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A Survey on Chemical, Biochemical and Microbiological Characteristics of a Traditional Dairy Product in Mediterrean Region: Kes

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Abstract: The study was conducted to determine the microbiological quality (total aerobic mesophilic bacteria, *Lactococcus* sp., *Lactobacillus* sp., Coliform bacteria, *Staphylococci-micrococci*, *Enterococci*, *Enterobacteriaceae*, yeast and mould) and biochemical properties (dry matter, fat, salt, acidity, pH, water activity, nitrogen, WSN/TN, TCA-SN/TN and PTA-SN/TN) of Kes. Total of 64 Kes samples were randomly collected from Burdur province of Turkey. According to the results of chemical analysis, Kes is included in the moisture food groups (a_w = 0.90) and less mature cheeses. Kes proteolysis were found to be low-level. The microbiological quality of Kes is not satisfactory because the total aerobic mesophilic bacteria, yeast and mould counts are high. Coagulase-positive staphylococci was detected in 12 of the 64 samples (25%) and *E. coli* was detected in 2 of 64 (3.1%). It was concluded that Kes may be contaminated with some microorganisms during production or selling.

Key words: Traditional products, dairy product, properties, Kes, bacteria, Turkey

INTRODUCTION

Kes is a Turkish dairy product produced and consumed in Anatolia, Black sea and the Mediterranean regions of Turkey. Kes is produced by small family businesses and in small commercial dairy processing plants. Kes is a dried dairy product originally brought from Central Asia to Turkey. Kes cheese is made from yogurt whose fat has been separated and is known as Kesk, Kesuk, Kis or Cokelek. This type of cheese is also known as Kurut (dried yogurt) in Van provinces because it is produced by means of air-drying. In small quantities (<200 g), it has an irregular cylindrical shape. It can be classified as hard cheese. This product is consumed directly or added to soups or pasta (Kirdar, 2004; Cakir *et al*., 2009). Kashk is a similar product that is available in Iran. It is made from yogurt. It is a dried product to which spices are added. Kashk is made in the form of cubic balls and conic forms. It can be added to soup. Khask is the primary protein source of the nomadic people of Iran. This product is also produced in Syria (Cakir *et al*., 2009; Kosikowski, 1982).

Kes still produced traditional way in Mediterrean region. The evening milk is left at room temperature. This practice is induced during coagulation to about 20°C. After coagulation, the curd is put into a cloth bag to drain for 24 h in a cool room (~15°C). Salting was carried out by adding solid salt (Approx. 2%). Afterwards, the curd was kneaded manually for 5 min to distribute the salt uniformly throughout the curd. After salting, black sesame (1%), capsicum (1%), black and red pepper (1%) were added to the curd and the curd was shaped into cubic or conical form and dried under room temperature conditions for 3-4 days. Kes was ripened at 6±1°C for 90 days (Kirdar, 2004). Few researchers have studied the chemical and biochemical characteristics of Kes cheese in different province. Tarakci determined some of the properties of Kes cheese in Ordu and reported the contents of dry matter 68.03%, moisture 31.97%, fat 11.35%, protein 42.34%, ash 8.33%, salt 7.08%, pH 3.9 and acidity 2.64% as lactic acid. They also reported the total bacteria as being 5.98 log cfu g^{-1}, molds and yeasts 4.68 log cfu g^{-1}, lactic acid bacteria 4.46 log cfu g^{-1} and lipolytic bacteria 3.93 log cfu g^{-1}.

No coliforms were observed in the samples. Dervisoglu *et al*. (2009) reported the mean values of dry matter, fat, salt, salt in dry matter, ash and pH of 41 different Kes cheeses in Ordu as 56.17±6.07, 8.79±2.84, 3.22±1.35, 5.68±2.19, 4.31±1.29 and 4.75±0.59%, respectively. Kirdar (2004) reported that Kes samples produced in Burdur province had dry matter 72.54%, fat 36.50%, fat in dry matter 50.31%, salt 4.68%, salt in dry matter 6.45%, acidity 1.85% and pH 4.31. Cakir *et al*. (2009) determined some of properties of Kes cheese in Bolu and

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reported dry matter 61.59%, ash 13.66%, protein 32.42%, fat 6.30%, salt 13.26%, acidity 0.22% and pH 3.81. The Kurut samples collected from Van province had 85.51%, fat 8.52%, protein 54.64%, ash 14.89%, salt 12.18% and titratable acidity 1.18% as lactic acid. The aim of this study was to determine chemical, biochemical, microbiological characteristics and proteolysis of the Kes traditionally produced in the Burdur province in Turkey.

MATERIALS AND METHODS

Total 64 ripened fresh Kes samples (~250 g each) were collected from the Burdur local bazaar in May, 2009 and September 2010 and put into in ice chest in sterile plastic bags in the laboratory under aseptic conditions. The microbiological and chemical analyses began immediately after the samples were brought to the laboratory under cold conversion condition (4°C). The analyses were carried out in triplicate.

Chemical analyses: The pH values of the cheeses were measured using a pH meter (HANNA). Titratable acidity was measured according to the methods of AOAC (2000). Fat was determined by the Gerber-Van Gulik Method (IDF, 1997). Total solids content was determined by the gravimetric method using oven drying in a laboratory oven at 105°C until constant weight (IDF, 1982). Salt content was determined using the method of the International Dairy Federation (IDF-FIL, 1979). The values of water activity (a_w) were measured using a water activity meter (TESTO-650). The Total Nitrogen (TN) content was determined using the Kjeldahl Method (IDF, 1993) and total protein was expressed as TN 6.38. The Total Nitrogen (TN), Water Soluble Nitrogen (WSN), Trichloroacetic Acid-soluble Nitrogen (TCA-SN, 12% w/v) and Phosphotungstic Acid-soluble Nitrogen (PTA-SN, 5% w/v) were determined using the Kjeldahl Method. The Ripening Index (RI) was also calculated as a percentage of the ratio of WSN to TN. WSN was expressed as a percent of TN (WSN/TN). TCA-SN was expressed as percent of TN (TCA-SN/TN). PTA-SN was expressed as percent of TN (PTA-SN/TN). All of the chemicals used for the analysis were of analytical grade and obtained from Merck or Sigma-Aldrich, Germany.

Microbiological analyses: The total number of mesophilic aerobic bacteria were enumerated on standard Plate Count Agar (PCA) incubated at 35±1°C for 48 h (Peeler and Maturin, 1992). Baird-Parker agar was used for enumeration of *Staphylococci-micrococci* at 35°C±2 for 48 h. Colonies were examined using gram stain, the Catalase test, anaerobic utilization of glucose and mannitol and coagulase test (BAM, 2001). VRBD Agar (Violet Red Bile Dextrose Agar) was used for isolation of Enterobacteriaceae at 37°C±0.1 for 48 h. The count of the total number of coliforms was performed on standard Violet Red Bile (VRB) agar incubated at 35±1°C for 24-48 h (APHA, 1978; ICMSF, 1983). Positive cultures were used to create sub-cultures on Eosin Methylene Blue lactose sucrose (EMB) agar.

They were incubated at 35±1°C for 24 h. *E. coli* isolates were biochemically characterized by IMViC tests. Yeasts and moulds were enumerated on Potato Dextrose Agar (PDA) following the Pour-plate method and incubated at 25°C for 5-7 days (BAM, 2001). *Enterococci* was grown on Slanetz-Bartley Agar plates (SBA) and subsequently incubated at 37°C±0.1 for 48 h (Facklam and Sahm, 1995). MRS agar was used for counting *Lactobacillus* sp., (Dupont *et al*., 2000) and M17 agar for *Lactococcus* sp., (Terzaghi and Sandine, 1975). Identification of the isolates was performed using the criteria of Bacteriological analytical manuel (Anonymous, 2001). All of the media were obtained from Oxoid (Unipath Ltd., Basingstoke, England). Microbiological analyses were carried out in triplicate.

Statistical analyses: All of the statistical calculations were performed using SPSS Statistical software and the obtained values were presented as the mean±SD. Evaluation of significance was performed by analyses of variance followed by Spearman correlation. The significance level of $p<0.01$ was used for determining statistical differences. Colony counts were converted to log cfu g^{-1} and means.

RESULTS AND DISCUSSION

The results concerning some of the chemical properties (mean values and associated standard deviations) of the Kes samples are shown in Table 1. In the Kes samples, the mean dry matter was 59.50%, nitrogen 3.38%, fat 25.00%, salt 2.96%, acidity 0.32% and the water activity (aw) 0.90. The ripening index (WSN/TN×100) values ranged from 1.45-28.21% with a mean of 7.6%.

The WSN/TN ratio of Kes samples ranged from 1.33-27.55% averaging 7.71%. The PTA-SN/TN values found in this research were determined to have an average of 2.92%. The TCA-SN/TN ratios of the samples were between 1.45-28.21%. There was a significant negative correlation between aw and titratable acidity ($r = -0.617$, $p<0.01$) between aw and salt ($r = -0.527$ $p<0.01$) and

between aw and dry matter (r = -0.631, p<0.01). The results of some microbiological quality of the Kes shown in Table 2. The correlation between the various microorganisms, acidity, pH, aw and salt values that are shown in Table 3. The average of total aerobic mesophilic bacteria, *Lactococcus* sp., *Lactobacillus* sp., Coliform bacteria, *Staphylococci-micrococci*, *Enterococci*, *Enterobacteriaceae* and Yeast-mould counts were 8.24, 7.63, 7.52, 2.44, 3.95, 2.95, 2.62 and 5.38 log cfu g^{-1} in success in cheese samples, respectively. Coagulase-positive staphylococci was detected in 12 of the 64 samples (25%) and *E. coli* was detected in 2 of 64 (3.1%). There was a significant positive correlation between TAMB and *Lactobasillus* sp., count (r = 0.520, p<0.01) and *Lactococcus* sp., counts (r = 0.513, p<0.01).

Table 1: Some chemical properties of Kes samples

Chemical parameters	Sample no.	Mean±SD	Minimum	Maximum
L.A. (%)	64	0.32±0.15	0.11	0.78
Fat (%)	64	25.00±7.22	7.00	39.00
Salt (%)	64	2.96±0.83	2.11	5.38
Dry matter (%)	64	59.50±9.82	40.56	83.10
Fat in dry matter (%)	64	42.03±8.99	16.36	66.23
Salt in dry matter (%)	64	5.07±1.50	3.64	10.97
Water activity (a_w)	64	0.90±0.03	0.84	0.94
Total nitrogen	64	3.38±0.81	2.07	5.45
Total protein (%)	64	19.52±5.01	13.23	34.78
WSN (%)	64	7.71±6.80	1.33	27.55
Ripening index	64	7.60±6.59	1.30	27.73
TCA (%)	64	7.67±7.24	1.45	28.21
PTA (%)	64	2.92±3.00	0.39	12.56

Table 2: Presence of microorganisms in the Kes samples (log cfu g^{-1})

Microorganisms	Sample no.	Mean±SD	Minimum	Maximum
Total aerobic mesophilic bacteria	64	8.24±0.95	6.00	9.95
Lactobasillus sp.	64	7.63±0.99	5.20	8.93
Lactococcus sp.	64	7.52±0.96	4.38	8.78
Enterobacteria	64	2.62±1.13	2.00	5.99
Staphylococcus-micrococcus	64	3.95±1.51	2.00	6.66
Enterococ	64	2.95±1.46	2.00	6.51
Coliform bacteria	64	2.44±0.87	2.00	5.53
Yeast-mould	64	5.68±1.40	2.00	9.00

The high content of dry matter and low amount of fat in the samples resulted in a hard texture. These results were in agreement with the findings of Cakir *et al.* (2009) and Dervisoglu *et al.* (2009). These results might be originating from the different production techniques and the different storage conditions that were used. Fat is an important constituent of milk and contributes quality, taste and flavor and nutritional value to the product. The fat ratio of Kes samples showed great variations. The differences in the fat content may exist because this product does not have a standard production technique and because different milks were used in cheese making. The results of the study are in accord with the studies by Kirdar (2004). Acidity has effect on the taste and flavor of the product. Kes samples low acidity (0.32%). Salt is an important ingredient that contributes to taste and flavor development. Great differences in salt content exist both among producers and the production periods. Low salt levels were recorded in Kes. Kes is hand-salted which probably indicates non-homogeneous salt distribution soon after cheese making a condition that may constrain the rate of proteolysis in the said cheeses during later ripening (Thomas and Pearce, 1981). Water activity (a_w) is an important physico-chemical parameter which influences microbiological and biochemical evolution during the cheese-ripening process.

According to the values of water activity, foods are divided into three groups; moisture food (a_w = 0.90-1.00), intermediate-moisture food (a_w = 0.60-0.85), low-moisture food (a_w<0.60). According to the study results, Kes is included in the moisture food groups. These results were in agreement with the findings of Ordu Kes cheese (Dervisoglu *et al.*, 2009). Proteolysis is probably the most important biochemical event during ripening of most cheese varieties. The nitrogen fractions are very important parameters for determining the extent of proteolysis (Fox, 1989). The amount of soluble nitrogen compounds was expressed as a percentage of total nitrogen to provide a ripening index for each of the samples.

Table 3: The correlation between the various microorganisms and chemical properties of Kes samples

	TAMB	MRS	M17	Enterobacter	Coliform bacteria	*Staphylococci -micrococci*	Enterococ	Yeast-mould	a_w	Salt
TAMB	1									
MRS	0.520**	1								
M17	0.513**	0.534**	1							
Enterobacter.	0.045	-0.228*	0.907**	1						
Coliform bacteria	0.021	-0.304**	0.021	0852.000**	1					
Staphylococci-Micrococci	0.150	-0.079	0.014	0.047	0.117	1				
Enterococcus	-0.0134	-0.392**	-0.223**	-0.572**	0.656**	0.368**	1			
Yeast-Mould	0.593**	0.468**	0.349**	-0.121	-0.038	0.487**	0.055	1		
a_w	-0.015	-0.020	0.080	0203.000*	0.631**	0.125	-0.009	0.236*	1	
Salt	-0.049	-0.065	-0.200	-0.404**	-0.326**	0.053	-0.074	-0.086	-0.527**	1

*p<0.05; **p<0.01

The ripening index values is higher than the results reported Cakir *et al*. (2009) in Bolu Kes cheese and lower than the results reported by Dervisoglu *et al*. (2009). The results of nitrogen fraction show that Kes does not undergo an excessive proteolysis. The reasons for this situation are the differences in milk quality and the composition of the milk used in cheese production; the differences in the proteolytic activity of microbial flora in the cheese samples; the compositional variations of the cheeses and the differences in the water, salt, protein and acidity of the samples (Fox, 1989). Total nitrogen was used to determine the evolution of the total protein content and the determination of the ripening index. Total nitrogen was correlated with total acidity, dry matter, fat in dry matter, water-soluble nitrogen and ripening index (Table 3).

The WSN/TN ratio of Kes samples is similar to those reported by Dervisoglu *et al*. (2009) for Ordu Kes. The TCA-SN is known to be an indicator of the amount of small peptides (<20 amimo acid residues) and amino acids present in cheese and its level is regarded as the ripening depth index (Fox, 1989). This value is similar to those reported by Tarakci. PTA-SN is known as the free amino acids index of proteolysis. These values are lower than those found by Dervisoglu *et al*. (2009). One possible reason for the high level of variation between the samples might be a lack of standardization of most of the parameters (milk quality and composition) and manufacturing steps (ripening and packaging). Salt concentration had the most highly negative effect on WSN and PTA-SN formation. It is well known that high concentration of NaCl in cheese inhibits proteolysis (Fox, 1993). TCA-SN/TN and PTA-SN/TN values also showed the differences among Kes samples. This can be explained by either different compositions of the raw milk or the lack of available standard production methods. Given the values obtained for the nitrogen fractions in Kes, it can be concluded that this cheese undergoes proteolysis that is high in extent but low to moderate in depth.

The TAMB counts of Kes were higher than the Bolu Kes samples found by Cakir *et al*. (2009). A high number of TAMB can be explained by sufficient change in the environmental conditions which occur during cheese storage and which allows for the growth and multiplication of microorganisms or it could also be due to unsanitary conditions during processing and handling of the cheese. The Lactic Acid Bacteria (LAB) count plays a fundamental role in the ripening of cheese due to the lactic acid fermentation development which is a required characteristic of dairy products. Lactic acid bacteria constitute the dominant flora of cheese (Sengul, 2006; Stiles and Holzapfel, 1997). The count of LAB is higher than the results obtained by other researchers. *Staphylococcus aureus* may cause food-borne poisoning when it is exceeds 1.0-10^6 cfu g^{-1}. Therefore, the presence of *Staphylococcus aureus* is undesirable. The *S. aureus* counts in Kes samples were also higher than for Turkish white cheese (1.30-1.70 log cfu g^{-1}) and Herby cheese (0.95 log cfu g^{-1}) (Kivanc, 1989). Coagulase-positive Staphylococci was detected in 12 out of 64 samples (25%). Coliform group bacteria in foods are an accepted hygiene index. The presence of coliform bacteria in cheeses is undesirable because they cause structural defects in cheeses. The Turkish Food Codex Microbiological Criteria Notification mandates that dairy products must contain no >100 cfu g^{-1} coliform bacteria and must exclude *E. coli*. All of the samples (except two) were legal in relationship to the Turkish Food Codex Microbiological Criteria Notification. In this study, average counts of TMAB and coliform bacteria in the analyzed Kes were found to be higher than the similar studies (Cakir *et al*., 2009). It was considered to be originated from bad raw material, production conditions which were neither modern nor hygienic, unsuitable conditions storage, low salt concentration, non-hygienic equipment and contaminations induced by the environment and personnel (Kivanc, 1989; Vural *et al*., 2010) The presence of Enterobactericeae in Kes cheeses may be due to the use of raw milk and/or contamination of the product during manufacturing from environment and/or personal related (Giraffa, 2002). The incidence of yeast and mold has been considered a common problem during the ripening and refrigerated storage stages of the cheese. According to the Turkish Food Codex, a maximum 100 cfu g^{-1} yeast and mould is allowed. The counts were lower than the limit (<100 cfu g^{-1}) in the Turkish Food Codex Microbiological Criteria Notification. The reasons include the higher levels of salt and dry matter. High number of yeast and molds shows contamination of the product which results in spoilage, undesired taste and flavor (Cakir *et al*., 2009).

CONCLUSION

The study has reported the chemical, biochemical and microbiological characteristics of traditional Kes. It has a high nutritional value to its higher dry matter, fat and protein. In conclusion, these variation could probably be due to the type and composition raw milk used for the production Kes and manufacturing techniques, drying and storage condition. The values obtained for the nitrogen fractions in Kes lead to the conclusion that this cheese undergoes proteolysis that is high in extent but

low to moderate in depth. The geographical zone of cheese making, season of production, ripening temperature and duration and type of dairy are factors which influence the proteolysis levels in cheeses. The microbiological quality of Kes is not satisfactory because TAMB, yeast and mould counts are high. Researchers concluded that the manufacturing technology should be standardized.

REFERENCES

AOAC, 2000. Official Methods of Analysis. 17th Edn., Association of Official Analytical Chemistry, Arlington, Virginia, USA.

APHA, 1978. Standard Methods for the Examination of Dairy Products 1970. American Public Health Association Inc., Broadway, New York.

Anonymous, 2001. Bacteriological Analytical Manual. AOAC International, Gaithersburg, MD., USA.

BAM, 2001. FDA Bacteriological Analytical Manual Online. AOAC International, Gaithersburg, MD., USA.

Cakir, I., H. Coskun, I.T. Akoglu, M.T. Isleyen, M. Kiralan and A. Bayrak, 2009. Introducing a traditional dairy product Kes: Chemical, microbiological and sensorial properties and fatty acid composition. J. Food Agric. Environ., 7: 116-119.

Dervisoglu, M., Z. Tarakci, O. Aydemir, H. Temiz and F. Yazici, 2009. A survey on selected chemical, biochemical and sensory properties of Kes cheese, a traditional Turkish cheese. Int. J. Food Prop., 12: 358-367.

Dupont, I., D. Roy and D. Lapointe, 2000. Comparison of exopolysaccharide production by strains of *Lactobacillus rhamnosus* and *Lactobacillus paracasei* grown in chemically defined medium and milk. J. Indian Microbiol. Biotechnol., 24: 251-255.

Facklam, R.R. and D.F. Sahm, 1995. Enterococcus. In: Manual of Clinical Microbiology, Murray, P.R. (Ed.). 6th Edn. ASM Press, Washington, DC., USA., pp: 308-314.

Fox, P.F., 1989. Proteolysis during cheese manufacture and ripening. J. Dairy Sci., 72: 1379-1400.

Fox, P.F., 1993. Cheese: An Overview in Cheese: Chemistry, Physics and Microbiology. 2nd Edn., Chapman and Hall, London.

Giraffa, G., 2002. Enterococci from foods. FEMS Microbiol. Rev., 26: 163-171.

IDF, 1982. Determination of the total solid content. IDF Standard 4A, International Dairy Federation, Brussels.

IDF, 1993. Milk determination of nitrogen content. IDF Standard 2/B. International Dairy Federation.

IDF, 1997. Milk and milk products: Determination of fat content (general guidance on the use of butyrometric methods). IDF standard No. 152A, Brussels, Belgium.

IDF-FIL, 1979. Cheese and processed cheese products/determination of chloride content potentiometric titration method. Standard 17, Brussels.

Kirdar, S.S., 2004. A research on kes cheese. Proceedings of the International Dairy Symposium on Recent Developments in Dairy Science and Technology, (RDDST'04), SDU Press, pp: 239-241.

Kivanc, M., 1989. A survey on the microbiological quality of various cheeses in Turkey. Int. J. Food Microbiol., 9: 73-77.

Kosikowski, F.V., 1982. Cheese and Fermented Milk Foods. 2nd Edn., FV Kosikowski and Associates, Brooktondale, New York.

Peeler, J.T. and L.J. Maturin, 1992. Aerobic Plate Count: Bacteriological Analytical Manual Online. United States Food and Drug Administration, Center for Food Safety and Applied Nutrition, USA.

Sengul, M., 2006. Microbiological characterization of Civil cheese, a traditional Turkish cheese: Microbiological quality, isolation and identification of its indigenous Lactobacilli. World J. Microbiol. Biotechnol., 22: 613-618.

Stiles, M.E. and W.H. Holzapfel, 1997. Lactic acid bacteria of foods and their current taxonomy. Int. J. Food Microbiol., 36: 1-29.

Terzaghi, B.E. and W.E. Sandine, 1975. Improved medium for lactic *Streptococci* and their bacteriophanges. Applied Environ. Microbiol., 29: 807-813.

Thomas, T.D. and K.N. Pearce, 1981. Influence of salt on lactose fermentation and proteolysis in Cheddar cheese. J. Dairy Sci. Technol., 16: 253-259.

Vural, A., M.E. Erkan and H.S. Guran, 2010. The examination of the microbiologic quality in orgu cheese (Braided Cheese) samples. Kafkas Univ. Vet. Fak. Derg., 16: S53-S58.