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Acidity, microbial, organic and free amino acids development during fermentation of skimmed milk, Kishk

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Six organic acids (butyric, propionic, acetic, formic, lactic and succinic) were produced during Kishk fermentation. Their levels increased up to the sixth day of fermentation, except succinic acid which decreased after the fifth and the third days of skimmed milk and Rayeb Kishk's fermentation, respectively. Lactic acid had the highest increment rate while formic acid had the lowest. The free amino acids content rose during the fermentation. These results correlated quite well with the growth of lactic acid and proteolytic bacteria as well as with acidity development during fermentation. Substituting Rayeb milk with skimmed milk in Kishk preparation produced an acceptable soup product. The development of organic acids during Kishk fermentation was compared with established values for Kishk soup acceptability.

INTRODUCTION

Kishk is an extremely popular fermented food in many parts of the Middle East. It is made by mixing wheat with fermented milk (Laban zear)† and sun-drying the mixture to 8–12% moisture content (Atia & Khattab, 1985). Methods for its preparation vary from one place to another, but parboiled wheat meal and yoghurt are almost always the two major components of Kishk (Van Veen & Graham, 1969). Long shelf life, safety and nutritional value are factors which are responsible for the continuing popularity of fermented foods in Asia and Africa (Wang & Hasseltine, 1981). Dried Kishk is not hygroscopic and can be stored in open jars for 2–3 years without deterioration, and is shelf-stable under ambient conditions (Morcos *et al.*, 1973). The low moisture content and the low pH of the final product are a safeguard against the growth of pathogenic microorganisms (Hamad & Fields, 1982). Kishk is a protein-rich product, and is a possible source of many vitamins and growth factors associated with the microbial fermentation process. It is used for feeding children, adults and elderly people (Van Veen & Graham, 1969). The chemical and microbial changes during preparation of wheat yoghurt, Kishk, have been studied (Demerdash, 1960; Van Veen *et al.*, 1969; Shaker *et al.*, 1980; Hamad & Fields, 1982). However, no information is published regarding the organic acid and free amino acid development during fermentation of Kishk.

Manufacturing Kishk from yoghurt during times when milk is surplus is a means of preserving it. This would be especially helpful in tropical and subtropical regions. Accordingly, efforts should be made to find new means to utilize skimmed milk, which is less expensive than Laban zear in Kishk preparations, and to supply information on the development of organic acids and free amino acids with regard to microbial changes as well as on the acceptability of Kishk soup.

MATERIALS AND METHODS

Polished wheat grains were bought on the Alexandria market. Cow liquid skimmed milk was obtained from the Dairy Department, College of Alexandria,

† Laban zear is prepared by collecting the sour milk (Laban Khad), resulting from churning of sour milk in goats pelts, in an earthenware container, (known as zear), and allowing further acidity to develop.

University of Alexandria. Laban Rayeb† was bought in the local market in Alexandria. All these raw materials were used for Kishk preparation.

Preparation of fermented skimmed milk

Cow skimmed milk was heated vigorously at 95°C for about 10 min, cooled to 45°C and immediately inoculated with a mixed starter culture (2·5%) of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. After mixing the milk was incubated at 40°C for 12 h and kept refrigerated until used.

Preparation of Kishk

Cleaned and washed polished wheat was cooked in water (1:3, w/v) for 15 min, cooled, spread over mats, sun-dried, and crushed in a hand mill. Coarse-grained parboiled wheat (bulgur) was gradually moistened with 3% salted water and then mixed with fermented skimmed milk or Laban Rayeb at a ratio of 1:2 (w/w). The porridge-like mix was covered with a thick cloth and incubated at 32°C for 24 h. The swollen paste was then thoroughly mixed, divided into small round pieces, spread over straw mats and incubated at 32°C for a further 5 days. Following incubation, the Kishk balls were kept at room temperature to remove more moisture and improve storage ability. When the samples were completely dry, they were stored in air-tight containers (Kilner jar) and/or milled to pass through a 45 mesh sieve for Kishk soup preparation.

Analytical methods

Moisture content was determined by drying the samples to constant weight at 105°C (AOAC, 1980). The pH values were measured using a pH meter (Orion Research I on analyser/model 407A, Cambridge, MA). The titratable acidity (calculated as % of lactic acid) was determined according to the method of Egan *et al.* (1981). The volatile acidity (as % of acetic acid) was estimated, using a modified version of the method used by Zein (1963).

For organic acids determination, 10 g of the Kishk sample was extracted with 0·2N sulphuric acid, centrifuged at 3000 rpm for 5 min, and filtered using Whatman No. 1 filter paper. The free acids in an aliquot of the extract were transformed into their sodium salts using 0·1N sodium hydroxide, then the solution was evaporated on a steam bath to dryness. Silica gel column chromatography was used for separation and determination of organic acids according to the modified method described by Ragaee (1984). A standard curve was constructed using a mixture of known amounts of butyric, propionic, lactic, acetic, formic and succinic acids. The amounts of acids obtained were corrected for percentage recovery of the corresponding acid for each determination (Salama, 1988). Free amino acids were determined by the sulphosalicylic acid method of Hamilton (1962).

† Laban Rayeb: fresh milk in an earthenware container is left undisturbed in a warm dark place until the cream rises and the milk coagulates. The cream layer is removed and beaten to butter and the curd is consumed fresh.

Microbiological methods

Twenty-five grams of the Kishk were weighed and transferred aseptically to flasks containing 225 ml sterile peptone water (0·1%), mixed thoroughly for 2 min and left to stand for 15 min to remove any foam (Fields *et al.*, 1981). The necessary dilutions were made and the pour-plate technique was used. Plate count agar, skim milk agar and lactic agar were used for counting total aerobic mesophilic bacteria, proteolytic bacteria and lactic acid bacteria, respectively (Specks, 1976). Plates were incubated at 30°C for 24 h in the incubator (Qualitemp 80 TC Laboratory Thermal Equipment, Greenfield, Oldham, UK).

Technological method

Preparation of Kishk soup

The ingredients for Kishk soup were: Kishk flour (140 g), salt (5 g), chopped onion (90 g), olive oil (40 g), black pepper (to season) and water (1200 ml). The Kishk flour was soaked in water for about 1 h. Onions were coated with olive oil and combined with the water–Kishk mixture and seasonings, then cooked over medium heat for about 10 min.

Organoleptic evaluation

Ten panellists were requested to evaluate the hot Kishk soup for consistency, colour and flavour according to Griswold (1962). Results were statistically analysed using the nonparametric analysis method of Hollander & Wolfe (1973) for comparison between the samples.

RESULTS AND DISCUSSION

The changes in moisture content, pH value, and titratable and volatile acidities during the fermentation of skimmed and Rayeb Kishks are given in Table 1. At the beginning of the experiment, the moisture content was higher in skimmed milk Kishk than in Rayeb Kishk, but they became equal after the sixth day of fermentation. There was a sharp rise in both titratable and volatile acidities on the first day, followed by a slow and gradual increase to reach the maximum on the sixth day of fermentation. The pH value decreased in a parallel manner from 5·03 and 4·74 to 3·75 and 3·30 in skimmed milk and Rayeb Kishks, respectively. These results correlate well with the changes in microbial count during the fermentation process (Table 2).

Table 1. Changes in moisture content, pH, titratable and volatile acidities in skimmed milk and Rayeb Kishks during fermentation

Fermentation time (days)	Skimmed milk Kishk				Rayeb Kishk			
	Moisture content[a] (%)	pH[a]	Titratable acidity[a] (%)	Volatile acidity[a] (%)	Moisture content[a] (%)	pH[a]	Titratable acidity[a] (%)	Volatile acidity[a] (%)
0	64·65 ± 0·34	5·03 ± 0·04	0·42 ± 0·021	0·16 ± 0·001	56·68 ± 0·27	4·74 ± 0·02	0·58 ± 0·01	0·18 ± 0·008
1	63·44 ± 0·10	4·80 ± 0·10	0·86 ± 0·032	0·24 ± 0·014	55·01 ± 0·30	4·45 ± 0·17	1·03 ± 0·05	0·20 ± 0·010
2	59·96 ± 0·21	4·66 ± 0·12	0·98 ± 0·046	0·26 ± 0·010	51·02 ± 0·41	4·10 ± 0·10	1·14 ± 0·02	0·27 ± 0·012
3	50·80 ± 0·07	4·34 ± 0·05	1·12 ± 0·017	0·29 ± 0·004	56·27 ± 0·33	4·00 ± 0·07	1·55 ± 0·03	0·32 ± 0·005
4	36·04 ± 0·24	4·22 ± 0·10	1·46 ± 0·070	0·31 ± 0·015	31·92 ± 0·18	3·76 ± 0·11	1·85 ± 0·05	0·38 ± 0·017
5	22·38 ± 0·30	3·98 ± 0·13	1·76 ± 0·040	0·33 ± 0·011	21·99 ± 0·23	3·40 ± 0·08	1·94 ± 0·09	0·43 ± 0·002
6	12·90 ± 0·06	3·75 ± 0·05	1·80 ± 0·082	0·34 ± 0·007	12·00 ± 0·17	3·30 ± 0·05	2·16 ± 0·11	0·45 ± 0·021

[a] Mean ± standard error.

Table 2. Progress of microbial count[a] of Kishk during fermentation

Fermentation time (days)	Skimmed milk Kishk			Rayeb Kishk		
	Total aerobic bacteria	Lactic acid bacteria	Proteolytic bacteria	Total aerobic bacteria	Lactic acid bacteria	Proteolytic bacteria
0	$2·9 \times 10^{6}$	$5·4 \times 10^{6}$	$2·1 \times 10^{4}$	$3·4 \times 10^{6}$	$6·0 \times 10^{7}$	$1·3 \times 10^{4}$
1	$6·2 \times 10^{7}$	$1·0 \times 10^{9}$	$3·0 \times 10^{6}$	$4·1 \times 10^{6}$	$1·5 \times 10^{11}$	$4·4 \times 10^{5}$
2	$4·9 \times 10^{8}$	$5·0 \times 10^{11}$	$8·2 \times 10^{6}$	$6·0 \times 10^{6}$	$7·0 \times 10^{12}$	$5·7 \times 10^{6}$
3	$6·0 \times 10^{8}$	$6·8 \times 10^{11}$	$2·6 \times 10^{7}$	$4·5 \times 10^{8}$	$1·5 \times 10^{10}$	$3·3 \times 10^{6}$
4	$2·7 \times 10^{7}$	$4·4 \times 10^{12}$	$4·3 \times 10^{7}$	$4·0 \times 10^{5}$	$1·0 \times 10^{9}$	$1·2 \times 10^{4}$
5	$3·9 \times 10^{4}$	$3·3 \times 10^{8}$	$1·6 \times 10^{4}$	$2·5 \times 10^{4}$	$5·1 \times 10^{8}$	$0·0 \times 10^{0}$
6	$3·1 \times 10^{4}$	$2·2 \times 10^{8}$	$0·0 \times 10^{0}$	$2·4 \times 10^{4}$	$4·5 \times 10^{8}$	$0·0 \times 10^{0}$

[a] Colony forming units (CFU) ml of Kishk slurry.

Table 3. Production of organic acids during fermentation of skimmed milk and Rayeb Kishks (g/100 g dry basis)

Organic acid	Skimmed milk Kishk (Fermentation time, days)							Rayeb Kishk (Fermentation time, days)						
	0	1	2	3	4	5	6	0	1	2	3	4	5	6
Butyric	0·016	0·022	0·034	0·068	0·089	0·112	0·122	0·019	0·027	0·062	0·084	0·146	0·156	0·162
Propionic	0·114	0·120	0·129	0·154	0·162	0·198	0·204	0·138	0·186	0·204	0·323	0·406	0·412	0·422
Acetic	0·097	0·114	0·120	0·138	0·142	0·148	0·152	0·100	0·124	0·135	0·140	0·175	0·186	0·198
Formic	0·044	0·051	0·062	0·074	0·078	0·080	0·081	0·049	0·050	0·068	0·084	0·092	0·094	0·095
Lactic	0·222	0·346	0·490	0·570	0·792	0·804	0·934	0·238	0·462	0·503	0·690	0·822	0·899	1·102
Succinic	0·050	0·125	0·148	0·164	0·196	0·112	0·102	0·058	0·138	0·190	0·218	0·186	0·181	0·156

The difference between Kishk sample fermentations can be attributed to the higher growth of lactic acid and other microbial species in Rayeb Kishk than in skimmed milk Kishk. Similar results were obtained by Zamora & Fields (1979), in their work on the fermentation of cowpeas and chickpeas, as well as by Regaee *et al.* (1986) on the fermentation of lentils.

Banigo & Muller (1972) correlated the production of carboxylic acids (development of acidity) with acceptability of fermented maize products. Table 3 lists the production of organic acids during fermentation of two Kishk samples. The six organic acids (butyric, propionic, acetic, formic, lactic and succinic) were detected at the zero time of fermentation. It can be seen that their amounts increased with prolonging the fermentation time, except for succinic acid which decreased after the third and fifth days of Rayeb and skimmed milk Kishks fermentation, respectively. The decrease in succinic acid may be due to the decrease in pH to lower than 4, which inhibited the succinic acid bacteria (Regaee *et al.*, 1986). Lactic acid was the highest component from the start to the end of fermentation, while

Table 4. Free amino acids content of skimmed milk Kishk during fermentation (g/16 g N)

Amino acid	Fermentation time (days)						
	0	1	2	3	4	5	6
Aspartic acid	0·017	0·024	0·034	0·054	0·062	0·059	0·056
Threonine	0·070	0·060	0·065	0·058	0·059	0·057	0·039
Serine	0·034	0·038	0·045	0·048	0·042	0·036	0·030
Glutamic acid	0·100	0·084	0·090	0·090	0·094	0·098	0·074
Proline	0·036	0·032	0·045	0·062	0·065	0·068	0·062
Glycine	0·031	0·016	0·024	0·032	0·040	0·046	0·029
Alanine	0·021	0·014	0·018	0·035	0·048	0·051	0·045
Cystine	—	—	—	—	—	—	—
Valine	0·014	0·024	0·016	0·021	0·011	0·009	0·006
Methionine	—	—	—	—	—	—	—
Isoleucine	0·012	0·018	0·024	0·018	0·016	0·014	0·014
Leucine	0·022	0·020	0·019	0·016	0·021	0·020	0·015
Tyrosine	0·012	0·016	0·021	0·025	0·027	0·029	0·016
Phenylalanine	0·012	0·014	0·015	0·017	0·013	0·010	0·008
Histidine	0·010	0·012	0·017	0·020	0·024	0·029	0·015
Arginine	0·017	0·019	0·020	0·022	0·018	0·012	0·008
Lysine	0·032	0·045	0·051	0·064	0·066	0·064	0·055
Total	0·340	0·436	0·504	0·582	0·606	0·608	0·472

Table 5. Free amino acids content of Rayeb Kishk during fermentation (g/16 g N)

Amino acid	Fermentation time (days)						
	0	1	2	3	4	5	6
Aspartic acid	0·015	0·054	0·039	0·061	0·044	0·050	0·058
Threonine	0·053	0·044	0·040	0·037	0·035	0·033	0·031
Serine	0·020	0·013	0·023	0·031	0·032	0·034	0·025
Glutamic acid	0·070	0·064	0·065	0·069	0·071	0·060	0·055
Proline	0·030	0·034	0·033	0·035	0·053	0·052	0·051
Glycine	0·022	0·011	0·013	0·014	0·017	0·015	0·014
Alanine	0·012	0·023	0·034	0·042	0·052	0·045	0·035
Cystine	—	—	—	—	—	—	—
Valine	0·011	0·012	0·013	0·016	0·032	0·022	0·020
Methionine	—	—	—	—	—	—	—
Isoleucine	0·021	0·012	0·013	0·014	0·015	0·013	0·010
Leucine	0·020	0·018	0·019	0·021	0·024	0·020	0·012
Tyrosine	0·021	0·011	0·033	0·024	0·013	0·011	0·009
Phenylalanine	0·006	0·008	0·012	0·014	0·009	0·007	0·006
Histidine	0·012	0·016	0·019	0·022	0·024	0·017	0·016
Arginine	0·051	0·055	0·070	0·060	0·040	0·035	0·029
Lysine	0·010	0·012	0·014	0·016	0·018	0·020	0·019
Total	0·374	0·387	0·440	0·476	0·479	0·434	0·390

butyric and formic acids remained at considerably lower levels than the other acids investigated. These results confirm that the fermentation of Kishk is mainly a lactic acid one, correlating quite well with the growth of bacteria during fermentation of Kishk. Production of butyric, propionic and succinic acids could be due to the activity of the microorganisms present in the fermentation medium along with the lactic bacteria (Mandelstam *et al.*, 1982). Acetic acid was developed as the fermentation advanced in Rayeb Kishk faster than in skimmed milk Kishk.

In the case of Rayeb Kishk, the organic acids produced were higher than in skimmed milk Kishk throughout fermentation, which was reflected in the levels of both the titratable and volatile acidities.

The free amino acids determined in skimmed milk and Rayeb Kishks before and during fermentation are given in Tables 4 and 5. It can be observed that both skimmed milk and Rayeb Kishks contain 15 free amino acids including all the essential ones except cystine and methionine, which were not detected and tryptophan, which was destroyed during acid hydrolysis. Skimmed milk Kishk had higher threonine, serine, glutamic acid, proline, glycine, alanine, valine, phenylalanine, and arginine and lower isoleucine, leucine, tyrosine, histidine and lysine than Rayeb Kishk. Most of the free amino acids were probably utilized by the lactic bacteria during their growth (Hamad & Fields, 1982), as they diminished on the first day of fermentation. Pederson (1971) reviewed the required amino acids for lactic acid bacteria and reported that 17 amino acids at least were essential for growth. However, all free amino acids tended to increase until the fourth and fifth days of Rayeb and skimmed milk Kishks fermentation, respectively, then followed a small drop. The increment rate in the amounts of free amino acids was higher in skimmed milk Kishk than Rayeb Kishk, and could be due to proteolysis as well as to the biosynthesis of the free amino acids by bacteria (Tongnual *et al.*, 1981). These results correlate well with the growth of proteolytic bacteria during fermentation of Kishk. The mentioned differences between skimmed and Rayeb Kishks of the results of free amino acids may be attributed to the differences in composition of the two sour milks and in the optimum pH of fermentation.

The statistical analysis of variance showed that there were no significant differences between the soup samples in consistency and colour scores (Table 6). However, significant difference existed with regard to flavour, with skimmed milk Kishk soup having the highest score. The relatively high acidity of Rayeb Kishk samples renders them too sour to be acceptable.

It can be concluded that substituting Rayeb milk with skimmed milk in Kishk preparations produced an acceptable product.

Table 6. Sensory evaluation of skimmed milk and Rayeb Kishk soups

Samples	Consistency	Colour	Flavour
Skimmed milk Kishk	6·4	6·1	6·8
Rayeb Kishk	6·5	5·9	5·3

LSD (0·05).

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