EVALUATION OF ANTIHYPERTENSIVE ACTIVITY OF PROTEINS IN FERMENTED TURKISH SUCUK

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Abstract: Angiotensin I Converting Enzyme (ACE) is the main constituent of Rennin Angiotensin System (RAS) which causes hypertension in blood vessels. Nowadays to overcome and inhibit ACE new aspects are in research interest. In this research Turkish sucuk types, fermented by *Lactobacillus sakei* and *Lb. plantarum* were used as potential source of ACE inhibitory peptide. Physicochemical and biofunctional properties were checked on 0, 7, 14 and 28 days of fermentation and storage. Sucuk samples were proteolytically hydrolyzed with pepsin and trypsin and total ACE inhibitory activity was evaluated. Control 0th day hydrolysate had lowest ACE inhibitory activity (IC_{50}) (3.46 mg/ml), meantime the highest inhibition was resulted in Control 28th day hydrolysate(1.96 mg/ml). Results displayed that the Turkish fermented sucuk could be a source of antihypertensive peptides even without starter culture inoculation (spontaneous microflora).

Keywords: ACE, hypertension, *Lb. sakei*, *Lb. plantarum*, sucuk

I. INTRODUCTION

Recently, individuals started to consider their foods and its nutritional quality. There is a public demand for exploring new nutraceutical materials from certain and renowned foods to overcome some life-style related diseases (LSRD) such as diabetic, obesity, cardiovascular diseases (CVD) and hypertension [1]. Hypertension a dreadful disease which results a reduction in patients' life quality. It can be described as high blood pressure inside the blood vessels (Blood pressure >120/ 80 mmHg) [2]. While the number of hypertension patients consist %14.9 of World population in 2007, it is estimated to reach higher levels through the 2020 [3]. Level of blood pressure in human body is being regulated by an endocrine system which is called Rennin Angiotensin System (RAS). Angiotensin I-converting enzyme (ACE), a dipeptidylcarboxypeptidase, is most important fragment of this system and plays a vital role in regulating blood pressure [4]. The ACE reacts on inactive Angiotensin I, and forms Angiotensin II, a vasoconstrictor, by cleaving His-Leu dipeptide [5]. Thus, Angiotensin II gives rise to constriction of blood vessels and as a result of it, causes hypertension. Consequently, ACE activity must be inhibited by different methods. Although there are pharmalogical drugs for curing hypertension, along with their ACE inhibition activity, drugs may also induce some side effects in human body [3]. With this justification, natural ACE inhibitors are requested to dominate activities associated with ACE. Hereby, during last decade much attention has been paid for safer applications to avoid from side effects of drugs and treat or minimize hypertension. Bioactive peptides from food sources, found to be promising approach to overcome this type of disorders.

Fermented Turkish Sucuk is a traditional meat product which is made of meat, fat, red and black pepper, cumin, pimento, salt and sugar. As a result of high amount of meat protein, sucuk is a great source of peptides. Throughout fermentation process, proteins in sucuk are being degraded into smaller peptides [6]. Consequently, newly generated biologically active peptides can be realized and therefore nutritional value of sucuk has to be determined especially against ACE action of mechanism to value the delicious meat product. In present study we determined effects of different starter cultures (*Lb. sakei* and *Lb. plantarum*) on some physiological properties and total ACE inhibition of Turkish fermented sucuk.

II. MATERIALS AND METHODS

Materials: Biceps femoris muscle of 2-2.5 years old Montofon bovine was purchased from Saray Çiftliği Meat and Meat Product Company, Kayseri. Sheep tail fat and all spices were purchased from local markets in Kayseri.

Sucuk Production: Lb. sakei and Lb. plantarum, isolated from traditional Turkish sucuk were used as starter culture in sucuk production. Four different types of sucuks such as; 1) control-C (no starter inoculation), 2) *Lb. sakei*-LS, 3) *Lb.*

plantarum-LP and 4) mixed starter type-LSP (inoculated with both *Lb. sakei* and *Lb. plantarum*) were produced. Experimental sucuk mixture was prepared according to Öz et al. [7]. Minced meat and fat along with the spices were mixed homogenously, and then starter cultures were inoculated into the mix separately at a ratio of 1 % (10^7 cfu/g). Sucuk mixtures were stuffed into synthetic collagen casings (36-mm Ø) and ripened in fermentation cabinet (Nüve/Turkey) at 24±1°C, 90±2 % relative humidity (RH) for 5 days, and then at 22±1°C, 85±2 % RH for 5 days and finally 18±1°C, 80±2 % RH for 4 days and subjected to following analysis.

Dry Matter and pH Values: Dry matter and pH analysis were done according to AOAC [8].

Protein Extraction: Guba-Straub-Adenosine Triphosphate (GS-ATP) Soluble Proteins and Water Soluble Proteins (WSP) were extracted by adding 28 mL of solution to 2 g of the samples [9].

SDS-PAGE Analyses: Extracts of GS-ATP and WSP were split up according to their molecular weight (MW) with SDS-PAGE technique. Samples were subjected to constant current of 30 mA/gel on gradient gel made of 7.5%–20% acrylamide. Analysis was carried out with a Mini protein II unit (Bio-Rad Laboratories, Inc., Richmond, CA, USA) [10].

Hydrolysis of Sucuk Samples: Pepsin from porcine gastric mucosa (37 ºC, pH=2.0, 2 hours and intestinal trypsin (37 ºC, pH=7.0, 2 hours (Sigma–Aldrich, Inc. St. Louis, MO, USA) were put in sucuk samples, respectively. Filtration process were applied to hydrolysates through A5 filter paper (Advantech Tokyo K. Ltd., Tokyo, Japan) and then 0.45µm filters were used and subsequently filtrates were collected for the ACE inhibitory assay.

Protein Concentration: Biuret method was used to check protein concentrations of samples[11].

ACE Inhibitory Activity Assay: The method of Cushman&Cheung [12] was used with slight modifications as described by Katayama et al. [13] to determine ACE inhibitory activity of samples.

III. RESULTS AND DISCUSSION

Dry Matter and pH values: In the course of fermentation, there was a remarkable increase in dry matter with the duration up to 28th days (Table 1). No significant differences observed between the sucuk types at 0th day, while at 7th days,*Lb. sakei* sample had the lowest dry matter compared to other 3 types. After 14 days of ripening, *Lb. sakei* and *Lb. sakei+Lb. plantarum* samples exhibited lower dry matter values ($p<0.05$). In the course of processing from 14th days to 28th days there was a remarkable increase, and as it was expected, all samples reached to the highest dry matter content which ranged between 83.61 ± 0.71 - 84.24 ± 0.27 % (Table 1). Microbial growth obviously induced accumulation of lactic acid which caused a significant differences ($p<0.05$) in pH values among the sucuk types within the time points. A significant decrease was observed in pH values between 0th day to 7th days. However a slight increase noted from 7th days to 14th days. Results suggested that due to higher microbial growth, accumulation of lactic acid was high during first 7 days of ripening. Up to 28th days, pH level changed significantly ($p<0.05$) in the starter inoculated samples except control sample.

Table 1. Some physicochemical properties of Sucuk samples in different time points.

	Parameter	Control	LS	LP	LSP
	Dry Matter				
Days	0	44.86^{Ca}± 0.18	44.86^{Ca} ±0.18	44.86^{Ca}± 0.18	44.86^{Ca} ±0.18
	7	64.13^{Bab} ±0.78	62.80^{Bb} ±0.01	65.04^{Bab} ±1.45	65.51^{Ba} ±0.09
	14	75.04^{Aab} ±0.40	73.80^{Ab} ±0.19	76.18^{Aa}± 0.63	74.31^{Ab} ±0.65
	28	83.61^{Aa}± 0.71	84.17^{Aa} ±0. 33	84.24^{Aa}± 0.27	83.65^{Aa} ±0.81
	pH				
Days	0	5.66^{Aa}±0 .00	5.66^{Aa}± 0.00	5.66^{Aa}±0 .00	5.66^{Aa}± 0.00
	7	4.66^{Ca}±0 .01	4.56^{Cb}± 0.00	4.50^{Cc}±0 .01	4.54^{Bb}± 0.03
	14	4.69^{Ba}±0 .02	4.60^{Bc}± 0.00	4.61^{Bc}±0 .03	4.65^{Bb}± 0.00
	28	4.69^{Ba}±0 .00	4.62^{Bb}± 0.01	4.51^{Cd}±0 .01	4.58^{Cc}± 0.01

Different uppercase letters in each column show statistically significant differences between the time points ($P<0.05$), and different lowercase letters in each row show statistically significant differences between the sucuk types ($P<0.05$). LS: *Lb. sakei*, LP: *Lb. plantarum*, LSP: *Lb. sakei+ Lb. plantarum.*

SDS-PAGE: Extracts obtained from sample at 0th day showed 13 protein bands on SDS-PAGE gel (Figure 1). Those bands showed native protein structures of non-fermented sucuk batch.

During the first 7 days, bands correspond to 95, 84, 75, 61, 42, 36 ,25, 14 kDa were disappeared and caused generation of a new and intense band with an approximate molecular weight of 40 kDa, 64, 54, 33 kDa bands protected its' structure (Figure 1). Apparently in samples at 7th, 14th and 28th days, 4 bands were detected.

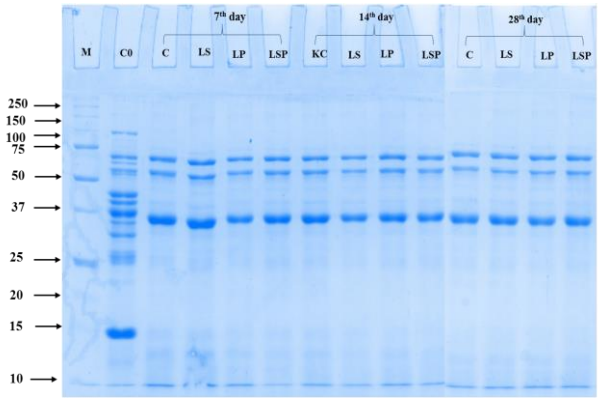


Figure 1. SDS-PAGE image of WSP in different time points. M: Marker, C: Control, LS: *Lb. sakei*, LP: *Lb. plantarum*, LSP: *Lb. sakei*+ *Lb. plantarum* Samples

At 0th day before inoculations and fermentation, GS-ATP extract of sucuk batch exhibited 16 different bands on gel ranges between 200 kDa-6.5 kDa (Myosin Heavy Chain (MHC) and Aprotinin, respectively) (Fig. 2). However at 7th days extracts for all samples showed very similar degradation progress to each other. Between the bands 250, 148, 95, 79, 43, 31, 26, 23 kDa were disappeared and smaller proteins or peptides were generated. Six bands were visible at 7th days and those bands maintained their existence during further fermentation period. SDS-PAGE images indicated that degradation of large proteins into smaller proteins occurred during first seven days. A few minor changes were observed in protein structure among the sucuk types perhaps due to similar degradation mechanism of the inoculated starter cultures. Due to cleavage pattern; at very beginning endopeptidases worked particularly on GS-ATP extract proteins and release smaller proteins. Then at advanced time of processing microbial exopeptidases worked on degradation products of GS-ATP extract and WSP, resulted in small peptides which may act like antihypertensive peptide, respectively.

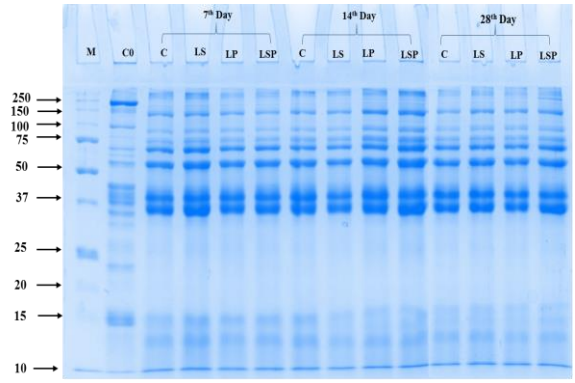


Figure 2. SDS-PAGE image of GS-ATP soluble proteins in different time points. M: Marker, C: Control, LS: *Lb. sakei*, LP: *Lb. plantarum*, LSP: *Lb. sakei*+ *Lb. plantarum* Samples

Protein Concentration: Protein concentration values of sucuk hydrolysates ranged between 14.92-15.67 mg/ml. As it is expected, control 0th day sample had the lowest protein concentration and other all samples had higher protein concentration than that of the control 0th. By the time even in control sample spontaneous microflora became dominant, caused fermentation and degradation which was also approved by SDS-PAGE images (Fig. 3). At 14th day, even though *Lb. sakei* is more proteolytic [14], *Lb. plantarum* sample had higher protein concentration. This can be attributed to the more aciduric behavior of *Lb. plantarum* and this can be supported with the pH results (Table 1).
At 28th days likewise 14th days *Lb. plantarum* was effective on protein concentration. S+P and P type sucuks had higher concentrations comparing to control and *Lb. sakei*.

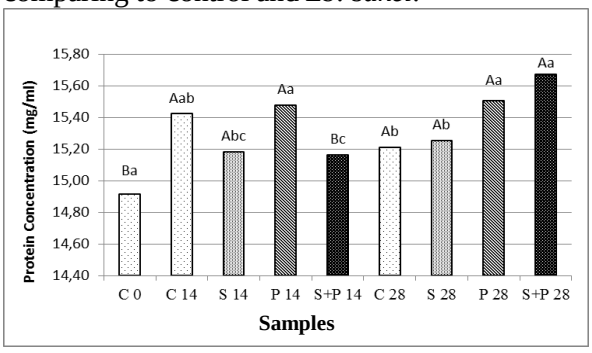
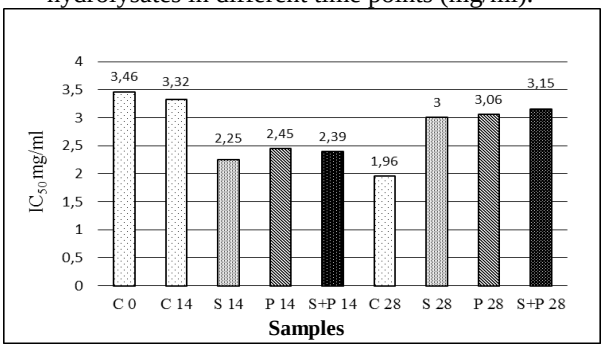


Figure 3. Protein concentration of Sucuk hydrolysates in different time points (mg/ml).
C: Control, S: *Lb. sakei*, P: *Lb. plantarum*, S+P: *Lb. sakei*+ *Lb. plantarum*

ACE Inhibitory Activity Assay: These results were expressed as IC_{50} value which is concentration of bioactive substance to inhibit

50% of an enzyme activity. Due to low degradation, control 0th day sample had higher IC_{50} value (3.46 mg/ml). Between 14th day samples *Lb. sakei* had the lowest IC_{50} (2.25 mg/ml). During the course of fermentation and storing period IC_{50} values of control type sucuk had sharp decrease and reached to the lowest value at 28th day (1.96 mg/ml) (Fig. 4). Results suggested that even without inoculation antihypertensive peptides can generation which may help inhibition of ACE action of mechanism to avoid formation of Angiotensin II, a vasoconstrictor in RAS.

Figure 4. ACE inhibitory activity of Sucuk hydrolysates in different time points (mg/ml).



C: Control, S: *Lb. sakei*, P: *Lb. plantarum*, S+P: *Lb. sakei+ Lb. plantarum*

IV. CONCLUSION

Antihypertensive peptides are promising qualifications for replacing pharmacological drugs to cure hypertension disease. Due to degradation behaviour during the course of fermentation, Sucuk is a probable source of different peptide sequences that may act like bioactive peptides. Further purification process with HPLC is needed to understand structure and action mechanism of these peptides. Also different biofunctional material (tea leafs etc.) added sucuks may be studied in future researches.

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