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EFFECT OF NaCl REDUCTION AND SUBSTITUTION WITH KCl ON BEHAVIOUR AND FUNCTIONAL CHARACTERISTICS OF *LACTOBACILLUS RHAMNOSUS* FERM P-15120 IN FERMENTED BEEF SAUSAGE

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ABSTRACT

The effect of NaCl reduction or using KCl as a salt substitute on some physicochemical and functional properties of fermented sausage with *Lactobacillus rhamnosus* FERM P-15120 at high and low temperature was evaluated. Sausage samples with 50 % salt reduction showed higher viable counts of probiotic cells than the other treatments with a slow growth rate at low temperature. The reduction of salt or addition of KCl did not exert a bad influence on pH value and taste parameters with some color differences. The 50 % salt reduction and/or substitution with 50 % KCl increased the angiotensin -I- converting enzyme inhibitory activity and tyrosine concentration compared with sausage containing the high percent of sodium chloride either at low or high fermentation temperature. The effect of reduction and/or substitution of salt on fermented sausage is of interest because this may help to increase the functional bioactive peptides and counteract the adverse effect of NaCl on the product, thus helping to serve the problem of hypertension and maintaining good human health.

Key words: Salt reduction, NaCl, KCl, *Lactobacillus rhamnosus* FERM P-15120, angiotensin -I- converting enzyme inhibitory activity.

INTRODUCTION

Reducing sodium levels in meat products has been one of the positive attitudes of the meat industry (WHO, 2012). Although NaCl is an essential ingredient in processed meat products, associated with the water-holding capacity, fat binding, color, flavor and also decreases water activity (aw), which significantly control the shelf life of these products (Wirth, 1989), A human diet high in sodium is the main risk factor for hypertension and the occurrence of cardiovascular disease. It is considered as an etiological factor for other diseases such as obesity, certain cancers, kidney stones and osteoporosis (Campagnol *et al.*, 2000; Desmond, 2006;He and MacGregor, 2010).Therefore, several recent studies paid their attention to assess the effect of salt reduction on the physicochemical and functional properties of fermented sausages(Gelabert *et al.*2003; Aaslyng *et al.*,2014; Dos Santos *et al.*, 2015a; Dos Santos *et al.*, 2015b).

NaCl is one of the most important ingredients affecting meat proteolysis and production of small bioactive peptides and free amino acids, because NaCl regulates the proteolytic enzymes activity, inhibiting their activity when its concentration increases during the late stage of fermentation (Toldrá, 2002).Therefore, the reduction of this ingredient may increase the activity of these enzymes, resulting in higher degradation of myofibrillar proteins(Toldrá, 2006). These peptides are mainly responsible for the development of sensory characters of these products such as texture, flavor, and odor and also have important bioactive functions such as antioxidant and antihypertensive activity(Escudero *et al.*,2013; Mora *et al.*,2014).

However, few studies showed that high sodium chloride in fermented food has stress effect leading to injury of the probiotic bacteria. It is still important to assess the degree of injury sustained by probiotic bacteria when subjected to increasing salt concentration stress(Gandhi and Shah, 2015).Nowadays, there is upsurge interest for manufacturing of low sodium food products either with sodium chloride reduction(Aaslyng *et al.*, 2014; Corral *et al.*,2013) or substitution with other chloride salts as potassium or calcium chloride with keeping the same function and sensory acceptance(Armenteros*et al.*,2012; Paulsen*et al.*,2014; Wu *et al.*, 2014).

This study aimed to clarify the impact of reducing sodium either by reduction of NaCl to 50 % or replacement with 50% of KCl on some properties of beef sausage fermented with intestinal lactic acid bacteria (i.e., *Lactobacillus rhamnosus* FERM P-15120). *L. rhamnosus* FERM P-15120 was isolated from human intestinal tract and is suitable for a probiotic meat starter

culture(Sameshima *et al.* 1998). The probiotic strain count, physicochemical parameters such as pH, color and taste and angiotensin -I-converting enzyme inhibitory activity during fermentation were measured.

MATERIALS AND METHODS

Reagents: Angiotensin converting enzyme (from rabbit lung) and substrate peptide hippuryl-L-histidyl-leucine (HHL) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Other chemicals and reagents used were of analytical grade.

Lactic acid bacteria for meat fermentation: The *Lactobacillus* strain used for meat fermentation was *L. rhamnosus* FERM P-15120. This strain was isolated from human intestinal tract and identified in our laboratory(Sameshima *et al.*1998).The strain was grown overnight in MRS broth (Oxoid, Basingstoke, UK) at 37 °C, harvested by centrifugation at 5000 rpm for 10 min at 6 °C, washed twice in saline solution (0.85% NaCl), resuspended in saline solution and stored at -80 °C with 20% of glycerol until further use.

Fermented model sausage preparation: The model sausage formulations were prepared with 50% reduction of NaCl or 50% substitution by KCl as shown in Table 1. The fermented sausages were manufactured using fresh beef trim obtained from local markets. The meat was ground and mixed with salt amount according to Table 1, glucose (10 g/kg) and the *Lactobacillus* strain at inoculation level (10^8 CFU/g). After complete homogenization, the meat batter stuffed into glass beaker to make individual pieces of sausage weighting approximately 200 g. The sausage pieces were incubated at controlled temperature and the fermentation occurred at either high temperature (35 °C) or low temperature (20°C). Samples for microbiological analysis, physicochemical analysis, measuring ACE inhibitory activity and tyrosine amino acid concentration were taken from sausages at 0, 24, 48 and 72 hrs of sausage kept at 20 °C fermentation and at 0, 24 and 48 hrs of sausage kept at 35 °C. The pH was measured with a combination electrode and pH meter.

Table 1. Levels of sodium chloride and potassium chloride used in fermented sausage formulations.

	Salt concentrations (%)		
	Control	**T1**	**T2**
Sodium chloride (NaCl)	3	1.5	1.5
Potassium chloride(KCl)	-	-	1.5

Control – 100 % NaCl; T1 – 50 % NaCl; T2 – 50 % NaCl and 50 % KCl.

Microbiological analysis: Twenty five grams sample of sausage was homogenized with 225 ml of 0.1 % peptone water and ten-fold serial dilutions were used for detection the viability of *Lactobacillus* strain. Viable count of *L. rhamnosus*FERM P-15120 was determined by enumeration of viable cells on MRS agar plates after incubation at 35 °C for 48 – 72 hrs.

Color measurement: The Color was measured at the end of fermentation using colorimeter model CR-200 (Konica Minolta) in terms of CIE L^*, a^*, b^* values. 3 pieces of each sausage samples were used for measuring the color of each sample.

Taste analysis: The taste parameters of fermented sausage samples were measured by taste sensory system SA402B (Intelligent Sensor Technology, Inc., Kanagawa, Japan), fitted with sensor probes for the different tastes and a reference probe according to the method of Hayashi*et al.*,(2013). The sensor measurement was automatically carried out at 25 °C. The taste parameters such as sourness, bitterness, umami, saltiness, and astringency were measured at the end of the experiment.

Assay for ACE inhibitory activity: ACE inhibitory activity of different treated sausage samples was measured according to the method of Cushman and Cheung,(1971) with the modifications by Arihara *et al.*,(2001).The sample solution (15 µL) was mixed with 125 µL of 100 mmol L^{-1} sodium borate buffer (pH 8.3) containing 7.6 mmol L^{-1} of Hip-His-Leu and 608 mmol L^{-1} of NaCl. Then it was centrifuged shortly for 2 minutes at 12000 rpm and kept at 37 °C for 5 min. The reaction was started by addition of 50 µL of enzyme solution (ACE dissolved in distilled water). Then the mixture was incubated at 37 °C for 30 min. For the blank, ACE was replaced by 50 µL of distilled water. The reaction was stopped by adding 125 µL of 1 M HCl. The hippuric acid produced by ACE was extracted by adding of 750 µL ethyl acetate to the mixture with vigorous shaking. After centrifugation at 12000 rpm for 10 min, 500 µL of the upper layer was collected and dried at 90 °C for 30 min. the residue (Hippuric acid) was dissolved in distilled water (1 ml) and its absorbance measured at 228 nm. The ACE inhibitory activity was calculated using the equation:

$$\text{Inhibitory activity (\%)} = (C-A)/(C-B)\times 100$$

Where A is the absorbance of sample reaction, B is the absorbance of the blank, and C is the absorbance of the control (distilled water).

Measurement of tyrosine concentration: The tyrosine concentration in fermented sausage samples was measured according to Hull, (1947) with some modifications. A 0.5 ml of sample extract is pipetted into a test tube; 0.5 ml of distilled water is added, followed by 2 ml of trichloroacetic acid. The tube is mixed and allowed to stand for 10 minutes then centrifuged at 3000

rpm for 10 minutes. After that 0.5 ml of the supernatant is added to 2 ml of the sodium carbonate reagent and mixed thoroughly before 300 µL of phenol reagent were added. The sample is mixed and 5 minutes allowed for the blue color to reach a maximum before any readings are taken and its absorbance measured at 650 nm. The concentration of tyrosine was calculated using standard tyrosine curve.

Statistical analysis: The analyses were made in triplicate. Statistical analysis of the data was performed using Graph Pad Prism 6 software (Graph Prism Software, La Jolla, California, USA). Two-way ANOVA followed by Dunnett's test at a 5 % significance level was used to examine the statistically significant differences of the treatment and time effects on the Physicochemical, microbiological and functional parameters. The results were stated as mean ± SEM. Differences were considered to be statistically significant ($P \leq 0.05^{*}$, $p \leq 0.01^{**}$, $p \leq 0.001^{***}$ and $p \leq 0.0001^{****}$.

RESULTS

Viability of lactic acid bacteria: We manufactured the fermented beef sausage with *L.rhamnosus* FERM P-15120. The effect of time of fermentation and treatment on viable cell counts of lactic acid bacteria are presented in Tables 2 and 3. Time effect on the growth of lactobacillus stain was statistically significant ($p \leq 0.0001$). At high temperature (35 °C), the viable counts of the probiotic strain ranged from 8.7 to 8.9 $\log_{10}$ CFU/g at the beginning of sausage processing. After 24 hrs of fermentation, the *L.rhamnosus* FERM P-15120 grew faster related to the faster drop in pH noted in Table 2 and the viable counts reached approximately 10.9, 10.6 and 11 $\log_{10}$ CFU/g in the control, T1 and T2 sausage samples, respectively. At the end of the fermentation process, the control samples showed the lower counts followed by sausages produced with 50% KCl substitution (T2) while the sausages with 50 % NaCl reduction (T1) showed the higher growth rate (11 $\log_{10}$ CFU/g) with significant differences to control samples ($p \leq 0.05$). On the other side at low fermentation temperature, the slower drop of pH led to the slower growth rate of the lactic acid bacteria (Table 3). At the beginning, the viable counts of *L. rhamnosus* FERM P-15120 were approximately 8 $\log_{10}$ CFU/g and then increased slowly reaching approximately 8.5 $\log_{10}$ CFU/g after 48 hrs of fermentation. At the end(after 72 hrs), the treatment effect was significant ($p \leq 0.01$) and the control samples showed the lower rate of growth (9.7 $\log_{10}$ CFU/g) compared with T2 and T1 with viable counts of 10.7 and 11.2 $\log_{10}$ CFU/g, respectively.

Physicochemical parameters: The results of physicochemical parameters are indicated in Tables 2 and 3. Reduction of NaCl or substitution with KCl did not show clear differences with control sausage samples in pH reduction at either high or low - temperature fermentation($p \geq 0.05$) with significant effect of time of fermentation ($p \leq 0.0001$). The drop in pH value to a level below 5 took place after three days for sausage samples fermented at 20 °C.

Table 2. Physicochemical and microbiological parameters of fermented sausage prepared at 35 °C.

		Hours of fermentation	Treatments		
			Control	T1	T2
pH		0	5.8 ± 0.00^{aA}	5.8 ± 0.00^{aA}	5.8 ± 0.00^{aA}
		24	4.9 ± 0.028^{aB}	4.7 ± 0.046^{aB}	4.8 ± 0.058^{aB}
		48	4.8 ± 0.028^{aB}	4.8 ± 0.035^{aB}	4.8 ± 0.052^{aB}
Color	L^{*}	48	50.40 ± 0.21^{a}	52.85 ± 0.31^{b}	50.31 ± 0.25^{a}
	a^{*}		7.74 ± 0.15^{a}	7.73 ± 0.17^{a}	7.67 ± 0.06^{a}
	b^{*}		5.70 ± 0.01^{a}	6.87 ± 0.07^{b}	5.76 ± 0.08^{a}
Viability of lactic acid bacteria ($\log_{10}$ CFU/g)		0	8.8 ± 0.17^{aA}	8.9 ± 0.058^{aA}	8.7 ± 0.23^{aA}
		24	10.9 ± 0.52^{aB}	10.6 ± 0.23^{aB}	11 ± 0.29^{bB}
		48	10.5 ± 0.17^{aB}	11 ± 0.40^{bB}	10.6 ± 0.12^{aB}

Data are shown as means values ± standard error. Mean values followed by different lowercase letters within the same raw and by the different uppercase letters within the same column are significantly different. The lowercase letters are indicator on the treatments differences and uppercase ones are for the time difference. Control – 100 % NaCl; T1 – 50 % NaCl; T2 – 50 % NaCl and 50 % KCl.

The effect of NaCl reduction and /or substitution with KCl on the color of fermented sausage was shown in Table 2 and 3. The color parameters, lightness (L^{*}), redness (a^{*}) and yellowness (b^{*}) were determined after 48 and 72 hrs for high and low - temperature fermented sausage samples, respectively. At 35°C, the addition of potassium chloride to fermented sausage didn't alter these parameters while, L^{*} and b^{*} of reduced salt sausages were significantly higher than those of control samples but a^{*} of the same samples was similar to the control. On the other hand, color parameters of sausages fermented at low temperature show some differences between reduced

salt sausages, sausage samples with 50 % added KCl and control samples ($p \leq 0.0001$). Lightness, redness, and yellowness of T1 and T2 sausage samples were higher than those of control samples.

Table 3 Physicochemical and microbiological parameters of fermented sausage prepared at 20 °C.

	Hours of fermentation	Treatments		
		Control	T 1	T 2
pH	0	5.6 ± 0.00^{aA}	5.5 ± 0.029^{aA}	5.6 ± 0.00^{aA}
	24	5.6 ± 0.00^{aA}	5.3 ± 0.058^{bB}	5.5 ± 0.017^{aA}
	48	5.1 ± 0.029^{aB}	5 ± 0.00^{aC}	5.1 ± 0.00^{aB}
	72	4.9 ± 0.00^{aC}	4.8 ± 0.00^{aD}	4.9 ± 0.00^{aC}
Color **L***		42.05 ± 0.31^{a}	50.10 ± 0.41^{b}	47.27 ± 0.21^{c}
a*	72	10.93 ± 0.26^{a}	13.08 ± 0.26^{b}	12.85 ± 0.21^{b}
b*		3.87 ± 0.09^{a}	7.31 ± 0.07^{b}	5.35 ± 0.21^{c}
Viability of lactic acid bacteria (log_{10} CFU/g)	0	8 ± 0.29^{aA}	8.2 ± 0.023^{aA}	8 ± 0.17^{aA}
	24	7.85 ± 0.087^{aA}	8 ± 0.057^{aA}	8.25 ± 0.27^{aA}
	48	8.5 ± 0.07^{aB}	8.6 ± 0.11^{aA}	8.5 ± 0.16^{aA}
	72	9.7 ± 0.28^{aC}	11.2 ± 0.17^{bB}	10.7 ± 0.12^{bB}

Data are shown as means values ± standard error. Mean values followed by different lowercase letters within the same raw and by the different uppercase letters within the same column are significantly different. The lowercase letters are indicator on the treatments differences and uppercase ones are for the time difference. Control – 100 % NaCl; T1 – 50 % NaCl; T2 – 50 % NaCl and 50 % KCl.

Taste parameters: The taste profile (Figure 1 and 2) showed that 50% reduction in NaCl and/or replacement with 50% KCl did not change the taste of fermented sausages manufactured at 35 °C or 20 °C except in saltiness with respect to control samples.

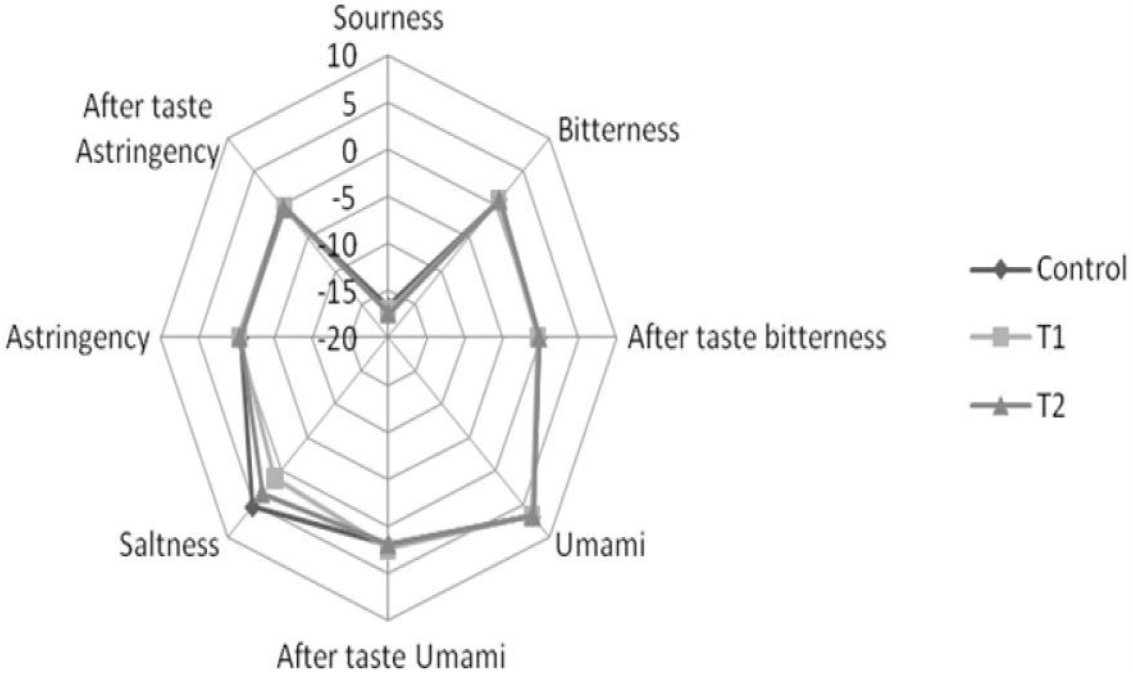

Figure 1. Taste profile of fermented sausage at prepared at 35 °C. Control – 100 % NaCl; T1 – 50 % NaCl; T2 – 50 % NaCl and 50 % KCl.

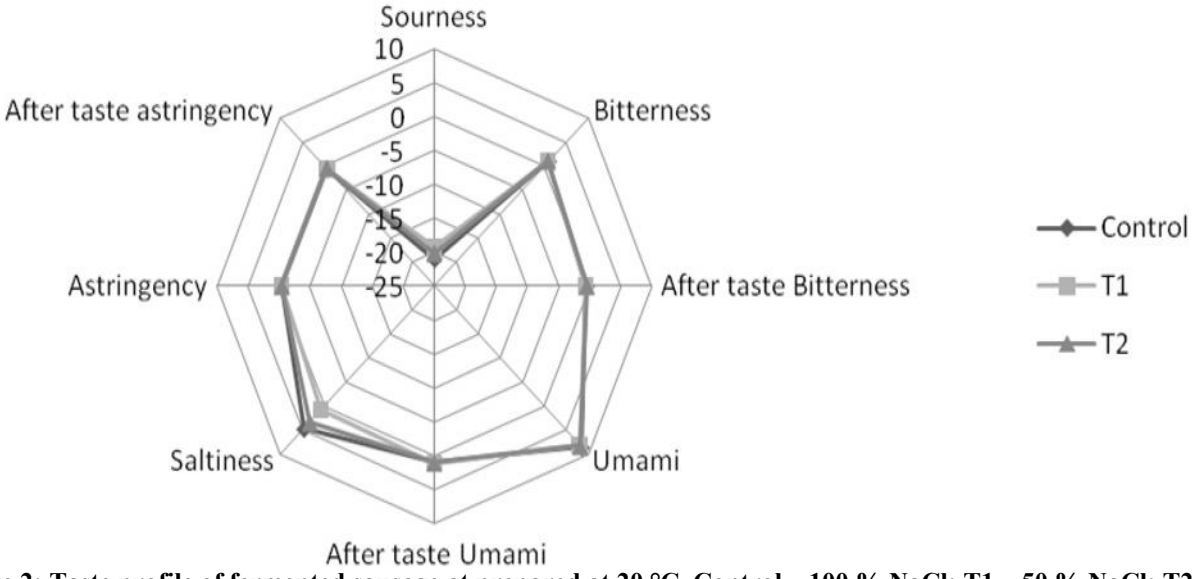

Figure 2: Taste profile of fermented sausage at prepared at 20 °C. Control – 100 % NaCl; T1 – 50 % NaCl; T2 – 50 % NaCl and 50 % KCl.

ACE inhibitory activity: Figure 3 showed the value of ACE inhibitory activity of sausage samples of different salt concentration during 48 hrs of fermentation at 35 °C. The time and treatment effects were statistically significant ($p \leq 0.0001$) with significant effect of their interaction ($p \leq 0.05$).The results showed that meat extract from all sausage samples had high ACE inhibitory activity increased gradually till reaching its peak at 48 hrs of fermentation comparing with the initial ACE. The reduced salt sausage (T1) had the highest activity of all tested treatments, and its activity differed significantly from the control ($p \leq 0.05$) followed by the sausage with of 50 % KCl. For low fermentation temperature, the time, treatment effects, and their interaction were statistically significant ($p \leq 0.0001$). The ACE inhibitory activity slowly increased recording the higher activity at 72 hrs for all treatments. The control samples had the significant lower activity compared with the other sausage treatments with the progress of fermentation (Figure4). This slow rate is suggested to be correlated with the slower growth rate of probiotic strain in these treatments (Table 3).

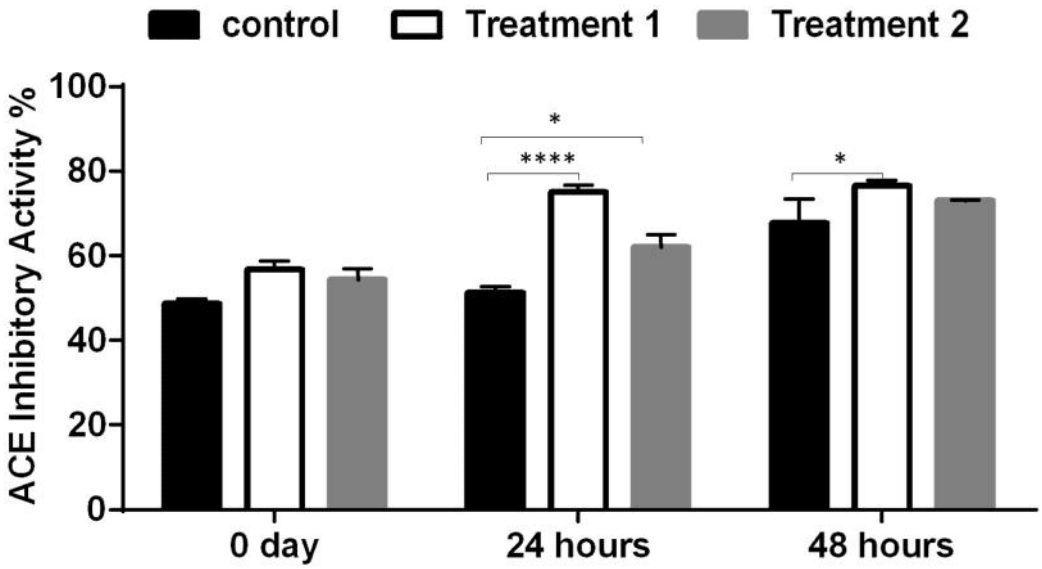

Figure 3: ACE inhibitory activity of meat extracts from fermented sausage samples prepared at 35 °C. Control – 100 % NaCl; T1 – 50 % NaCl; T2 – 50 % NaCl and 50 % KCl. * means significantly different compared to control ($p \leq 0.05$), **** means significantly different compared control ($p \leq 0.0001$).

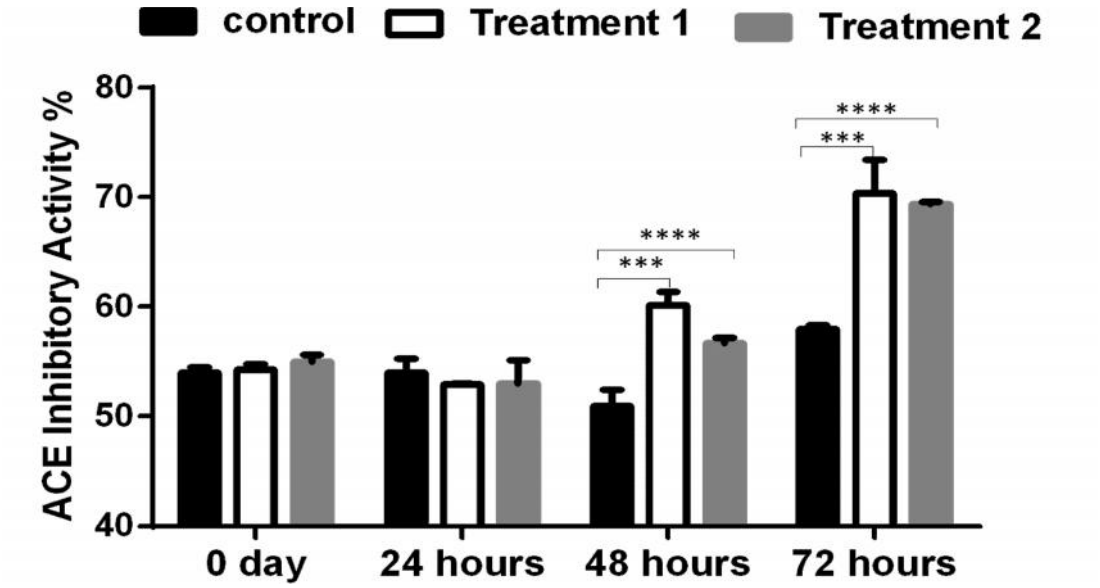

Figure 4: ACE inhibitory activity of meat extracts from fermented sausage samples prepared at 20 °C. Control – 100 % NaCl; T1 – 50 % NaCl; T2 – 50 % NaCl and 50 % KCl. *** means significantly different compared to control (p≤ 0.001), **** means significantly different compared control (p≤ 0.0001).

Tyrosine concentration: The time, treatment effects, and their interaction on tyrosine concentration were statistically significant (p≤0.0001).The tyrosine concentration (µg/ml) in meat extract of sausage samples manufactured at 35 °C (Figure5) was higher after 48 hrs compared with the first day of fermentation for all treatments but the concentration of tyrosine in control samples is lower than of those in reduced salt sausages and sausages with added 50% KCl (p≤ 0.0001) .On the other hand, the tyrosine concentration in the control sausage samples fermented at low temperature was declined after 24 hrs of fermentation. The 50% salt reduction or replacement with 50% KCl significantly increased the concentration giving the higher tyrosine concentration at 72hrs (p≤ 0.0001) (Figure 6).

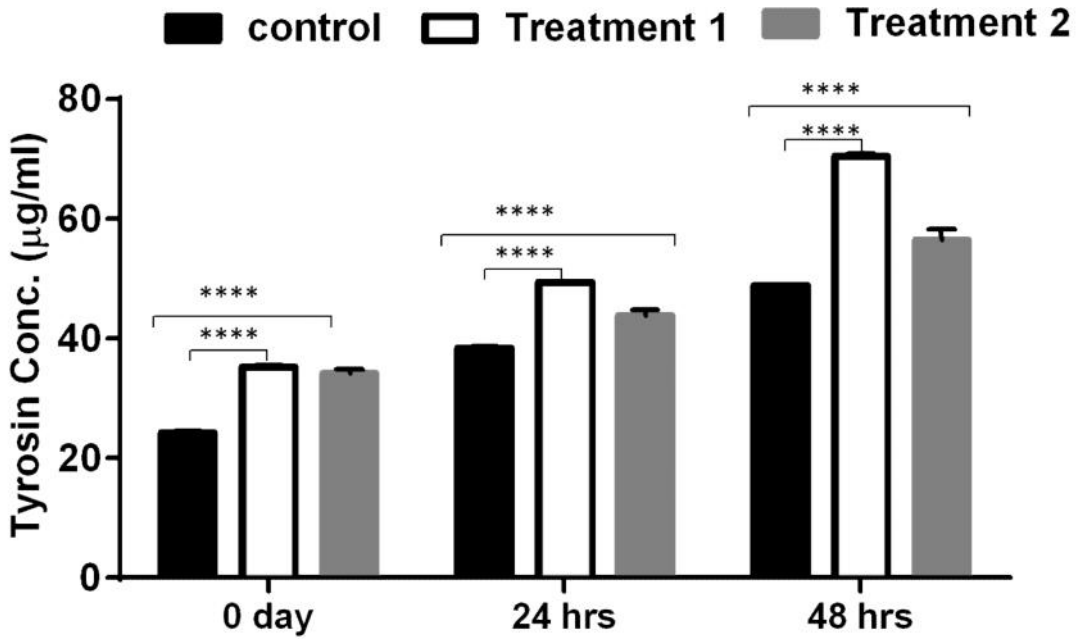

Figure 5: Tyrosine concentrations (µg/ml) in meat extracts of fermented sausage samples prepared at 35 °C. Control – 100 % NaCl; T1 – 50 % NaCl; T2 – 50 % NaCl and 50 % KCl. **** means significantly different compared to control (p≤ 0.0001).

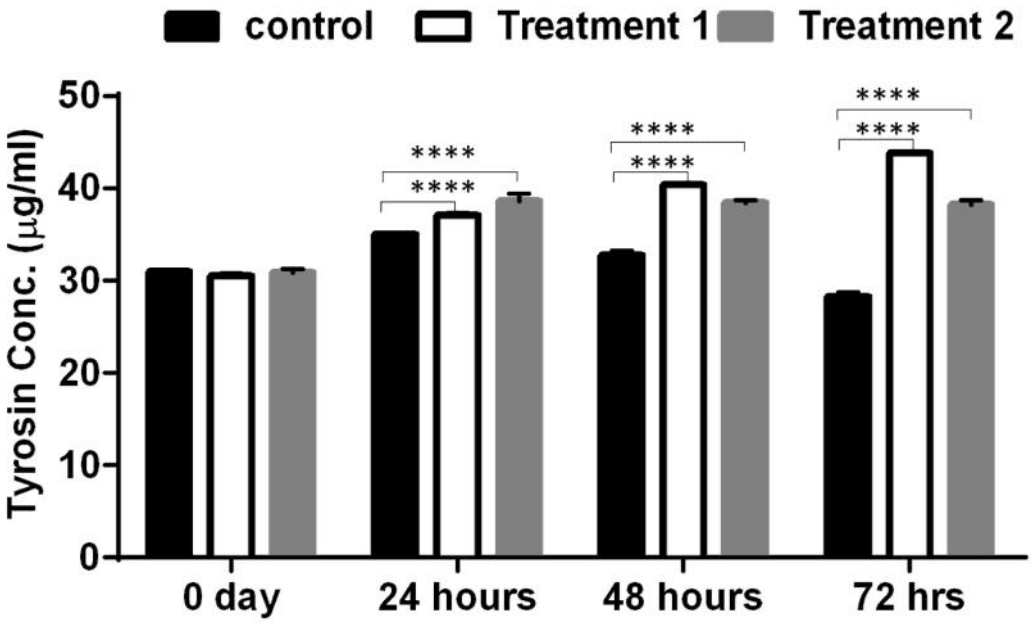

Figure 6: Tyrosine concentrations (µg/ml) in meat extracts of fermented sausage samples prepared at 20 °C. Control – 100 % NaCl; T1 – 50 % NaCl; T2 – 50 % NaCl and 50 % KCl. **** means significantly different compared to control ($p \leq 0.0001$).

DISCUSSION

Beef meat is a good source of protein which is highly responsible for the nutritive value and functional properties. Functional foods are processed foods with additional extra functions associated with the promotion of human health or prevention of some diseases by functional ingredients (Arihara, 2014). One of the most important ingredients is the bioactive nitrogen compounds, these compounds are inactive in their primary structure in the original meat protein. The liberation of the active form of these compounds achieved by the enzymatic proteolysis producing small active peptides and free amino acids (Nalinanon *et al.*, 2011; Torres-Fuentes *et al.*, 2011). NaCl is one of the most important additives in fermented meat products for maintaining the product quality and enhancing the functional and sensory characters. However, the excessive use of this salt lowers the growth rate of lactic acid bacteria during the fermentation process(Takeda *et al.*, 2017). In our study, the lower growth rate of lactic acid bacteria in control samples at high and low temperature compared with the other treatments could be regarded to that that higher concentration of sodium chloride in control sausage samples. Gandhi and Shah, (2015)reported that excess NaCl may decrease the cell viability and metabolic activity of LAB due to its effect on esterase activity and cell membrane integrity. Also, Gelabert *et al.,* (2003) studied the effect of replacement of sodium chloride with 40 % KCl on microbial parameters of fermented sausage and found that the growth of lactic acid bacteria increased during fermentation. The similarity of *Lactobacillus* count between the control samples and sausages with 50 % KCl at 35 °C or increasing in the same sausage samples than control at 20 °C suggest that KCl is good replacer for NaCl at the point of growth of lactic acid bacteria.

The functional properties of meat proteins are related to their contribution to sensory and physiochemical properties of meat and their products (Sikorski, 2006). The differences in color parameters observed in sausage samples (Tables 2 and 3)may be caused by color heterogeneity which is a character of the typically fermented sausage (Campagnol *et al.*, 2011) and also the meat products color mostly due to chemical reaction between muscle sarcoplasmic myoglobin (unstable protein) and oxygen, this reaction is mainly related to oxygen availability on meat surface(Girolami *et al*, 2013). These color differences also observed in the previous study by Dos Santos *et al.*,(2015b). Askar *et al.*, (1994) reported that the replacement of NaCl with KCl in meat products is primarily limited due to the bitter taste of KCl. Our results showed that the taste parameters of fermented sausage manufactured with three different salt concentrations were nearly similar (Fig. 1 and 2). These results were agreed with the results obtained by Wu *et al.*,(2014).

One of the most important bioactive peptides is angiotensin converting enzyme (ACE) inhibitory peptides which are responsible for blood pressure control (Decker and Park, 2010). There are many previous researchers studying the ACE inhibitory activity produced in vitro from animal muscle protein hydrolysates (Fernández *et al.*,2016;Jang and Lee, 2005; Takeda *et al.*,2017). The slow rise of ACE inhibitory activity of meat extracts of sausage samples fermented at 20°C (Figure 4) may be attributed to the slower growth rate of *Lactobacillus* strain in these treatments (Table 3). These LAB have shown good protein degradation ability (Fadda *et al.*, 1999; Fernández *et al.*,2016). The lower values achieved by control samples at high and low fermentation temperatures could be due to the higher concentration of sodium chloride which decreases the proteolytic enzymes activity(Toldrá, 2006). The elevation of ACE inhibitory activates in our samples is directly related to the increased time of fermentation and decreased sodium chloride concentration ($p \leq 0.0001$). This may be attributed to the increasing activity of microbial proteases during fermentation leading to higher rate of proteolysis and production of peptides as ACE inhibitory peptides (Fadda *et al*., 1999; Martín, *et al.,* 2007). Castellano *et al.*, (2013) used *Lactobacillus* species for meat fermentation and found that these bacteria were able to generate functional peptides with remarkable ACE inhibitor activity. Vaštag *et al.*, (2010) found that higher amount of peptides were released during Petrovac sausage ripening, the ACE inhibitory peptides was one of the most significant peptides produced. Further studies are needed to identify these peptides with ACE inhibitory activity and apply in vitro on SHR to assess its antihypertensive activity.

The liberation of free amino acids is the end product of proteolysis of muscle protein in fermented sausage (Sun *et al.*, 2009). The concentration of free amino acid obtained during the processing of fermented sausage associate with the pH drop, salt concentration, use of probiotics and the processing condition such as temperature and time, as all these factors mainly affect the aminopeptidase enzymes activity(Sanz and Toldrá, 2002).We found that the concentrations of tyrosine increased within the treatments 1 and 2 compared with control samples (Figure 5 and 6), this could be regarded to the high sodium chloride content of the control sausage samples. Dos Santos *et al.*, (2015a) also found that 50% salt reduction give the highest amount of free amino acids. This may be attributed to that the salt reduction below 2 % increase the activity of muscle proteases which increase the proteolytic activity and so the formation of free amino acids (Toldrá, 1992).

In conclusion, the reduction of sodium chloride in beef fermented sausage can be done either by reduction of 50% of the salt or substitution with 50 % potassium chloride without changing the overall physical and sensory characters of the product. The viability of *lactobacillus* strain used as probiotic culture increased by sodium reduction. In addition, the functional properties of reduced NaCl fermented sausages by the two ways obviously increased giving high ACE inhibitory activity and tyrosine concentration. The obtained results may be used to develop healthy functional food with low salt and high antihypertensive activity.

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Conflict Of Interest: The authors declare that they have no conflict of interest.

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