PARTIAL REPLACEMENT OF NITRITE WITH BETANIN, CHITOSAN AND ROSEMARY AS A CURING MIXTURE IN DRY FERMENTED SAUSAGE

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ABSTRACT

The effect of some nitrite-reduced meat curing mixtures on the keeping quality of dry fermented sausage during ripening and storage was investigated by measuring certain physicochemical, microbiological and sensory changes in experimental sausage samples. The data showed that pH, color, residual nitrite level and sensory attributes (except taste) were affected by curing mixtures. The statistical analysis showed that curing mixtures which included 40-80 mg nitrite/kg, 3.6-14.4 mg betanin/kg, 10 g chitosan/kg and 2 g rosemary/kg (samples B and C) had the color, presumably flavor acceptability and oxidative stability and microbial quality which are imparted by nitrite.

Keywords: Curing mixtures, Residual nitrite, Betanin, Chitosan, Rosemary

INTRODUCTION

Nitrite was and still is, one of the most widely used of all food additives. Nitrite as a traditional agent for curing meat products has multifunctional properties: it imparts the characteristic pink color to cooked cured meat products, it contributes to the typical flavor of cured meat products, it acts as an antioxidant and most importantly, it has a strong antimicrobial effect for microorganisms in general, particularly against the growth and toxin formation of *Clostridium botulinum* in meats (Honikel, 2008). However, unfortunately, during the last 30 years nitrite has become the source of serious concerns. Nitrite may react with amines, amides and amino acids present in meats, leading to the formation of carcinogenic N-nitrosamines in meat. Moreover, the residual nitrite present in cured meat may also lead to the formation of carcinogenic N-nitrosamines in the gastrointestinal tract (Marco *et al.,* 2006). Schweinsberg and Bürkle (1985) reported that nitrite enhances the carcinogenic action of N-nitroso-N-methylbenzylamine in the production of esophageal tumors.

To overcome these potentially serious problems, several approaches have been considered by researchers. Because the rate, and therefore the amount, of nitrosamine production depends on the square of the concentration of the residual nitrite in meats (Shahidi and Pegg, 1992), a reduction in the level of nitrite addition to meats has proved to be an effective measure in reducing the risk of nitrosamine formation. Therefore, it is desired goal to find suitable alternatives for nitrite.

Because the possibility of finding a single compound to mimic all functions of nitrite is remote, Shahidi and Pegg (1992) proposed the use of composite non-nitrite curing mixtures for duplicating the cumulative action of nitrite.

The aim of the present research was to develop a multi-component curing system in which individual constituents are used to produce the color, and flavor imparted by nitrite and to reproduce its antioxidant and antimicrobial effects.

MATERIALS AND METHODS

Materials

Betanin was obtained from Sensient Food Colors (Geesthacht, Germany). Chitosan 75-85% deacetylation was obtained from Sigma-Aldrich GmbH (Steinheim, Germany) and Rosemary was purchased from local market (Hannover, Germany). All media used in microbiological analysis were obtained from Merck (Darmstadt, Germany).

Preparation of dry fermented sausage

Sausage samples were manufactured according to the method described by Ansorena and Astiasaŕan (2004). sausages were made from lean beef (75%) and beef fat (25%). The following additives were added in g per kg quantities to the meat mixture: sodium chloride (28), sucrose (2), white pepper (2), black pepper (1), coriander (0.5) and commercial starter culture (1) (S-B-61, Bactoferm, Germany) which containing *Staphylococcus carnosus*. Ingredients were mixed in a cutter (Seydelmann, Stuttgart, Germany), particle size being reduced to about 3 mm, and the sausage mixture was divided into four batches (15 kg each). A=sodium nitrite 125 mg/kg which used as a control, B=sodium nitrite 80 mg/kg, betanin 3.6 mg/kg, chitosan 10 g/kg and rosemary 2 g/kg, C=sodium nitrite 40 mg/kg, betanin 14.4 mg/kg, chitosan 10 g/kg and rosemary 2 g/kg and D=betanin 21.6 mg/kg, chitosan 10 g/kg and rosemary 2 g/kg. After mixing meat mixture with treatment additives, the sausage mixture was stuffed into collagen casings (Naturin R2, Naturin GmbH & Co., Weinheim, Germany) of 60-64 mm diameter under aseptic conditions using a filling maschine (Heinrich Frey Maschinenbau GmbH, Herbrechtingen, Germany), the final weight for each sausage being 900 to 950 g. The sausages were fermented and matured in a chamber (Wilhelm Fessmann GmbH U. Co., Winnenden, Germany), at 80-96±1% relative humidity (RH %) and 14-20±0.5 ºC during 28 days, then stored at 4±1 ºC for 120 days.

Sampling

Proximate analyses (moisture, protein, fat and ash) were determined only in the initial meat mixture. For tested parameters (moisture, pH, TBA, color, and nitrite), determinations were performed on 0, 1, 7, 14, 21 and 28 days of ripening and 30, 60, 90 and 120 days of cold storage, microbial examinations were performed on 0, 1, 4, 7, 14, 21 and 28 days of ripening and 30, 60, 90 and 120 days of storage and sensory evaluation was performed directly after ripening

and after 30, 60, 90 and 120 days of storage. All analyses were carried out in duplicate.

Physicochemical analysis

Moisture, crude protein and ash contents were determined according to AOAC (1990). The fat content was determined according to soxhelt method as described by Amtliche Sammlung von Untersuchungsverfahren nach § 64 Lebensmittel und Futtermittelgesetzbuch (LFGB) (1980). pH value was determined according to the method of Bozkurt (2006). TBA value was determined according to the method described by Shahidi *et al.* (1987). The color of the sausage samples was measured using Minolta Spectrophotomater CM-2002 (Minolta Camera Co. Ltd., Osaka, Japan), the measurements were repeated on five randomly selected locations of each sample. Residual nitrite was determined according to the method of Amtliche Sammlung von Untersuchungsverfahren nach § 64 LFGB (1990).

Microbiological analysis

Ten grams of the sample was aseptically weighed into a sterilized plastic bag containing 90 ml diluent (8.5 g sodium chloride and 1 g casein peptone diluted to 1 L distilled water). Each sample was homogenized with a stomacher 400 lab blender (Fa. Seward Medical, London, UK) for 2 minutes and the suspension was used as 10^{-1} dilution. Serial decimal dilutions were made of sample solution (10^{-2} to 10^{-7}). Total plate count was counted on standard nutrient agar, incubated at 37 ºC for 2 days. Dilutions were spread-plated in duplicate on crystal-violet neutral-red bile dextrose agar (VRBD) for *Enterobacteriaceae*, de Man, Rogosa and Sharpe (MRS) for lactic acid bacteria, *Escherichia coli* direct agar (ECD) for *E. coli* and yeast extract glucose chloramphenicol agar (YGC) for yeast and mould. Plates were incubated at 30 ºC for 2 days for *Enterobacteriaceae*, 25 ºC for 3 days for lactic acid bacteria, 37 ºC for 2 days for *E. coli* and 25 ºC for 4 days for yeast and mould.

Sensory evaluation

The organoleptic quality attributes (appearance, color, texture, odor and taste) of the control and treated samples were evaluated initially and periodically during storage. Sensory evaluation was performed with 10 trained panel members, Institute of Food Quality and Food Safety, University of Veterinary Medicine, Hannover, Germany. The panelists were asked to evaluate each attribute on a 5-point scale: 1, very poor; 2, poor; 3, acceptable; 4, good; 5, very good according to the method described by Byun *et al.* (2001).

Statistical analysis

Significant differences between the mean values of estimated testes were measured according to Strotmann *et al.* (2008).

RESULTS AND DISCUSSION

Physicochemical analysis

Mean percent contents of moisture, protein, fat and Ash for the meat mixture used for the preparation of experimental sausages were 59.5%, 16.3%, 20.1% and 3.0%, respectively. These results are in accordance with the range of values reported by Ambrosiadis *et al.* (2004) for Greek traditional sausages.

Results in Fig. (1a) show that moisture content rapidly decreased during ripening period from 59.3% (day of preparation) to 34.5% (28^{th} day). Afterwards it decreased slowly and rather linearly to 25.9% on the 120^{th} day of storage. No significant differences ($p>0.05$) were found between treatments throughout the ripening storage periods. Similar results were observed by Bozkurt and Erkmen (2007) for Turkish dry fermented sausage.

Results in Fig. (1b) show that samples containing chitosan (B, C and D) showed similar ($p>0.05$) pH values throughout the ripening and storage periods, but significantly ($p<0.05$) higher compared to the control (A). This could be attributed to chitosan's pH value of 7.2 (Georgantelis *et al.,* 2007a). During the first 14 days of ripening, the pH values of all sausage samples decreased from 5.54 to 5.01 owing to the increase in lactic acid content, as a result of carbohydrate (dextrose and lactose) breakdown by microbial metabolism (Komprda *et al.,* 2001). Thereafter, the pH values increased during further ripening and storage. This increase could be explained by an accumulation of non-protein nitrogen and amino acid catabolism products (Pérez-Alvarez *et al.,* 1999). These results coincided with those described by Fernández-López *et al.* (2008).

Results in Fig. (1c) show that the TBA value of all sausages increased during ripening and storage indicating a progressive oxidation of lipids. Bozkurt and Erkmen (2007) found that the TBA value of dry sausages increased during ripening period. Aguirrezabal *et al.* (2000) found that the TBA value increased to 7 mg/kg after 30 of storage. The results for B and C samples reflect the effect of combination between nitrite, chitosan and rosemary on delaying the increase of TBA value comparing with the results of control and D sample.

Data in Fig. (1d) indicate that at any sampling time during ripening and storage the control sample (A) showed significantly higher ($p<0.05$) levels of residual nitrite than those observed in the other samples (B, C and D). The residual nitrite levels decreased sharply during the first 7 days of ripening and decreased slowly during further ripening and storage. This result might be due to the reduction of nitrite to nitric oxide and to the oxidation of nitrite to nitrate. Alley *et al* (1992) observed that in the first stages of fermentation, more than 50 % of nitrites that disappeared were transformed into nitrates. The rapid decrease in nitrite level observed in dry sausages is well documented (Samelis *et al.,* 1998 and Bozkurt and Ermen 2004).

Changes of *L*, *a* and *b* values in sausage samples during ripening and storage are shown in Table (1).

Fig. (1). Effect of different curing mixtures on physicochemical properties of dry fermented sausage during ripening and storage.
A: control sodium nitrite 125 mg/kg, B: sodium nitrite 80 mg/kg + betanin 3.6 mg/kg + chitosan 10 g/kg + rosemary 2 g/kg, C: sodium nitrite 40 mg/kg + betanin 14.4 mg/kg + chitosan 10 g/kg + rosemary 2 g/kg, D: betanin 21.6 mg/kg + chitosan 10 g/kg + rosemary 2 g/kg.
*** Ripening time (fermentation stage for 7 days and drying stage for 21 days)**

The control sample (A) had the highest *L* values among all treatments during ripening and storage, while sample (D) had the lowest values. This finding is mainly due to the effect of betanin on *L* values. Similar results were observed by Bloukas *et al.* (1999). A decreasing trend was observed as regards to *L* values during ripening and storage periods. These results are in agreement with those observed by Chouliara *et al.* (2006) who mentioned that *L* values decreased with time of ripening. Also, Zarringhalami *et al.* (2009) found that *L* values of sausages decreased during storage. Significant differences ($p<0.05$) between the experimental treatments (B, C and D) and control (A) for *a* values during the whole ripening period. On the day of stuffing sample (D) had the highest *a* value, while control sample had the lowest value. During ripening the *a* values of the control increased as the time of ripening increased. This might be due to the reaction of nitric oxide with myoglobin to form the red nitrosylmyoglobin (Honikel, 2008). In contrast, *a* values of samples B, C and D decreased as the time of ripening increased, which is attributed to betanin degradation in the presence of oxygen (Herbach *et al.,* 2006). From the data presented in (Table 1) it could be noticed that *b* values were higher when betanin level was higher. This behavior is similar to that observed by Bloukas *et al.* (1999). During ripening and storage the *b* values of all samples were found to progressively decrease reaching its maximal decrease after 120 days of storage. The obtained results are in good agreement with those of Georgantelis *et al.* (2007b).

3.2. Microbiological analysis

The microbiological analysis of sausages was determined during ripening and cold storage to evaluate its keeping quality.

Data presented in Table (2) indicate that TPC in all treated samples was gradually increased during the first 21 days of ripening. Thereafter, the TPC decreased during further ripening and storage. Similar results were observed by Bozkurt and Erkmen (2004) who reported that aerobic plate count increased during the first 8 days ripening owing to the high relative humidity RH (75-90%) and temperature (18-25 ^{o}C). After 8 days of ripening, APC decreased as RH decreased from 60-75 %. The decreasing trend of APC continued during storage because of the low RH, which was kept constant at 50% in this period. The counts of *Enterobacteriaceae* increased during ripening process from initial values 2.35 to 3.75 log CFU/g (day 14). Thereafter, *Enterobacteriaceae* counts decreased during further ripening. These increases could be related to the availability of nutrients and with pH, a_w and the ratio of salt/moisture being favorable for the growth of these organisms. The counts of *Enterobacteriaceae* showed a continuous decrease during storage reaching its maximal decrease after 30 days of storage. *Enterobacteriaceae* was not observed in any samples taken at 60, 90 and 120 days of storage. At the beginning of fermentation counts of LAB were lower than count of TPC, at the end of ripening LAB were the predominant bacteria.

T1

Table (2). Effect of different curing mixtures on microbiological profiles of dry fermented sausage during ripening and storage.

Time (days)	Treatment	Total plate count	Enterobacteriaceae	Lactic acid bacteria	Yeast and mould	*E. coli*
Ripening*						
0	A	5.76±0.078a	2.35±0.028a	3.90±0.049a	3.02±0.028a	1.85±0.212a
	B	5.70±0.042a	2.32±0.042a	3.72±0.085a	3.04±0.021a	1.78±0.424a
	C	5.80±0.085a	2.33±0.021a	3.80±0.099a	3.08±0.049a	1.80±0.424a
	D	5.72±0.035a	2.40±0.035a	3.88±0.078a	3.07±0.042a	1.90±0.212a
1	A	6.33±0.071a	2.58±0.127a	4.00±0.092ab	3.12±0.021a	1.70±0.283a
	B	6.28±0.035a	2.56±0.044a	3.88±0.035a	3.14±0.035a	1.50±0.071a
	C	6.42±0.057a	2.62±0.021a	3.98±0.092ab	3.15±0.035a	1.65±0.354a
	D	6.49±0.085a	2.70±0.057a	4.18±0.077b	3.17±0.014a	1.75±0.071a
4	A	7.14±0.099a	2.95±0.057a	8.09±0.050a	3.28±0.035a	1.40±0.212a
	B	7.09±0.085a	2.87±0.078a	8.07±0.042a	3.20±0.042a	1.35±0.212a
	C	7.13±0.057a	3.04±0.078a	8.06±0.057a	3.22±0.113a	1.38±0.283a
	D	7.18±0.078a	3.07±0.085a	8.11±0.042a	3.31±0.014a	1.43±0.106a
7	A	7.68±0.453a	3.20±0.149a	8.28±0.028a	3.45±0.113a	1.15±0.085a
	B	7.66±0.389a	3.14±0.099a	8.17±0.014b	3.40±0.099a	1.10±0.212a
	C	7.70±0.141a	3.24±0.078a	8.18±0.028a	3.43±0.085a	1.20±0.198a
	D	8.00±0.085a	3.28±0.127a	8.35±0.028b	3.52±0.198a	1.12±0.127a
14	A	8.00±0.276a	3.71±0.099a	8.30±0.014a	2.21±0.071a	1.05±0.099a
	B	7.93±0.198a	3.57±0.050a	8.20±0.007bc	2.03±0.046a	1.00±0.112a
	C	8.03±0.297a	3.73±0.085ac	8.25±0.050ac	2.28±0.212a	1.03±0.127a
	D	8.16±0.149a	3.87±0.057bc	8.41±0.057b	2.59±0.665a	1.10±0.212a
21	A	8.11±0.057a	3.37±0.092a	8.38±0.028a	1.98±0.071a	ND
	B	8.13±0.071a	3.30±0.042a	8.30±0.042a	1.80±0.050a	ND
	C	8.15±0.050a	3.41±0.014a	8.31±0.035a	1.89±0.092ab	ND
	D	8.33±0.099a	3.52±0.085a	8.35±0.007b	2.14±0.092b	ND
28	A	8.06±0.149a	2.47±0.127a	8.40±0.014a	1.96±0.092a	ND
	B	8.04±0.240a	2.43±0.045a	8.35±0.021a	1.75±0.085a	ND
	C	8.11±0.219a	2.52±0.035a	8.39±0.035a	1.83±0.064a	ND
	D	8.24±0.106a	2.53±0.042a	8.62±0.028b	2.00±0.092a	ND
Storage	A	8.06±0.149a	2.47±0.127a	8.40±0.014a	1.96±0.092a	ND
	B	8.04±0.240a	2.43±0.045a	8.35±0.021a	1.75±0.085a	ND
0	C	8.11±0.219a	2.52±0.035a	8.39±0.035a	1.83±0.064a	ND
	D	8.24±0.106a	2.53±0.042a	8.62±0.028b	2.00±0.092a	ND
30	A	8.08±0.092a	ND	8.40±0.042a	1.51±0.085a	ND
	B	8.00±0.156a	ND	8.32±0.035a	1.41±0.177a	ND
	C	8.14±0.057a	ND	8.37±0.021a	1.62±0.085a	ND
	D	8.21±0.050a	ND	8.60±0.007b	1.69±0.106a	ND
60	A	7.93±0.085a	ND	8.38±0.035a	1.21±0.078a	ND
	B	7.86±0.127a	ND	8.27±0.042a	1.08±0.106a	ND
	C	7.92±0.035a	ND	8.35±0.071a	1.22±0.085a	ND
	D	8.00±0.035a	ND	8.41±0.021a	1.28±0.113a	ND
90	A	7.89±0.120a	ND	8.15±0.042a	1.00±0.028a	ND
	B	7.82±0.099a	ND	8.07±0.021a	1.00±0.021a	ND
	C	8.00±0.014a	ND	8.13±0.007a	1.08±0.106a	ND
	D	7.98±0.014a	ND	8.18±0.035a	1.14±0.198a	ND
120	A	7.75±0.064a	ND	8.00±0.120a	ND	ND
	B	7.74±0.064a	ND	7.85±0.099a	ND	ND
	C	7.86±0.021a	ND	7.89±0.113a	ND	ND
	D	7.94±0.099a	ND	7.97±0.106a	ND	ND

Values with different letters in each column are significantly different ($p<0.05$) from one to another. (A: control sodium nitrite 125 mg/kg, B: sodium nitrite 80 mg/kg + betanin 3.6 mg/kg + chitosan 10 g/kg + rosemary 2 g/kg, C: sodium nitrite 40 mg/kg + betanin 14.4 mg/kg + chitosan 10 g/kg + rosemary 2 g/kg, D: betanin 21.6 mg/kg + chitosan 10 g/kg + rosemary 2 g/kg).

*** Ripening time (fermentation stage for 7 days and drying stage for 21 days)**

During the first 4 days LAB count increased rapidly from 3.83 to 8.08 log CFU/g. Thereafter, the LAB count increased gradually to 8.44 log CFU/g at the of ripening. These results are in accordance with those observed by Bozkurt and Erkmen (2007) who mentioned that the count of LAB increased from 4.62 log CFU/g at the beginning of ripening to 5.47 at the end of ripening. Yeast and mould count increased during the first 7 days of ripening and decreased during further ripening and storage. No yeast and mould count observed at the end of storage (120 days). These results might be due to the effects of drying and curing agents. The obtained counts of yeast and mould were lower than those observed by Fernández-López *et al.* (2008) who mentioned that the counts of yeast and mould found in the final products (4.26 log CFU/g; day 30) are normal for this type of meat products. These results are in accordance with those observed by Bozkurt and Erkmen (2004) who mentioned that yeast and mould of sausage samples decreased during storage from 2.84-3.26 at zero time to 2.40-3.00 at the end of storage. No significant differences ($p>0.05$) for *E. coli* count were observed between the investigated samples. *E coli* counts decreased from 1.83 to 1.05 log CFU/g after 14 days of ripening. Thereafter, no *E coli* colonies were observed during further ripening and storage. Komprda *et al.* (2001) reported that *E coli* did not observed in any of seven samples taken during the ripening of the sausages. The presence of salt, sodium nitrite, the growth of starter bacteria to significant amounts and the concomitant production of organic acids and pH reduction coupled with reduction in moisture content may result in inhibition of *E coli* (Barbut and Parolari, 2002).

Sensory evaluation

Results presented in Fig. (2) show that no significant differences ($P > 0.05$) in the scores of appearance, odor and taste were observed between the investigated samples. While, significant differences ($P < 0.05$) in the scores of color, texture and overall acceptability were found between samples (A, B and C) and sample (D) at the end of ripening (zero time storage). As the storage time proceeded the scores of all tested parameters decreased in all investigated samples. This decrease might be attributed to the oxidative changes (lipid oxidation, oxidation of betanin, Mb and accumulation of metmyoglobin) and microbial activities. The control sample had the highest score values for all tested attributes at any sampling time during storage, followed by samples (B and C). While, sample (D) recorded the lowest score values.

These results are in agreement with those reported by Bozkurt and Erkmen (2004) who found that the overall sensory quality of sausages increased as the nitrite concentration increased. Also, the results of sensory evaluation demonstrated the positive effects of combination of chitosan and rosemary on both retarding lipid oxidation and improving color retention of sausage samples during cold storage. The same results were observed by Georgantelis *et al.* (2007b) for beef burgers.

Fig. (2). Effect of different curing mixtures on organopleptic properties of dry fermented sausage during storage.
A: control sodium nitrite 125 mg/kg, B: sodium nitrite 80 mg/kg + betanin 3.6 mg/kg + chitosan 10 g/kg + rosemary 2 g/kg, C: sodium nitrite 40 mg/kg + betanin 14.4 mg/kg + chitosan 10 g/kg + rosemary 2 g/kg, D: betanin 21.6 mg/kg + chitosan 10 g/kg + rosemary 2 g/kg.
Sensory scale: 1, very poor; 2, poor; 3, acceptable; 4, good; 5, very good.

From the above mentioned results, it could be concluded that the curing systems which included 40-80 mg nitrite/kg, 3.6-14.4 mg betanin/kg, 10 g chitosan/kg and 2000 mg rosemary/kg have the color, oxidative stability, presumably flavor acceptability and microbial stability which are imparted by nitrite to cured-meat products.

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الإحلال الجزئى للنيتريت بالبيتانين، الشيتوزان والحصالبان فى مخاليط المعالجه للسجق الجاف المتخمر

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يهدف هذا البحث الى دراسة تأثير بعض مخاليط المعالجة منخفضة المحتوى من النيتريت على جودة السجق الجاف المتخمر خلال فترتى التسوية والتخزين بدراسة التغيرات فى الخصائص الكيموطبيعية, الميكروبيولوجية والحسية فى العينات المختبرة. وقد أوضحت النتائج أن مخاليط المعالجةالمستخدمة كان لها تأثير على قيم الـ pH, اللون, مستوى النيتريت المتبقى والخصائص الحسية (فيماعدا الطعم). أوضح التحليل الاحصائى أن استخدام مخاليط المعالجة المكونة من المخلوط المكون ٤٠-٨٠ ملجم نيتريت/كجم, ٣,٦-١٤,٤ ملجم بيتانين/كجم, ١٠ جم شيتوزان/كجم و ٢ جم حصالبان/كجم أعطى نفس اللون، النكهة المقبولة الثبات التأكسدى والميكروبيولوجى ودرجة القبول العام المتحصل علية بواسطة النيتريت.

Table (1). Effect of different curing mixtures on *L*, *a* and *b* values of dry fermented sausage during ripening and storage.

Days / Treatments	Parameter	Ripening time* (days)						Storage time (days)				
		0	1	7	14	21	28	0	30	60	90	120
A	*L*	50.20±0.42a	50.18±0.49a	49.83±0.46a	49.01±0.31ab	48.73±0.16a	47.20±0.47a	47.20±0.47a	45.92±0.55a	44.61±0.57a	43.83±0.51a	42.82±0.24a
	a	9.39±0.26a	9.41±0.28a	10.67±0.19a	11.10±0.19a	11.40±0.36a	12.92±0.11a	12.92±0.11a	12.98±0.13a	12.80±0.57a	12.78±0.42a	12.37±0.21a
	b	8.83±0.20a	8.85±0.28a	8.10±0.52a	7.51±0.57a	7.13±0.45a	6.56±0.43a	6.56±0.43a	6.51±0.49a	6.13±0.17a	6.09±0.15a	6.10±0.01a
B	*L*	49.57±0.14a	49.50±0.14a	49.15±0.11a	48.70±0.06ac	46.93±0.91ac	46.26±0.13ac	46.26±0.13ac	44.97±1.29ac	43.90±0.27a	43.70±0.11a	43.24±0.33a
	a	13.14±0.12b	13.12±0.14b	13.37±0.04b	13.19±0.28b	12.68±0.40b	12.86±0.71a	12.86±0.71a	13.17±0.55a	12.96±0.21a	12.87±0.42a	12.77±0.14a
	b	8.96±0.30a	8.90±0.33a	8.66±0.47a	8.18±0.71ab	7.97±0.77ab	7.58±0.42ab	7.58±0.42ab	7.33±0.28a	7.17±0.11b	7.27±0.33b	7.15±0.28b
C	*L*	49.25±0.35ac	49.20±0.42ac	49.59±0.66a	48.19±0.41ac	47.58±0.59ac	46.54±0.99ac	46.54±0.99ac	45.37±0.38a	43.27±0.35a	42.95±0.42a	42.30±0.46a
	a	14.80±0.20c	14.72±0.28c	14.12±0.06c	14.01±0.14c	13.62±0.20c	13.50±0.14a	13.50±0.14a	13.37±0.18a	13.02±0.14a	12.76±0.71a	12.73±0.14a
	b	10.09±0.17b	10.05±0.14b	8.62±0.14a	8.93±0.62ab	8.65±0.70ab	7.90±0.17b	7.90±0.17b	7.48±0.13a	7.47±0.51bc	7.65±0.22b	7.49±0.24b
D	*L*	48.89±0.07bc	48.80±0.07bc	48.15±0.06b	47.60±0.57bc	47.03±0.17bc	45.38±0.62bc	45.38±0.62bc	43.10±0.71bc	41.02±0.71b	40.79±0.17b	39.90±0.06b
	a	15.09±0.04c	15.07±0.14c	14.74±0.08d	12.04±0.59ab	10.19±0.21d	9.83±0.32b	9.83±0.32b	9.71±0.39b	9.20±0.06d	9.07±0.14b	8.95±0.16b
	b	10.59±0.21b	10.53±0.28b	9.77±0.26b	9.35±0.32b	9.17±0.43b	8.10±0.14b	8.10±0.14b	8.11±0.14b	7.96±0.06c	7.86±0.08b	7.87±0.57b

Values with different letters in each column are significantly different ($p<0.05$) from one to another. (A: control sodium nitrite 125 mg/kg, B: sodium nitrite 80 mg/kg + betanin 3.6 mg/kg + chitosan 10 g/kg + rosemary 2 g/kg, C: sodium nitrite 40 mg/kg + betanin 14.4 mg/kg + chitosan 10 g/kg + rosemary 2 g/kg, D: betanin 21.6 mg/kg + chitosan 10 g/kg + rosemary 2 g/kg).

*** Ripening time (fermentation stage for 7 days and drying stage for 21 days)**