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ARTICLE

Quality Characteristics of Functional Fermented Sausages Added with Encapsulated Probiotic *Bifidobacterium longum* KACC 91563

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Abstract The present study aimed at evaluating the utilization possibility of encapsulated probiotic *Bifidobacterium longum* for production of functional fermented sausages. The *B. longum* isolated from the feces samples of healthy Korean infants encapsulated with glycerol as a cryprotectant was used for fermented sausages production as a functional bacterial ingredient, and its effect was also compared with those inoculated with commercial starter culture (CSC). Results showed that most inoculated encapsulated *B. longum* (initial count, 5.88 Log CFU/g) could survive after 4 days fermentation (5.40 Log CFU/g), and approximately a half (2.83 Log CFU/g) of them survived in the products after 22 days of ripening. The products inoculated with encapsulated *B. longum* presented the lowest lipid oxidation level, while had higher total unsaturated fatty acid content and more desirable *n*-6/*n*-3 fatty acids than those inoculated with CSC or non-inoculated control. Moreover, the odor and taste scores in the samples made with *B. longum* were comparable to those in the treatment with CSC. The inoculation with the *B. longum* had no effects on the biogenic amine contents as well as did not cause defects in color or texture of the final products. Thus, the encapsulation could preserve the probiotic *B. longum* in the meat mixture, and the encapsulated *B. longum* could be used as a functional ingredient for production of healthier fermented meat products.

Keywords fermented sausages, probiotics, biogenic amine, sensory quality

Introduction

Bifidobacteria are Gram-positive bacteria which are commonly present in healthy human's intestinal tract; especially they occupy approximately 90% of total bacteria in feces of breast-fed infants and about 3% to 5% in adult fecal microbiota (Ham et al., 2011). Through the application of genomic sequencing technique, it has been shown that *Bifidobacterium longum* is absent from virulence and pathogenicity factors and it is considered as the safest bacteria without health hazards (Meile et al., 2008). The bifidobacteria have been proven to produce beneficial effects on the human host

through the prevention of intestinal colonization of pathogens and production of bioactive fatty acids etc. (Kwon et al., 2017). Till now, a significant number of human *Bifidobacterium* genera (e.g., *B. longum* and *B. infantis* etc.) have been used as the potential probiotics (Masco et al., 2005; Srůtkova et al., 2011). In our previous study, a *Bifidobacterium* strain (KACC 91563) has also been proven to show its probiotic effects such as foods allergy alleviation (Kim et al., 2013).

In the dairy products such as yogurt, cheese and fermented milk etc., the probiotic *B. longum* has been included as a functional ingredient or starter culture in order to improve the health beneficial effects and technological quality characteristics of the products (Albenzio et al., 2013; Sabikhi et al., 2014; Song et al., 2017).

One of the crucial criteria in production of probiotic products or foods containing the probiotic bacteria is that the microorganisms included must successfully survive in the undesirable conditions (e.g., acidic and salty environments) of the gastrointestinal tract or food matrix, and this is the biggest challenge for the producers. Regarding the probiotic bifidobacteria, studies conducting on the effects of various environmental conditions have reported that the environment containing greater 3.5% NaCl significantly reduced the esterase activity, and the salt resistance of *B. longum* has been found to be lower than other bacterial species such as lactobacilli (Gandhi and Shah, 2015). Also, the major challenge to the bifidobacteria is acid stress which significantly reduces their viability and probiotic effects (Wang et al., 2015).

In recent time, in order to protect the probiotic microorganisms against such adverse environments of the gastrointestinal tract and foods, the microencapsulation techniques have been applied for the probiotic products production. The nature of this technique is packaging cells in small capsules by using some coating materials which provide the living cells with physical barrier (Chavarri et al., 2010). Studies have reported that the application of encapsulation resulted in improved viability and stability as well as functionality of *B. longum* cultures during freeze-drying and storage as well as gastro-intestinal conditions (Amine et al., 2014; Chavarri et al., 2010). Alginate is among the most commonly used coating materials for encapsulating probiotic microorganisms due to its non-toxic nature (Amine et al., 2014; Chavarri et al., 2010); however, it still shows limitations such as not always improving the probiotic microorganism's survival (Chandramouli et al., 2004). In order to overcome the problem with alginate encapsulation, researchers have recently used fatty acids, glycerol or chitosan as the cryoprotectants to improve the survival of probiotic bacteria by reducing acid diffusion (Amine et al., 2014).

Though a great amount of information regarding the health beneficial effects of the probiotic bifidobacteria has been published and available, also many attentions have been paid to its applications in the dairy food products production as described above. To the best of our knowledge, however, there is no study focusing on evaluation of the applicable efficiency of the probiotic *B. longum* in fermented meat products production. Since the *B. longum* has shown its potential probiotic effects and also quality improvements in fermented dairy products (Albenzio et al., 2013; Song et al., 2017), therefore, the evaluation for utilization possibility of the probiotic bacteria for the fermented meat products production is necessary.

Thus, the main objective of this work was to evaluate the utilization possibility of encapsulated *B. longum* KACC 9156 for production of healthy fermented sausages as a functional starter culture.

Materials and Methods

Probiotic *B. longum* KACC 9156

In the present study, the probiotic *B. longum* KACC 9156 used was isolated from fecal samples of healthy Korean neonates. The whole genomic sequences and proteins-encoding sequences as well as total G+C content of the bacteria were determined as described in our previous study (Ham et al., 2011).

Preparation of microencapsulated *B. longum* starter

The *B. longum* (0.5 mL of approximately 10^6 CFU/mL stock frozen in –80°C deep freezer) was first cultured in 40 mL of Man, Rogasa & Sharp (MRS) broth supplemented with L-cysteine at 37°C for 48 h. Thereafter, the 40 mL of MRS containing the *B. longum* were sub-cultured in 3 L of the MRS supplemented with L-cysteine at 37°C for 48 h in a fermenter. The bacteria were harvested by centrifuging at 3,000×g for 10 min, and twice washed with 0.85% sodium chloride solution. The washed *B. longum* were then freeze-dried using a vacuum-freezing dryer. Finally, the freezing-dried bacteria (in powder form) were used for encapsulating using the protocol as described by Amine et al. (2014).

Fermented sausages production

Four different treatments of fermented sausages: 1 control (C: non-inoculated), 1 added with 0.02% (w/w) commercial starter culture (CSC) containing *Staphylococcus carnosus* and *Lactobacillus sakei* (Oftering, Austria), 1 inoculated with 0.02% (w/w) encapsulated *B. longum* (T1) and 1 added with 0.02% (w/w) encapsulated *B. longum* and 0.02% (w/w) *B. longum* in saline solution (T2) were prepared. In our previous examinations (data not shown), non-encapsulated *B. longum* could not survive in the fermented sausages after 2 days of fermentation. In this work, therefore, in order to determine whether the non-encapsulated *B. longum* can survive in the products during processing, the non-encapsulated *B. longum* in saline solution was also added into the T2 and considered as an extra control. All treatments (3 batches/treatment) were prepared with about 10 kg meat mixture including: 80% pork ham, 20% pork fat, 2% NaCl, 1.5% sugar, 0.2% black pepper, 0.2% polyphosphate, 0.2% sodium ascorbate, 0.01% sodium nitrite and 0.005% sodium nitrate. All the treatments were prepared on the same day and in an identical manner, following our previous procedures (Ba et al., 2016). The humidity levels set in the fermenting rooms were same for all the treatments and control as following: 15°C/90% for 18 h, 23°C–25°C/90% for 48 h, 15°C/80%–90% for 10 days and 14°C–15°C/75%–80% until the samples reached a water activity of about 0.81–0.83. At the end of the ripening/drying (22^{nd} day), the samples were collected and used for analyses.

Technological quality (pH and water activity) assessment

The pH values of the samples during processing were measured in triplicates using a pH meter (Model 340, Mettler-Toledo GmbH, Ohio, USA). Water activity (a_w) was determined using a water activity measuring instrument (Model AW SPRINT-TH 300, Novasina Co, Lachen, Switzerland) The procedures used for determinations of pH and (a_w) were the same as described in our previous work (Ba et al., 2016).

Proximate composition

The proximate compositions (protein, fat, and moisture) were analyzed using a Food Scan™ Lab 78810 (Foss Tecator Co., Ltd., Hillerod, Denmark), according to the procedure as described in our previous work (Ba et al., 2016).

Instrumental color measurement

The instrumental color was determined at different areas on the freshly cut surface of each sample using a Minolta Chroma Meter CR-400 (Minolta Camera Co., Ltd., Osaka, Japan) that was standardized with a white plate (Y=86.3, X=0.3165, and y=0.3242). Color was expressed according to the Commission International de l'Eclairage (CIE) system and reported as CIE L*(lightness), CIE a*(redness), CIE b*(yellowness), chroma and hue angle (h°). In which the chroma and hue angle were

calculated as $(a^{*2}+b^{*2})^{0.5}$ and $\tan^{-1}(b^*/a^*)$, respectively.

Textural profile analysis (TPA)

The TPA was performed using a puncture probe (7 mm diameter) attached to a texture Analyzer (Model 4465, Instron Corp., Norwood, USA). Each sample was cut into 2.54-cm long pieces; each the cube was axially compressed twice to 80% of its original height. The speed of load cell was set at 120 mm/min and the following textural parameters were calculated: hardness (kg), cohesiveness (kg*mm), gumminess (kg) and chewiness (kg*mm).

Microbiological analysis

The total lactic acid bacteria (LAB) and bifidobacteria were determined during processing (0, 4, and 22nd day). The LAB and bifidobacteria were cultured on MRS (Difco) agar and *Bifidobacterium* Selective Medium, respectively. Briefly, after removing the casing, about 10 g of each sample was taken aseptically and placed into sterile stomacher bag containing 90 mL of saline solution. The samples were then homogenized for 1 min using a stomacher (Model: Bagmixer 400 W, Interscience, France). Before plating, appropriately serial dilutions were made using saline buffer solution. Approximately 100 μL of each diluted solution in each sample was plated on the agar plate. The plates were then incubated in a 37℃- incubator for 24–48 h. Each sample was done in duplicates and total count was expressed as log numbers of colony forming units/gram (CFU/g).

Lipid oxidation

The lipid oxidation was determined by measuring the thiobarbituric acid reactive substances (TBARS) content as described by Pikul et al. (1989). Particularly, each sample (10 g) was homogenized with 35 mL of 4% perchloric acid and 1 mL of 7.5% butylated hydroxyansole (BHA) at 13,000 g for 20 s using a homogenizer (Polytron MR-2100, Kinematica AG, Luzern, Switzerland). Prior to filter through No. 1 Whatman filter paper, the volume of the homogenate was adjusted to 50 mL with 4% perchloric acid solution. Thereafter, 5 mL of each filtrate was taken and transferred to separate 50 mL- tubes and added with 5 mL of 0.02 M thiobarbituric acid solution. After mixing, the reaction mixture was heated at 80℃ for 1 h in a water-bath. Finally, approximately 1.5 mL of each sample was taken and the absorbance was measured at 532 nm using an UV-visible spectrophotometer (ProteomeLab Du-800, Brea, USA). The TBARS values were expressed as mg malonaldehyde/kg (MAD/kg) of sample. Three repetitions were applied for each sample in each treatment.

Biogenic amine

Biogenic amines were determined using the protocol as described in detail in our previous study (Ba et al., 2016). The samples were derivatized by dansyl chloride (5-dimethylaminonaphthalene-1-sulfonyl chloride, DCl) and then separated onto an Eclipse XDB-C_8 column (150 mm×4.6 mm×5 μm particle size, Supelco) connected to a high performance liquid chromatography (HPLC, Agilent 1100, Agilent Technologies, Inc., Waldbronn, Germany). The separated amines were identified by comparison of the retention times of known standards. The concentrations of the identified amines were expressed in mg/kg of sample.

Fatty acid profiles

Fatty acid compositions in samples were extracted using a solvent mixture of chloroform: methanol (2:1, v/v) as described

by Folch et al. (1957) and then the extract was methylated using the procedure of Morrison and Smith (1964). The fatty acids were separated on a capillary column (30 m×0.32 mm i.d.0.25 μm film thickness) connected to a Gas Chromatography (GC, Model Star 3600, Palo Alto, USA). The GC condition was set as follows: 250℃ for injection port and 300℃ for detector. The free fatty acids in samples were identified by comparing their retention times with those obtained from standard fatty acids. The results were expressed as relative percentages based on total peak area.

Sensory evaluation

Ten randomly-selected sausages from each the treatment were used. The panelists used were the members of Animals products Development Division, National Institute of Animal Science, Korea, and they were chosen on the basis of previous experiences in sensory evaluation of fermented meat products. The sensory samples were prepared as described in by Ba et al. (2017). Briefly, each sausage sample from the treatments was tested by 6 panelists. And six 0.3-cm thick pieces were taken from each the sausage, placed onto dishes and coded with random numbers. The sensory samples were randomly allotted into sessions and each session had 6 panelists. The panelists were served with the sensory samples in a random manner. The panelists evaluated 4 major sensory traits such as color, odor, taste and overall acceptability for each samples and rated using a 7-point scale (7=extremely like; 6=like very much; 5=like moderately; 4=neither like nor dislike; 3=dislike moderately; 2=dislike very much and 1=dislike extremely).

Statistical analysis

The obtained data was statistically analyzed using a Statistic Analysis System (SAS) package (SAS, 2007). The data were analyzed by using the General Linear Model procedure considering treatment/or processing time as the main effect. The differences between means were compared by using Duncan's Multiple Range Test, and significance was defined at ($p<0.05$).

Results and Discussion

The proximate compositions of the fermented sausage samples at the end of ripening were analyzed and found as follows: The levels of fat, moisture and protein among the treatments ranged from 37.27% to 39.93%, 19.42% to 23.37% and 28.20% to 32.02%, respectively. Statistical results showed that these compositions were not significantly different among the treatments, as the same raw materials and formulation were used for their production. In general, the levels of fat and protein contents in all the samples in this study were higher than those reported for the same product type in our previous works (Ba et al., 2016; Ba et al., 2017). These contrasting results could be related to the moisture content differences among the studies.

Technological quality traits (water activity, weight loss and pH)

Table 1 presents the weight loss percent and water activity (a_w) of the samples at the end of ripening, and the pH values in the samples during the processing periods (0, 4, and 22 days). The a_w-values among the treatments ranged from 0.80 to 0.81, and the values in all treatments were not significantly different from the control ($p>0.05$). It is well known that the water activity largely influences the shelf-life stability of food products; low a_w can increase the shelf-life stability (Fernandez-Salguero et al., 1993). The a_w-values in all the samples in the present study were lower than the values (0.85–0.87) reported for the same product type in literature (Corral et al., 2016) but similar to the a_w value reported by Ba et al. (2016). The contrasting results could be related to the differences in moisture contents in samples among the studies. The weight loss ranged among the

Table 1. Water activity, weight loss and pH in fermented sausages inoculated with the encapsulated probiotic *B. longum*

Treatment	Water activity (a_w)	Weight loss (%)	pH		
			0 day	4 day	22 day
C	0.80±0.00^{A}	51.19±0.52^{A}	6.36±0.03^{aA}	6.07±0.01^{bA}	6.03±001^{bA}
CSC	0.80±0.00^{A}	50.78±0.56^{A}	6.23±0.01^{aA}	4.86±0.01^{bD}	4.81±0.01^{bD}
T1	0.81±0.00^{A}	49.29±0.56^{B}	6.23±0.01^{aA}	5.29±0.02^{bB}	5.24±0.01^{cB}
T2	0.81±0.00^{A}	49.52±0.42^{B}	6.21±0.01^{aA}	4.98±0.01^{cC}	4.88±0.01^{bC}

$^{A–C}$ Means in the same column with different letter are significantly different ($p<0.05$).
$^{a–c}$ Means in the same row within each parameter with different letter are significantly different ($p<0.05$).
C, non-inoculated control; CSC, added with commercial starter culture; T1, added with 0.02% encapsulated *B. longum*; T2, added with 0.02% encapsulated *B. longum*+0.02% *B. longum* in saline.

treatments from 49.29% to 51.19%. The control or the treatment with CSC had approximately 1% higher weight loss level in comparison to those made with encapsulated *B. longum,* while the similar loss level was found for the two treatments made with the encapsulated *B. longum* (T1 and T2). Corral et al. (2016) reported lower weight loss levels (37%–38%) for the same product type; this could be due to the higher moisture contents (37%–38%) in samples in their study.

Regarding the pH values, no statistical differences occurred among the treatments at the processing day (0 day) ($p>0.05$). At 4th day, the pH values significantly ($p<0.05$) differed among the treatments in the following order: Control>T1 (encapsulated *B. longum*)>T2 (encapsulated *B. longum*+*B. longum* in saline)>CSC, being their mean values of 6.07, 5.29, 4.98 and 4.86. The pH values continuously declined with prolonged ripening time up to 22nd day with the same order; the values were found as 6.03, 5.24, 4.88 and 4.81 for the control, T1 (encapsulated *B. longum*) and T2 (encapsulated *B. longum*+*B. longum* in saline) and CSC, respectively. The pH variations could be related to the differences in the levels of fermenting activities by LAB or other bacterial species among the treatments. In general, the control presented the highest pH values whereas the treatment with CSC showed the lowest value after 4–22 days fermenting/ripening. These results are in agreement with the findings of Ba et al. (2016) and Sun et al. (2016), who also reported lower pH values for samples inoculated with microbial starter cultures. However, when compared to the pH values (6.03–6.07) of control samples during processing (4–22nd day) the samples made with encapsulated *B. longum* presented significantly lower values (4.84–5.29). This means that the samples inoculated with encapsulated *B. longum* showed the higher acidifying activity in comparison with the control, suggesting the fermentative efficiency of the bifidobacteria in the products. Regarding this, Nguyen et al. (2009) also reported the strong fermentative activities of *Bifidobacterium* strains in fermented milk. Similar findings have also been reported for other food products such as dairy products (e.g., cheese) inoculated with *B. longum* (Albenzio et al., 2013; Sabikhi et al., 2014; Song et al., 2017). These authors have reported that the cheese samples inoculated with *B. longum* had significantly lower pH values throughout the processing periods as compared to non-inoculated samples. In general, the pH values in all the samples inoculated with the encapsulated *B. longum* in the present study were almost similar to values (4.5–4.9) reported for the dairy products added with *B. longum* as mentioned above, and were similar to pH values (4.66–5.24) in fermented sausages made with starter cultures containing LAB (Ba et al., 2016; Kaban et al., 2009; Sun et al., 2016).

Lactic acid bacteria, *B. longum* and aerobic plate count (APC)

The changes in number of LAB and APC as well as the viable counts of *B. longum* during processing are presented in Table 2. The LAB count showed differences among the treatments on all days determined. The LAB counts ranged among the treatments from 4.83 to 6.97, 7.87 to 8.73 and 7.95 to 8.88 Log CFU/g at 0, 4, and 22nd day, respectively. It was observed that the number of LAB significantly ($p<0.05$) increased after 4 days of fermentation and then did not significantly increase

Table 2. Lactic acid bacteria, *B. longum* and aerobic plate counts (Log CFU/g) in the experimental fermented sausages during processing

	Lactic acid bacteria			*B. longum*			Aerobic plate count		
	0 d	4 d	22 d	0 d	4 d	22 d	0 d	4 d	22 d
C	4.83±0.01^{bD}	8.58±0.04^{aA}	8.68±0.04^{aB}	NA	NA	NA	5.03±0.01^{cC}	8.06±0.02^{bA}	8.61±0.02^{aA}
CSC	6.68±0.02^{cB}	8.73±0.02^{bA}	8.88±0.01^{aA}	NA	NA	NA	6.39±0.01^{aB}	6.63±0.08^{aC}	5.81±0.02^{bC}
T1	5.83±0.00^{bC}	8.37±0.03^{aA}	8.44±0.06^{aC}	5.88±0.04^{aB}	5.4±0.07^{aA}	2.83±0.05^{bB}	4.33±0.01^{cD}	7.75±0.03^{bB}	8.72±0.09^{aA}
T2	6.97±0.04^{bA}	7.87±0.05^{aB}	7.95±0.02^{aD}	7.05±0.02^{aA}	5.08±0.03^{bA}	3.17±0.07^{cB}	6.45±0.01^{cA}	7.53±0.09^{bB}	7.87±0.01^{aB}

$^{A–C}$ Means in the same column with different letter are significantly different ($p<0.05$).
$^{a–c}$ Means in the same row within each parameter with different letter are significantly different ($p<0.05$).
C, non-inoculated control; CSC, added with commercial starter culture; T1, added with 0.02% encapsulated *B. longum*; T2, added with 0.02% encapsulated *B. longum*+0.02% *B. longum* in saline; NA, not added.

during the ripening process for all the treatments, except the treatment with CSC. At the end of ripening, the LAB counts were in the following order: CSC>Control>T1 (encapsulated *B. longum*)>T2 (encapsulated *B. longum*+*B. longum* in saline), being their mean values of 8.88, 8.68, 8.44, and 7.95 Log CFU/g, respectively. In general, the samples added with CSC presented the highest LAB count after 4–22 days of ripening; and this may be responsible for the lowest pH values (Table 1) in these samples. Although the control samples presented the second highest LAB count (after the CSC treatment), however, they had higher pH values than the samples inoculated with the encapsulated *B. longum* (T1 and T2) (Table 1). This suggests that there were a high number of spontaneous non-starters LAB in the control samples (Leroy et al., 2006).

The viability of the bifidobacteria was determined during fermenting/ripening process. Our results showed that the initial viable counts of bifidobacteria (day 0) were 5.88 and 7.05 Log CFU/g for the T1 (added with 0.02% encapsulated *B. longum*) and T2 (added with 0.02% encapsulated *B. longum* and 0.02% *B. longum* in saline solution), respectively. At 4th day, the numbers of bifidobacteria tended to decrease (e.g., 5.40 and 5.08 Log CFU/g in the T1 and T2, respectively), and no significant differences occurred between these two treatments ($p>0.05$). These results signified that the non-encapsulated *B. longum* (in saline solution) added into the T2 could not survive in the meat mixture after 4 days of fermentation. After 22 days of ripening the viable counts of the bifidobacteria were found at 2.83 and 3.17 Log CFU/g for the T1 and T2, respectively and no statistical differences were found between these two treatments. When compared to the viable counts of probiotic *B. longum* in the fermented meat products in the present study, other researchers have reported higher number of added probiotic bifidobacteria (6.50–7.55 Log CFU/g) in dairy products (Albenzio et al., 2013; Song et al., 2017). The probiotic bifidobacteria are known to sensitive to the unfavorable conditions (e.g., high salt content or acidity) (Gandhi and Shah, 2015; Wang et al., 2015), and the viabilities of encapsulated bifidobacteria were significantly reduced in fruit juices or after freezing storage (Amine et al., 2014; Wang et al., 2015). This study for the first time, the bifidobacteria were encapsulated and added as a functional starter culture in the meat products, and the results confirmed that a certain number (approximately 50%) of encapsulated probiotic *B. longum* could survive in the final products. The intake of probiotic bifidobacteria or foods containing these probiotic bacteria has been associated with a variety of health-promoting benefits (Sarkar and Mandal, 2016). Moreover, fermented sausage is the most popular and widely consumed meat product in many countries (Oliveira et al., 2018). Therefore, further solutions are needed to improve the survival rate of the probiotic bifidobacteria in the fermented sausages.

APC is generally used as an important indicator indicating the microbiological quality of food products during processing and storage. Results showed that the APC showed significant differences among the treatments on all days examined, and all the control and treated samples significantly increased in the APC with increased ripening time, except the treatment with

CSC which showed a decrease. At the end of ripening, the samples made with CSC had the lowest APC (5.81 Log CFU /g), followed by the T2 (7.87 Log CFU/g), while the T1 (encapsulated *B. longum*) and the control had the highest APCs (approximately 8.6–8.7 Log CFU/g). The lower APC in the samples made with CSC or encapsulated *B. longum* (T2) could be related to their higher acidic environment (as indicated by lower pH in Table 1) because pH in an important quality trait, and in order to be considered "shelf-stable" the ultimate pH of finished products must be 5.3 or lower (Ba et al., 2016). Chaves-Lopez et al. (2015) also reported the APC of about 8–9 Log CFU/g for the same product type ripened for 28 days.

Lipid oxidation and biogenic amine

The levels of TBARS and biogenic amine in the samples determined at the end of ripening are shown in Fig. 1A and Fig. 1B, respectively. Regarding the TBARS content, its values significantly ($p<0.05$) differed among the treatments, in the following order: CSC>Control>T1 (encapsulated *B. longum*)>T2 (encapsulated *B. longum*+*B. longum* in saline), being their mean values of 1.42, 2.53, 1.11, and 0.99 mg MDA/kg sample, respectively. This means that the treatment with CSC presented the highest lipid oxidation level, followed by the control. These results agree with the findings of Ba et al. (Ba et al., 2016; Ba et al., 2017). Interestingly, both the treatments (T1 and T2) with the encapsulated *B. longum* presented the lowest TBARS contents in comparison to the control or CSC treatment, suggesting that the oxidation of lipid probably was inhibited by the added *B. longum* in these samples. Regarding this, previous studies have also reported the strong antoxidation activities of *Bifidobacterium* species isolated from infant's feces (Kim et al., 2003). When compared to the TBARS levels (1.5–3.16 mg MDA/kg) reported for the same product types (Ba et al., 2016; Chaves-Lopez et al., 2015), the samples made with the encapsulated *B. longum* (T1 and T2) presented much lower values (0.99–1.11 mg MDA/kg sample). Since the lipid oxidation has been found associated with quality deterioration and health risks (Grun et al., 2006), the inoculation of the probiotic *B. longum* therefore could enhance the lipid oxidation stability of the fermented meat products.

It has been reported that the ingestion of foods containing high amounts of biogenic amines can cause hazard to consumer's health (Latorre-Moratalla et al., 2012). In the present study, cadaverine was the unique biogenic amine found in all the samples at the end of ripening (Fig. 1B). Our finding agrees well with that of Latorre-Moratalla et al. (2017), who

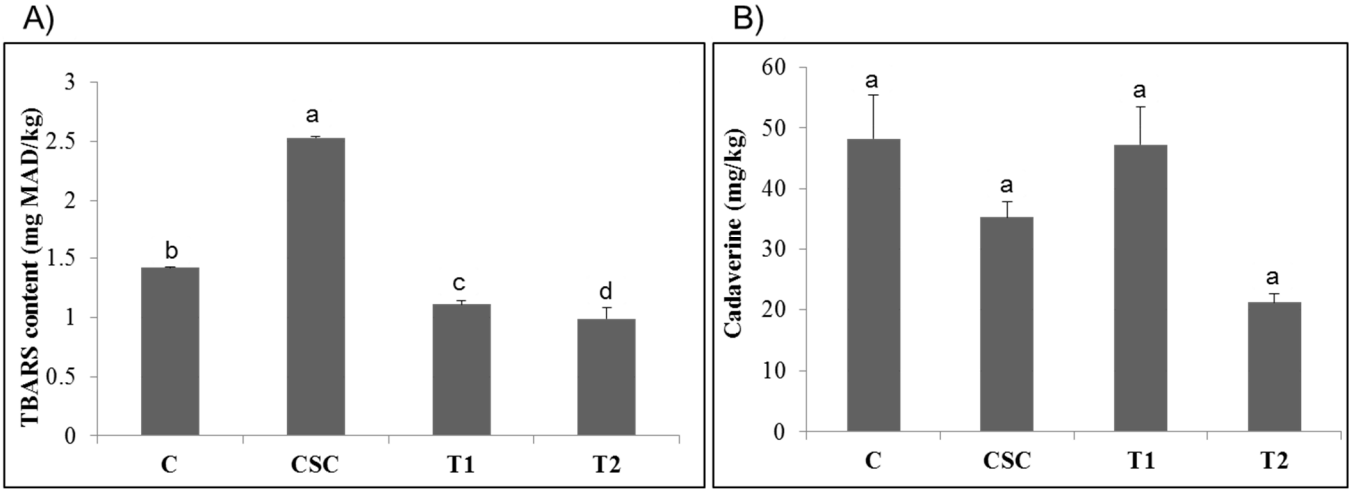

Fig. 1. The TBARS concentration (mg MDA/ kg sample) (A) and the amounts (mg/kg) of biogenic amine (cadaverine) (B) in fermented samples at the end of ripening period. [a–d] Different letters indicate significant differences between the treatments ($p<0.05$). TBARS, thiobarbituric acid reactive substances; C, non-inoculated control; CSC, added with commercial starter culture; T1, added with 0.02% encapsulated *B. longum*; T2, added with 0.02% encapsulated *B. longum*+0.02% *B. longum* in saline.

reported that cadaverine is the most frequently found biogenic amine in dry fermented sausages. The levels of the amine were 48.27, 47.26, 35.30, and 21.23 mg/kg for the control, T1 (encapsulated *B. longum*), CSC and T2 (encapsulated *B. longum*+*B. longum* in saline), respectively and no statistical differences were found among the treatments ($p>0.05$). Histamine and tyramine are the main dietary biogenic amines associated with health hazard (Latorre-Moratalla et al., 2012), however, none of them were found in all the samples in the present study, probably due to their absences or produced levels were too low under the detection limit. In general, the levels of the cadaverine detected in all the samples in the present work fell within the limits (17.12–50 mg/kg) for fermented sausages by Sun et al. (2016) and Ba et al. (2017), but were much lower than the levels (100–250 mg/kg) reported by Tabanelli et al. (2012). Although the cadaverine itself has been proven as a non-toxic compound however its presence may enhance the toxicity of other biogenic amines (Ruiz-Capillas and Jimenez-Colmenero, 2005). The biogenic amines are mainly produced from the bacterial decarboxylation of amino acids (Latorre-Moratalla et al., 2012) and a variety of microbial starter cultures such as LAB, enterococci and staphylococci etc. have been reported as the biogenic amines producers (Tabanelli et al., 2012). From the obtained results, therefore, it may be said that the *B. longum* is not an amines-producing LAB strain.

Color and textural traits of fermented sausages

The color and textural traits of fermented sausages inoculated with encapsulated *B. longum* measured at 22nd day of ripening are presented in Table 3. The samples added with encapsulated *B. longum* (T1 and T2) presented L* (lightness), a* (redness) and b* (yellowness), Chroma and hue angle - values of 49.79–51.29, 12.65–13.86, 8.27–9.48, 15.12–16.79 and 33.14–34.40, respectively. Of which the values for lightness, redness and yellowness were not significantly different from those of the control or the treatment with CSC ($p>0.05$). Additionally, the values of lightness, redness and yellowness in all the samples were almost similar to those reported for the same product type added with other CSCs (Ba et al., 2016).

Table 3. Color and textural traits of fermented sausages inoculated with encapsulated probiotic *B. longum* at the end of ripening

Items	Treatment			
	C	CSC	T1	T2
Color traits				
L* (lightness)	52.65±0.84^{a}	48.40±1.69^{c}	49.79±1.30B^{c}	51.29±1.11^{ab}
a* (redness)	13.44±1.06^{ab}	13.57±0.88^{ab}	13.86±0.49^{a}	12.65±0.61^{b}
b* (yellowness)	11.29±1.62^{a}	7.72±0.93^{c}	9.48±0.19^{b}	8.27±0.72^{Bc}
Chroma	17.56±1.82^{a}	15.62±1.14^{ab}	16.79±0.51^{Bc}	15.12±0.82^{c}
Hue angle	39.86±2.19^{a}	29.59±2.08^{c}	34.40±0.51^{b}	33.14±1.78^{b}
Textural profile				
Hardness	1.83±0.67^{c}	3.32±0.46^{a}	1.91±0.27^{c}	2.63±0.34^{b}
Cohesiveness	1.16±0.98^{a}	0.88±0.66^{a}	0.95±0.82^{a}	1.25±0.57^{a}
Springiness	18.99±0.85^{b}	20.45±0.66^{a}	20.57±0.93^{a}	21.49±0.53^{a}
Gumminess	2.43±2.62^{a}	2.95±2.34^{a}	1.81±1.59^{a}	3.19±1.43^{a}
Chewiness	44.83±7.13^{a}	60.03±7.26^{a}	37.87±4.18^{a}	68.09±9.90^{a}

$^{a–c}$ Means in the same row with different letter are significantly different ($p<0.05$).
C, non-inoculated control; CSC, added with commercial starter culture; T1, added with 0.02% encapsulated *B. longum*; T2, added with 0.02% encapsulated *B. longum*+0.02% *B. longum* in saline.

Contrastingly, Lorenzo et al. (2013) reported higher values while Ba et al. (2017) reported lower values for all these color traits of the fermented sausages samples. These contrasting results could be related to the differences in formulations, conditions and starter contents used among the studies. From the obtained results it might be said that the addition of *B. longum* did not cause any defects in the color characteristics of the final products.

Regarding the textural profiles, our results showed that except the hardness, the values for the other remaining traits such as cohesiveness, springiness, gumminess and chewiness in the samples added with the encapsulated *B. longum* (T1 and T2) were not significantly different from those of the control or the treatment with CSC ($p>0.05$).

Fatty acid profiles of the probiotic fermented sausages

In the present study, the total fatty acid compositions were determined in order to characterize and determine whether the added *B. longum* affects the lipolysis that alters the fatty acid compositions of the final products. The fatty acid profiles are presented in Table 4. The outcome of our analysis showed that fourteen fatty acids were detected on all the samples. Significant differences in levels of some fatty acids including: stearic acid (C18:0), linolenic acid (C18:3n3), erucic acid (C20:1n9) and arachidonic acid (C20:4n-6) occurred among the treatments ($p<0.05$). In particular, the level of the C18:0 was highest in the control samples whereas it was lowest in the samples inoculated with encapsulated *B. longum* (T1 and T2) or CSC. While, the polyunsaturated fatty acids (PUFA) such as C18:3n3 and C20:4n6 whose levels were significantly higher in the samples inoculated with encapsulated *B. longum* (T1 and T2) in comparison to the control or CSC treatment ($p<0.05$). On the other hand, the total unsaturated fatty acids (UFA) and *n*-6/*n*-3 ratio also showed differences among the treatments, with higher UFA levels were found in the samples made with *B. longum* (T1 and T2) than the control ($p<0.05$). From the point of view of health and nutrition, the UFA especially PUFA (e.g., C18:3n3) exert the significant effects on physiological processes and human health (Jump, 2002). According to the recommendations for the healthy diet by the American Heart Association (Krauss et al., 1996), the lower the *n*-6/*n*-3 fatty acids the healthier the diet. Our analysis showed that the *n*-6/*n*-3 ratio was significantly lower in the samples made with encapsulated *B. longum* (T1 and T2) than that in the control or the treatment with CSC ($p<0.05$). Fatty acids in fermented sausages are derived mainly from the muscle tissues and fat, and though the same raw materials, formulation and production conditions were used for all the treatments in the present study, however, the differences in levels of the fatty acids still occurred among them. This could be due to the differences in lipolysis activities by the microbial lipase (Chen et al., 2017) and lipid oxidation degrees (Fig. 1A) among the treatments during fermenting/ripening process, and the samples made with encapsulated *B. longum* (T1 and T2) presented the more desirable fatty acid profiles than those inoculated with CSC.

Sensory quality

Table 5 shows the scores for the color, odor, taste and acceptability of the samples made with probiotic *B. longum* at the end of ripening. Results showed that the panelists gave significantly ($p<0.05$) higher scores for color and odor traits for the inoculated samples than for the non-inoculated control. Especially, the treatments with *B. longum* (T1 and T2) had the color, odor and taste scores comparable to those of the ones made with CSC. Regarding the acceptability, the similar scores were given for the samples added with *B. longum* (T1 and T2) and those added with CSC ($p>0.05$). Only the treatment that showed significantly ($p<0.05$) higher acceptability score than the non-inoculated control was T2 (0.02% encapsulated *B. longum*+0.02% *B. longum* in saline solution). This could be associated with the synergistic effects of their higher odor and taste scores. From the obtained results, it may be said that the inoculation with the probiotic *B. longum* did not cause adverse

Table 4. Relative percentages of total fatty acids in fermented sausages inoculated with encapsulated probiotic *B. longum* at the end of ripening

Variable	Treatment			
	C	CSC	T1	T1
C14:0	1.51±0.11[a]	1.46±0.08[a]	1.50±0.05[a]	1.51±0.10[a]
C16:0	25.35±0.94[a]	24.86±0.51[a]	24.9±0.52[a]	24.83±0.57[a]
C16:1n7	1.23±0.06[a]	1.57±0.79[a]	1.23±0.36[a]	1.64±0.10[a]
C18:0	13.06±0.48[a]	12.2±0.14[b]	12.47±0.32[b]	12.52±0.07[ab]
C18:1n9	40.18±0.80[a]	40.56±0.49[a]	40.87±0.39[a]	40.17±0.83[a]
C18:1n7	0.17±0.02[a]	0.18±0.02[a]	0.17±0.02[a]	0.19±0.01[a]
C18:2n6	16.51±0.23[a]	17.18±0.54[a]	16.86±0.76[a]	17.2±0.31[a]
C18:3n6	0.06±0.00[a]	0.06±0.01[a]	0.05±0.01[a]	0.06±0.01[a]
C18:3n3	0.54±0.01[b]	0.54±0.01[b]	0.57±0.01[a]	0.58±0.02[a]
C20:1n9	0.87±0.01[ab]	0.89±0.01[a]	0.84±0.02[b]	0.85±0.03[ab]
C20:4n6	0.35±0.01[b]	0.32±0.01[b]	0.38±0.00[a]	0.37±0.00[c]
C20:5n3	0.01±0.00[a]	0.01±0.00[a]	0.01±0.00[a]	0.01±0.00[a]
C22:4n6	0.13±0.01[a]	0.13±0.01[a]	0.12±0.01[a]	0.13±0.01[a]
C22:6n3	0.01±0.00[a]	0.01±0.00[a]	0.01±0.00[a]	0.01±0.00[a]
SFA	39.92±0.99[a]	38.52±0.61[a]	38.89±0.64[a]	38.86±0.58[a]
UFA	59.71±0.60[b]	61.48±0.61[a]	61.11±0.64[a]	61.14±0.58[a]
MUFA	42.45±0.82[a]	43.2±0.30[a]	43.11±0.35[a]	42.84±0.76[a]
PUFA	17.42±0.12[a]	18.28±0.56[a]	18.00±0.77[a]	18.3±0.32[a]
n6/n3	30.29±0.53[ab]	31.63±0.44[a]	29.63±1.16[b]	29.67±1.11[b]
MUFA/SFA	1.06±0.05[a]	1.12±0.02[a]	1.11±0.02[a]	1.10±0.04[a]
PUFA/SFA	0.44±0.02[a]	0.47±0.02[a]	0.46±0.03[a]	0.47±0.01[a]

[a–c] Means in the same row with different letter are significantly different ($p<0.05$).
C, non-inoculated control; CSC, added with commercial starter culture; T1, added with 0.02% encapsulated *B. longum*; T2, added with 0.02% encapsulated *B. longum*+0.02% *B. longum* in saline.
SFA, saturated fatty acid; UFA, unsaturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

Table 5. Scores (7-points scale) for sensory quality of fermented sausages inoculated with encapsulated probiotic *B. longum* at the end of ripening

Traits	Treatment			
	C	CSC	T1	T2
Color	4.49±0.30[b]	5.40±0.07[a]	5.08±0.07[a]	4.98±0.13[ab]
Odor	4.19±0.27[b]	5.12±0.24[a]	5.11±0.24[a]	5.05±0.19[a]
Taste	4.25±0.25[b]	4.92±0.38[ab]	5.07±0.24[ab]	5.22±0.31[a]
Acceptability	4.19±0.33[b]	5.11±0.31[ab]	4.97±0.27[ab]	5.22±0.30[a]

[a–c] Means in the same row with different letter are significantly different ($p<0.05$).
C, non-inoculated control; CSC, added with commercial starter culture; T1, added with 0.02% encapsulated *B. longum*; T2, added with 0.02% encapsulated *B. longum*+0.02% *B. longum* in saline.

effects whereas it partially improved the eating quality of the final products.

Conclusion

The bifidobacteria were encapsulated and added as a functional ingredient and starter culture for production of functional fermented sausages. The inoculation with *B. longum* resulted in the greater acidifying activity compared to the non-inoculated control but lower than the CSC. In general, the application of encapsulation successfully preserved approximately a half of bifidobacteria viability in the final products. It was observed that the products made with *B. longum* showed lower lipid oxidation levels, and presented more desirable fatty acid profiles. Especially, the addition of the *B. longum* partially improved the eating quality, without adverse effects on technological quality of the final products. However, further study on the modifications of encapsulation technology or formulations (e.g., reducing salt level and increasing the initial added count of bifidobacteria) and processing condition etc. is needed in order to improve the viability of the probiotic bifidobacteria, thus improving the functionality of the final fermented meat products.

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