TURKISH FERMENTED SUCUK: A SOURCE OF ANTIHYPERTENSIVE PEPTIDES

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Abstract: In search of new approaches against Angiotensin I Converting Enzyme (ACE) action of mechanism in Turkish sucuk, fermented by *Lactobacillus sakei* and *Lb. plantarum* used as material. Physicochemical and biochemical analysis were carried out at 0., 7. and 14. days. Sucuk samples were subjected to hydrolyzation process with pepsin and trypsin and total ACE inhibitory activity was evaluated. At 0th day ACE inhibitory activity (IC_{50}) of hydrolysates was the lowest in control sample (3.46 mg/ml), while the highest inhibition was resulted in *Lb. sakei* (2.25 mg/ml) sample. Our results exposed that the hydrolysate of Turkish fermented sucuk by lactic acid bacteria especially by *Lb. sakei* could serve as a source of peptides with antihypertensive activity.
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. Hypertension 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, most important fragment of this system 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 hypertension.

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 are being degraded into smaller peptides inside of sucuk [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 that 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: Four days vacuumed *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 for sucuk production. Four different types of sucuks such as; control (no

inoculation), *Lb. sakei*, *Lb. plantarum* and combination type (inoculated with both *Lb. sakei* and *Lb. plantarum*) were produced and subjected to following analysis. Experimental sucuk dough 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 dough separately at a ratio of 1 % (10^7 cfu/g). Sucuk dough mixtures were stuffed into synthetic collagen casings (36-mmØ) 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.

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].

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE): GS-ATP and WSP extracts were separated according to their molecular weight (MW) by using SDS-PAGE technique. Samples were run on gradient gel made of 7.5%–20% at a constant current of 30 mA/gel 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 applied on sucuk at an appropriate enzyme /substrate, respectively. Hydrolysates were filtrated trough A5 filter paper (Advantech Tokyo K. Ltd., Tokyo, Japan) and then 0.45µm filter and filtrates were collected for the ACE inhibitory assay.

Protein Concentration: Protein concentrations *were* determined by using the Biuret method [11].
*ACE inhibitory activity assay:*The ACE inhibitory activity of samples were determined according to the method of Cushman&Cheung [12], with slight modifications as described by Katayama et al. [13].

III. RESULTS AND DISCUSSION

Dry Matter and pH values: In the course of fermentation, there was a remarkable increase in dry matter (Table 1). No significant differences observed between the sucuk types at 0th day. However, at 7th days *Lb. sakei* sample was statistically different ($p<0.05$) compared to other 3 types and had the lowest dry matter. After 14days of ripening *Lb. sakei* and *Lb. sakei + Lb. plantarum* samples differed and had lower dry matter values ($p<0.05$). A significant decrease was observed in pH values between 0thday to 7th days. However a slight increase noted from 7th day to 14th day. Results suggested that due to higher microbial growth, accumulation of lactic acid was high during first seven days of ripening.

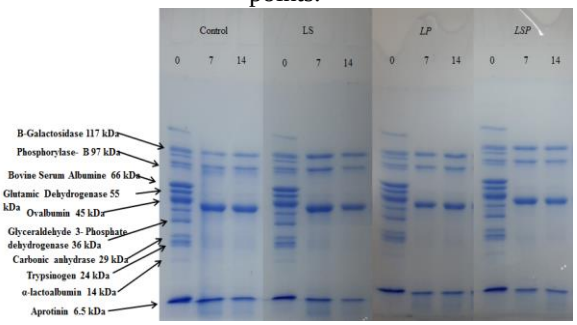
Table 1. Physicochemical properties of Sucuk samples in different time points.

Parameter	Control	LS	LP	LSP
Dry Matter				
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
pH				
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

Different capital letters in each column show statistically significant differences between the time points ($P<0.05$), and different small 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: WSP extracts of 0th day sample, before inoculations and fermentation, showed 13 protein bands on SDS-PAGE gel (Figure 1). During the first 7 days, bands correspond to Bovine Serum Albumine (BSA) 66 kDa, Glutamic Dehydrogenase 55 kDa, Ovalbumin 45 kDa disappeared and caused generation of a new and intense band with an approximate molecular weight of 40 kDa (Figure 1). Apparently in samples at 7th and 14th days, 4 bands were observed.

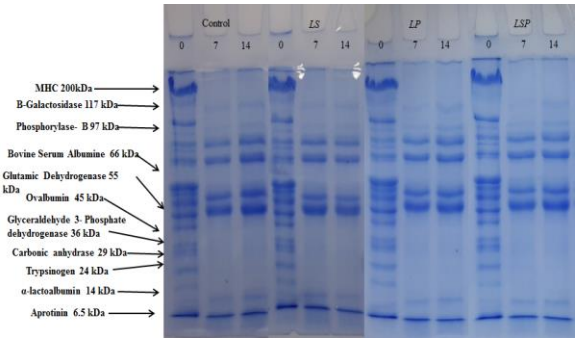
Figure 1. SDS-PAGE image of WSP in different time points.



LS: *Lb. sakei*, LP: *Lb. plantarum*, LSP: *Lb. sakei*+ *Lb. plantarum*

GS-ATP extract of 0th day exhibited 16 different bands (Fig. 2). High molecular weight bands such as Myosin Heacy Chain (MHC) -200 kDa, B-Galactosidase-117 kDa, Phosphorylase- B 97 kDa and also Actin-45 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.

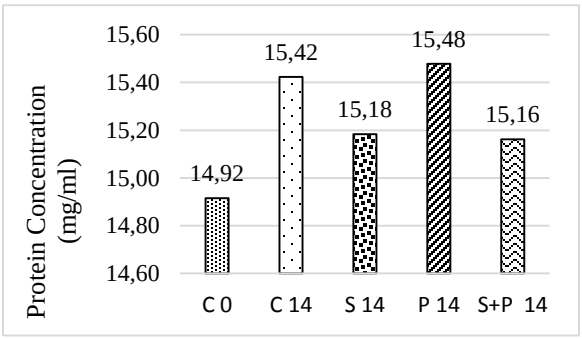
Figure 2. SDS-PAGE image of GS-ATP soluble proteins in different time points.



LS: *Lb. sakei*, LP: *Lb. plantarum*, LSP: *Lb. sakei*+ *Lb. plantarum*

Protein Concentration (P.C.): P.C. values of sucuk hydrolysates ranged between 14.92-15.67 mg/ml. As it is expected, control 0th day sample had the lowest P.C. and other all samples had higher P.C. than 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 P.C..

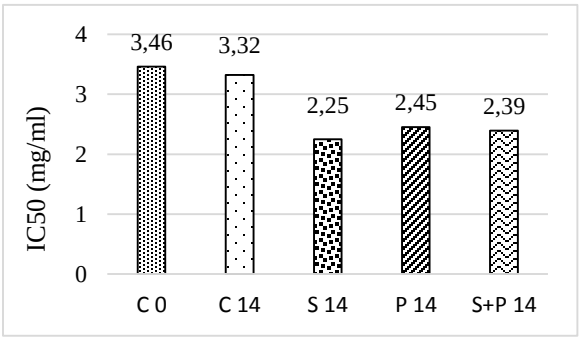
Figure 3. Protein concentration of Sucuk hydrolysates in different time points (mg/ml).



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

ACE inhibitory activity assay: Results were expressed as IC_{50} value which is concentration of bioactive substance to inhibit 50% of an enzyme or a radical. 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) (Fig. 4). Results suggested that inoculation of *Lb. sakei* into sucuk may cause generation of more antihypertensive peptides that 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 bioactive peptides is a promising approach for replacing pharmacological drugs to deal with hypertension. Because of degradation during the course of fermentation, Sucuk becomes a valuable source of different peptide sequences which may act like bioactive peptides. A purification process is currently ongoing by HPLC as an attempt to reach and identify the bioactive peptides.

ACKNOWLEDGEMENTS

C.B. Author thanks to The Scientific and Technological Research Council of Turkey (TUBITAK) and Scientific Research Project Found Office of Erciyes University for their supports.

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