins are closer to their isoelectric point, resulting in lower water retention capacity in fermented sausage samples (Drosinos et al., 2007).

Table 1. Evolution of physical parameters during the ripening of sausage samples.

Ripening (days)	Samples	Parameters				
		pH	a_w	Color		
				L*	a*	b*
0	SC	6.1±0.2^{aA*}	0.979±0.1^{aA}	44.7±0.02^{aA}	11.02±0.16^{aA}	29.33±0.1^{aA}
	S*LP*	6.05±0.14^{aA}	0.971±0.13^{aA}	44.±0.07^{aA}	11.04±0.1^{aA}	29.3±0.02^{aA}
7	SC	6±0.21^{aA}	0.977±0.28^{aA}	45±0.04^{aA}	26.89±0.12^{aB}	30.97±0.9^{aA}
	S*LP*	5.45±0.1^{bA}	0.976±0.4^{aA}	44.8±0.05^{aA}	26.83±0.5^{aB}	30.92±0.07^{aB}
14	SC	5.9±0.8^{aB}	0.9±0.7^{aB}	61.64±0.02^{aA}	28.6±0.17^{aC}	33.21±0.2^{aAB}
	S*LP*	5.2±0.04^{bB}	0.9±0.09^{aB}	61.58±0.03^{aB}	28.7±0.15^{aC}	33.21±0.3^{aB}
21	SC	5.87±0.2^{aB}	0.9±0.1^{aB}	57 ±0.1^{aA}	19.45±0.07^{aD}	18.44±0.2^{aB}
	S*LP*	5.08±0.3^{bB}	0.9±0.2^{aB}	57.1±0.1^{aC}	19.43±0.02^{aD}	18.41±0.1^{aC}
28	SC	5.34±0.7^{aB}	0.82±0.3^{aB}	52.2±0.2^{aA}	17.02±0.16^{aD}	17.35±0.2^{aB}
	S*LP*	4.8±0.18^{bB}	0.87±0.17 ^{aB}	52.22±0.1^{aD}	16.98±0.2^{aD}	17.35±0.1^{aC}

*: Mean values±SD (n=3);SC:sausage inoculated with starter culture (control); SLP:sausage inoculated with *L.plantarum*; Lower-case letters show the differences between the samples in the same ripening time and upper-case letters indicate differences between the ripening times of samples ($p < 0.05$).

Color

Color parameters of goat sausage samples are presented in Table 1. The inoculation of probiotic strain *L.plantarum* CT28 did not affect the color changes ($p > 0.05$).However, color parameters were affected by the ripening time of the goat sausage samples ($p < 0.05$).These results are similar to those of Ben Slima et al. (2018). For redness (a*) values,an increase ($p < 0.05$) was observed during the first two weeks of ripening of dry fermented sausage samples followed by a significantly decrease which probably due to partial or total denaturation of nitrosomyoglobin because of the production of lactic acid.In relation to L* values,an increasewas observed during the first two weeks followed by a significantly decrease which due to weight loss and higher myoglobin content (Huang et al., 2023).Considering yellowness, this parameter is associated with oxidation processes of the lipid fraction. In our study, the b* values decreased durin repening period ($p > 0.05$). The presence of probiotic and standard culture may have limited the

increase of b* index, thanks to its antioxidant effect. The observed values of yellowness agreed with those found by other authors for sausages enriched with olive leaf extract (Totaro et al., 2024).

Compositional experiments

Changes in moisture, fat andprotein contents of dry fermented sausage samplesduring ripeningare summarized in Table 2.

Table 2. Evolution of proximate composition during the ripening of sausage samples.

Ripening (days)	Samples	Parameters (%)		
		Moisture	Protein	Fat
0	SC	56.6±0.12 ^{aA*}	16.5±0.22 ^{aA}	18.05±0.22 ^{aA}
	S*LP*	56.5±0.17^{aA}	16.5±0.34^{aA}	18.2±0.34^{aA}
7	SC	53.2±0.2^{aB}	20.4±0.1^{aB}	19.86±0.62^{aA}
	S*LP*	53.4±0.21^{aB}	20.6±0.1^{aB}	19.88±0.2 ^{aA}
14	SC	44.1±0.13 ^{aB}	21.3±0.1^{aB}	26.20±0.33^{aB}
	S*LP*	44.09±0.1^{aB}	21.35±0.1^{aB}	26.21±0.2^{aB}
21	SC	39.08±0.08 ^{aB}	22.3±0.1^{aC}	32.22±0.6^{aC}
	S*LP*	39.07±0.15^{aB}	22.75±0.1^{aC}	32.25±0.3^{aC}
28	SC	22±0.4 ^{aB}	25.88±0.15 ^{aC}	35.88±0.15 ^{aC}
	S*LP*	22±0.1 ^{aB}	25.89±0.1 ^{aC}	35.89±0.1^{aC}

*: Mean values±SD (n=3);SC:sausage inoculated with starter culture (control); SLP:sausage inoculated with *L.plantarum*; Lower-case letters show the differences between the samples in the same ripening time and upper-case letters indicate differences between the ripening times of samples ($p < 0.05$).

The moisture content decreased significantly ($p < 0.05$) in all goat sausagesamples, from the initial values of about 56.6±0.12to22±0.1duringripening (Table 2). However, no significant difference ($p > 0.05$) was observed between the inoculated and the control sausage samples during ripening. These results are in line with the study of Ayyach et al.(2019) who found that moisture in fermented camel sausagewas not affected by the incorporation of probiotic strainsand it may be attributed to the lower emulsification of fat which in turn released more water during ripening.Also, Dalmis and Soyer (2008) reported that moisture content through ripening of sausage samples could be affected by both processing method and processing time.

In another hand, results showed that protein and fat contentsin control and probiotic goat sausage samplesincreasedduring ripening ($p< 0.05$). However, no significant differences ($p > 0.05$) were showed between control cases and those inoculated. The current results were similar to those obtained by Ben Slima et al.(2018) who reported that no significant effect was observed between control and probiotic samples. These data were confirmed by LAB not considered as strong proteolytic bacteria (Washington et al., 2015). Also, Ayyach et al.(2019) and Kargozari et al.(2014) reported a high fat and protein contents in camel sausage samples.

Textural profile

Five textural parameters were examined such as, hardness, chewiness, cohesiveness and gumminess, as summarized in the Table 3. The hardness, chewiness, flexibility, cohesiveness and gumminess values increased significantly during ripening period ($p<0.05$)which may be due to the lower moisture content and water activity (Hu et al., 2022). Similar results were found by Gonzalez-Fernandez et al. (2006) who reported that the increase of hardness could be explained by the elevated temperature during fermentation (24°C).

Table 3. Changes in textural parameters during the ripening of sausage samples.

Ripening (days)	Samples	Texture parameters				
		Hardness (Kg)	Chewiness	Gumminess	Cohesiveness	Flexibility
0	SC	5.2±0.3^{A}	0.4±0.1^{A}	0.57±0.7^{A}	0.5±0.1^{A}	0.47±0.2^{A}
	S*LP*	5.21±0.2^{A}	0.4±0.1^{A}	0.58±0.2^{A}	0.53±0.1^{A}	0.47±0.33^{A}
7	SC	10.4±0.3^{A}	1±0.15^{B}	1.56±0.7^{B}	0.65±0.1^{A}	0.54±0. ^{B}
	S*LP*	10.6±0.17^{A}	1.023±0.1^{B}	1.55±0.2^{B}	0.64±0.1^{A}	0.52±0.1^{A}
14	SC	16.02±0.6 ^{A}	1.2±0.1^{B}	2.3±0.2^{C}	0.88±0.1^{AB}	0.66±0.28^{C}
	S*LP*	16.72±0.2^{A}	1.27±0.1^{B}	2.6±0.1^{C}	0.9±0.1^{B}	0.63±0.16^{B}
21	SC	19.7±0.3^{B}	2±0.1^{C}	2.71±0.14^{C}	1.2±0.1^{B}	0.7± 0.2^{D}
	S*LP*	19.9±0.19^{C}	2.12±0.1^{C}	2.78±0.3^{B}	1.3±0.1^{BC}	0.7±0.1^{D}
28	SC	30.36±0.2^{B}	2.21±0.1^{C}	2.92±0.1^{C}	0.91±0.1^{B}	0.8±0.1^{D}

	SLP	30.66±0.4^{B}	2.35±0.1^{C}	2.97±0.15^{B}	0.92±0.1^{C}	0.78±0.1^{D}

*: Mean values±SD (n=3);SC:sausage inoculated with starter culture (control); SLP: sausage inoculated with *L.plantarum*. Upper-case letters indicate differences between the ripening times of samples (p<0.05).

Results showed that there were no significant differences between batches in any of the textural parameters studied(p >0.05).These results were in agreement with Ben Slima et al.(2018)and who reported that probiotics have a significant effect on the textural profile of beef sausage sample.In this context, Afraei et al. (2022) mentioned that the inoculation of *L.plantarum* and *L.fermentum* as probiotic cultures improved the texturesausages.

Sensory attributes

Sensory evaluation is essential to estimate the acceptability of a product.The median scores of the sensorial characteristics of dry-fermented goat sausage samples are shown in Figure 2. Overall, significant differences were observed between the probiotic cases and the control, in the attributes of overall acceptance, odor, red color, hardness and acidity (p <0.05). Scores of all attributes in goat sausage samples were higher than 7. The sour taste was slightly more intense in the probiotic samples. In this context, Shao et al. (2024) reported that probiotic bacteria have a positive impact on sensory characteristics of fermented sausage samples. Overall, the *L.plantarum* culturecontributed more to the development of favorable sensory properties of the fermented sausages and was suitable as a meat starter culture.

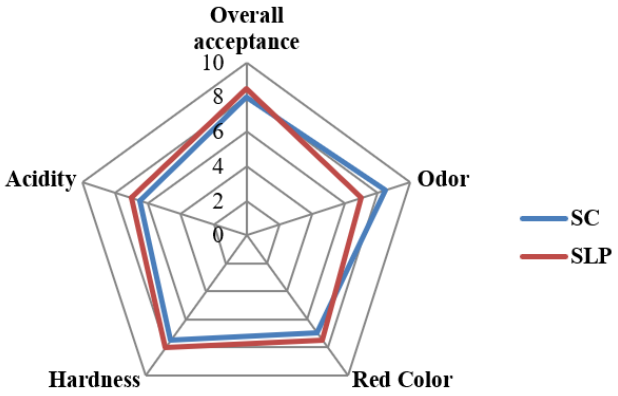

Figure2. Sensory evaluation of sausage samples.SC: sausage inoculated with starter culture (control); SLP: sausage inoculated with *L.pl*; Lower-case letters show the differences between the samples in the same ripening time (p <0.05).

Conclusion

The results of the current study demonstrated that the potential probiotic strain *L. plantarum* CT28 can be used in the fermented goat sausage samples manufacture, since it leads to similar or betterquality of sausage samples with functional properties. The probiotic strain survivedand competed well with the starter cultures and it wasdetected in adequate amounts during the ripening period (>10^{7} CFUg^{-1}). Furthermore,the probiotic sausage samples received similar or higher sensory scores compared tothe control. Inoculation of probiotic strains could be used as bioprotective strains to extend shelf-life of sausage samples.

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