SUCUK; SOME CHEMICAL AND MICROBIOLOGICAL CHARACTERISTICS AND LACTIC ACID BACTERIA HAVING ANTIMICROBIAL ACTIVITY

ÇON,A.H. and GÖKALP, H.Y.

Pamukkale Uni., Engineering Coll., Food Eng. Dept.,20017 Çamlık-Denizli/TURKEY

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INTRODUCTION

In Turkey, typically fermented and spicy, un-heated, semi-dry, predominant and one of the oldest fermented sausage is "sucuk". It is processed from beaf, and/or mutton and water buffalo meat, usually contain 10% tail-fat of fat-tailed sheep. Minced and casing stuffed material is ripened at 18-27°C under 80-95% relative humidity. Marketed product contains approximately 35-40% water, 28-40% fat, 28-32% protein and pH values varying between 5.00 and 5.60. Product is consumed as is (uncooked), broiled, fried, or toasted. Starter lactic cultures are not commonly practiced in the processing of sucuk, and the production is usually done by "chance inoculation" (Gökalp et al., 1995). Due to this chance inoculation and other factors, there is a lack of uniformity in standards and quality (Gökalp et al., 1988; Atala, 1992).

The objective of this study was to determine some of the chemical and microbiological characteristics of sucuk samples obtained from the markets of different areas of Turkey and to isolate and identify bacteriocin and/or bacteriocin-like metabolites producing lactic acid bacteria. Additionally, characterization of the other types of antimicrobial metabolites of these lactic acid bacteria and the use of the appropriate isolates, as sucuk starter cultures, have been planned for future research works.

MATERIAL and METHODS

Material, Bacterial Strain and Culture Mediums

Total of 51 sucuk samples were obtained from various parts of Turkey, within a period of a year. Total of 424 lactic acid bacteria (LAB) were isolated from the samples. *Lactobacillus sake* Lb706 (bacteriocin producing strain), *L.sake* Lb706-A (none-bacteriocin producing strain), and indicator sensitive strains *L.sake* Lb790, *Listeria monocytogenes* Li6 and *Staphylococcus aureus* St44 were obtained from the Federal Center For Meat Research, Kulmbach, Germany, and *Escherichia coli* NRRL B-3704 from the USDA Agriculture Research Service., Illinois,USA. All the LAB stock cultures were maintained in 11% non-fat milk powder medium, supplemented with 15% glycerol and stored at -20°C (Beyatlı, 1990). Working cultures were propagated in de Man, Rogosa and Sharp (MRS) broth, at 30°C and stored in MRS agar as stab cultures at 4°C (Schillinger and Lücke, 1989). *L. monocytogenes* Li6, *S. aureus* St44 and *E. coli* NRRL B-3704 were cultivated in Tryptone Soy Broth (TSB), which was supplemented with 0.6% Yeast Extract (YE) at 30°C. These organisms were maintained as slant cultures on Tryptone Soy Agar (TSA) supplemented with 0.6% YE at 4°C (Lewus et al., 1991).

Microbiological Analyses, Isolation, Antagonistic Effects and Identification of LAB

25g of ground sample was aseptically homogenized in 225 ml physiological saline solution in a sterile stomacher bag (Stomacher Lab Blender, 400-BA 7021, Seward Medical) for 90s. Further decimal dilutions were prepared . Following media and incubation conditions were used to enumerate the specific classes of microorganisms: Plate Count Agar (PCA, Oxoid) for total counts (32°C, 48 h) (Speck,1976); MRS Agar (Oxoid) and Lactic Agar (LA) (prepared in our laboratory) for lactic acid bacteria (30°C, 72 h) in an anaerobic incubator (10% CO_2, 90% N_2) (Silla et al., 1989); Violet Red Bile Dextrose Agar (VRBD, Oxoid) for *Enterobacteriaceae* (37°C, 24 h) (Silla et al., 1989).

Lactic acid bacteria (LAB) were isolated and some tests were carried out (Benson, 1983; Vanderzant and Splittstaesser, 1992). For determination of the antagonistic activity of LAB, agar spot and well diffusion assay tests were used (Schillinger and Lücke, 1989). Identification of the LAB was done as outlined (Schillinger and Lücke, 1987; Anon.,1991). Carbohydrate fermentation profiles were determined by using API 50 CH strips and API 50 CHL Medium (bio Meriux, sa 6928 Marcy L'Etoile, France), results were checked after 24, 48 and 72 hours. Moisture, fat content and pH values of the sucuk samples were determined as indicated Gökalp et al. (1993).

RESULTS and DISCUSSION

pH, moisture and fat content of the samples exhibited very wide variations (Table 1). Premium quality sucuks should have pH values in the range of 5.10.-5.20 and it should not exceed 5.40 (Gökalp et al.,1995). The Turkish Standards (TS 1070 Sucuk) limits both the moisture and fat contents of marketed sucuk to maximum 40%. A large number of the samples had moisture and fat content greater than 40%. Results of similar research show similar findings (Aytekin, 1986).

Table 1. Results of the Chemical and Microbiological Analyses of the Sucuk Samples

	Values				Microbiological Counts (CFU/g)		
	Min.	Max.	Ave.		Min.	Max.	Ave.
pH	4.10	6.31		Total Aerobic Mesophilic Bacteria	$4.2x10^4$	$3.8x10^9$	$5.3x10^8$
Water (%)	20.96	50.49	35.01	Lactic Acid Bacteria (MRS)	$5.7x10^4$	$1.6x10^9$	$4.6x10^8$
Fat (%)	21.0	51.0	34.4	Lactic Acid Bacteria (LA)	$4.8x10^4$	$1.9x10^9$	$4.3x10^8$
				*Enterobacteriaceae	1.0	$9.2x10^4$	$3.9x10^3$

*Entrobacteriaceae counts of <10 CFU/g were found in 10 samples; one sample had a count of > $3x10^5$ CFU/g

Total aerobic mesophilic counts were within the range of the results obtained in previous studies (Aytekin,1986; Gökalp et al.,1988). LAB counts were very similar to those presented by Kaya (1992). There are no published literature regarding *Enterobacteriaceae* counts from sucuk samples (Table 1). *Enterobacteriaceae* counts which include broad bacteria groups, are lower than the *coliform*

counts of some research findings (Aytekin, 1986; Gökalp et al., 1988). This is somewhat encouraging, but its presence in the samples is nonetheless significant and worrisome, since this group includes pathogenic bacteria. However, the fact that 19.61% of the samples had *Enterobacteriaceae* counts of <10 CFU/g proves that use of proper technological and hygienic techniques will reduce these counts.

After isolation, antagonistic effect tests and identification, 57 LAB isolates were selected for their antimicrobial activity. The selection based on an isolate exhibiting an inhibition zone of at least 0.5 mm against at least one indicator organism as a results of the agar spot and/or well diffusion test. The following isolates were identified: 19 *Lactobacillus plantarum*, 4 *L. curvatus*, 2 *L. pentosus*, 1 *L. rhamnosus*, 1 *L.delbrueckii*, 3 *P. acidilactici*, 4 *P.pentosaceus*. Of the remaining 23 isolates, 21 were not species identifiable, and 2 were identified as *L. sake* based on its carbohydrate fermentation characteristics as outlined in the Bergey's Manual of Systematic Bacteriology (Kandler and Weis, 1984). The inhibitory ability of each of these 57 isolates are listed in Table 2. A total of 9 isolates exhibited very significant antimicrobial activity against the indicator organisms.

Table 2. Antimicrobial Activity of LAB Isolates of Sucuk Against Indicator Bacteria

		L. sake Lb790		*L.monocytogenes* Li6		*S. aureus* St44		*E. coli* NRRL B-3704	
		Spot	Well	Spot	Well	Spot	Well	Spot	Well
L. plantarum	452	+	+	+	+	-	-	++	-
	495	++	+	++	-	-	-	++	-
L. curvatus	348	++	-	+	+	+	-	+	-
Lactobacillus spp.	411	+++	+++	+++	+++	+	-	++	-
	517	++	+	++	-	+	-	++	-
P.pentosaceus	416	+++	+++	+++	+++	-	-	+	-
P. acidilactici	413	+++	++	+++	++	-	-	+	-
	419	+++	+++	+++	++	-	-	-	-
	446	+++	++	+++	++	-	-	+	-

-:< 0.5mm; +: 0.5-1.0mm; ++: 1.1-3.0mm; +++: > 3.0mm

The antimicrobial effects of the species isolated in this study have previously been determined by numerous other researchers (Schillinger and Lücke, 1989; Lewus et al., 1991; Kaya, 1992; Fricourt et al., 1994).

CONCLUSIONS

High water content and high pH values of some samples indicates that sucuk is being marketed before it is adequately ripened. This coupled with high fat content of the product, causes economic losses to the consumer and also results in shortened shelf life of the product . Results of the study show that the samples analyzed exhibited somewhat acceptable levels of total aerobic mesophilic and LAB counts. *Enterobacteriaceae* counts of many samples were found to be at unacceptable levels. These findings suggest the cause to be due to a lack of application of standard methods, not using starter culture, lack of uniformity of raw materials, and a wide diversity in production conditions. In order to ensure production of premium quality sucuk which is in compliance with accepted standards and safe for human consumption it is imperative that the industry comply with the accepted methods and standards in regards to use of appropriate starter culture, raw materials and production guidelines. It is recommended that future research to be conducted to further investigate the appropriateness of using the following isolates, which were identified out of 57 isolates as having antimicrobial activity, for production of sucuk and other fermented products: *P. pentosaceus* 416; *P. acidilactici* 413,419,446; *L. curvatus* 348; *L. plantarum* 452,495; and *Lactobacillus spp.* 411 and 517. There is on-going research by our group to determine production ability of acid and flavor metabolites, and whether producing undesirable metabolites or not in fermented products and sucuk of these identified and recommended species as starter culture.

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