EFFECT OF FERMENTATION ON SOME CHEMICAL COMPONENTS OF THE SUDANESE FERMENTED BUTTER MILK, ROBE

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KEYWORDS: Milk, Fermented milk, Chemical composition, Free amino acids, Macro and microelements.

ABSTRACT

Changes following the fermentation of the Sudanese sour milk product (Robe) were investigated. The average total protein, whey protein, casein and non-protein nitrogen contents of fresh milk were 3.1 ± 0.14%, 2.24 ± 0.16%, 2.0 ± 0.01% and 0.22 ± 0.01%, respectively. A comparison of free amino acid profiles of fresh milk indicates that the fermentation causes increase in accumul-ation of these free amino acids. In addition, the fermentation increases the availa-bility of some free amino acids, particularly histidine, isoleucine and methionine. The concentrations of most amino acids of Robe are about twice as that of fresh milk. The average value for ash content of fresh milk (0.81 ± 0.02%) is slightly different from that of Robe (0.76 ± 0.05%). The fermentation of milk into Robe increases the contents of most macro-and microelements. However, the contents of sodium and manganese are decreased. Generally, the concentrations of microelements are very low and hardly detectable in the analyzed samples.

الملخص:

تم في هذه الورقة التحري عن التغيرات التي تلي تخمر منتج الروب السوداني. كان متوسط محتويات البروتين الكلي، بروتين الشرش، الكيزين والنيتروجين غير البروتيني في اللبن الطازج 3.1 ± 0.14%، 2.24 ± 0.16%، 2.0 ± 0.01% و 0.22 ± 0.01%، علي التوالي. مقارنة محتوي اللبن الطازج من الأحماض الأمينية الحرة أظهر أن التخمر نتج عنه زيادة ملحوظة في تراكم الأحماض الأمينية الحرة. إضافة لذلك فان التخمر زاد من تيسر بعض الأحماض الأمينية الحرة، خصوصا الهستيدين، الايسوليوسين والمثيونين. تركيز معظم الأحماض الأمينية في منتج الروب مساوي تقريبا لضعف الأحماض الأمينية في

اللبن الطازج. متوسط محتوي اللبن الطازج من الرماد (0.81 ± 0.02%) اختلف اختلافا طفيفا عن متوسط محتوي الروب 0.76 ± 0.05%. تخمر اللبن إلى روب زاد من محتويات معظم العناصر الكبرى والصغرى، بينما أدى إلى تناقص محتوي كل من الصوديوم والماغنسيوم. عموما تركيز العناصر الصغرى كان قليلاً جداً وكانت هنالك صعوبة كبيرة في التحري عن وجودها في العينات المحللة.

INTRODUCTION

Fermented milk products are known for their taste, nutritive value and therapeutic properties. According to the International Dairy Federation (1969), fermented milks are "products prepared from milks, whole or fully skimmed, concentrated or milk substituted from partially or full skimmed dried milk, either homogenized or un-homogenized, pasteurized or sterilized and fermented by means of specific microorganisms. Milk from eight species of domesticated mammals (cow, buffalo, sheep, goat, horse, camel, yak and zebu) have been used to make traditional fermented milk products throughout the world. The people who had domesticated these milk animals usually accepted fermented milks by necessity (Kroger *et al*. 1989).

Steinkraus (1987) and Campbell-Platt (1987) have made extensive attempts in bringing into focus the large number of fermented foods of varying sensory attributes, specific to their places of origin. Besides, a few research workers have also presented food reviews relating to those fermented foods of their respective regions

(Oberman, 1985; Wood and Hodge-Robinson, 1985; Odunfa, 1985; Stanton, 1985; Kosikowski, 1977; and Soni & Sandhu, 1990).

Traditional fermented foods have existed in Sudan for a long time, of which fermented milks are widely popular and consumed by a large section of the human population. In general, these fermented milks are a result of natural fermentation. The quality and safety of the final product is dependent upon various influencing factors, especially the hygienic status of raw milk used and the presence of lactic acid bacteria and yeasts in good numbers and their ability to grow and bring in desirable fermentation.

Robe, a traditionally fermented milk product is the most widely produced product. Nomadic tribes produce the entire Robe during the rainy season (2-4Months), particularly in central and western Sudan. The bulk of Robe is made from cow's milk, while a smaller proportion is prepared from either goat's or sheep's milk or a mixture of these two milks (Abdelgadir *et al.,* 1996).

Many uses have been attributed to Robe. Freshly prepared Robe which is available early in the morning is a pleasant sour product with a characteristic buttery flavour. As the day wears on, the product losses its original pleasant flavour and turns more sour till then the whey separates from the curd which floats on top being fully impregnated with gas. Such a Robe is put to a number of uses. In hot climatic conditions, Robe is diluted with 2 or 3 volumes of water to give Gubasha, a thirst quencher (Dirar, 1997).

Saeed (1981) and (El-Mardi, 1988) reported that market samples of Robe was found to contain 7.2% total solids, 3.3% protein, 2.0% lactose, 0.16% fat, 1.9% total acidity (as lactic acid) and a pH value of 3.5. It seems that little work was accomplished regarding the nutritional quality of Robe. Bearing this in mind, the present study aimed to determine of the protein content, amino acid composition, macro-and microelements of this ferment-ed milk product.

MATERIALS AND METHODS

Materials: Five samples of fresh cow's milk and Robe from each site were collected in 250mL sterilized screw capped bottles. Fresh samples were collected immediately after milking. Each sample was transferred to a picnic thermoflask containing ice cubes to suppress microbial growth. The sources of fresh milk and Robe samples included five different sites in Gezira province (Central Sudan) named Barakat, El-Mekki, Darweesh, Atra and Nesheshiba. In these areas pastoralists graze their cows which belong to Butana and Kenana types (local breeds) with a few being crossed with Friesian breeds. Most of the cows were in second or third lactation. The cows were under autumn feeding conditions, and mainly upon grass. The analyses were conducted on samples upon arrival to the laboratory. Samples were kept in a refrigerator at 6°C pending other analyses.

Methods: Protein and protein fractions, amino acid composition, ash, macro-and microelement contents of fresh milk and Robe samples were determine-ed. Kjeldahl apparatus was used to determine the nitrogen content (N) according to

AOAC method (1990) the protein % was calculated by multiplying N×6.38. The separation of protein fractions was performed as described by (Csapo, 1984). The amino acid composition was determined by HPLC, post column derivitization with ninhydrin and UV detection. The concentration of free amino acids in the different samples was determined from averages of duplicate injections of the extract preparations. The results were reported as free amino acids per 100g sample.

Ash content was determined by AOAC (1984) method. The macro- and micro-elements, present in the ash as metallic oxides, were converted to chlorides by HCl and diluted. Flame Atomic Absorption Spectrophotometer (FAAS, Waldbronn, Germany) with a variant spectrometer (SPECTR AA-10, Sigma-Aldrich, Germany) was used to determine calcium, magnesium, sodium, potassium, iron, zinc, copper and manganese. Phosphorous content was determined using a Spekol Photometer by measuring the blue colour created by ammonium molybdenate.

RESULTS AND DISCUSSION

The concentration of protein and protein fractions and their changes in Robe and fresh milk from different sites are shown in (Table 1 and 2). The free amino acids of fresh milk and Robe samples expressed as mg/100 gram sample. Ash content and concentrations of macro- and microelements are shown in (Table 3). The source of fresh milk was found to have no influence in the content and distribution of protein fractions and the amino acid composition of fresh milk and Robe samples.

The total protein content of fresh milk ranged from 3.4% and 3.8% and averaged $3.5 \pm 0.14\%$. This value increased to $4.04 \pm 0.2\%$ average total protein content of Robe. Owing to the fact that the true protein content was calculated by subtraction of non-protein nitrogen (NPN) from total protein, the changes in true protein coincides with those of total protein. There was a similar large change in whey protein contents in fresh milk compared to Robe. The whey protein increased from $0.6 \pm 0.1\%$ in milk to $1.86 \pm 0.1\%$ in Robe, respectively. The changes in casein content are also relatively large, but changes in NPN content were much smaller. The casein content of milk was $2.86 \pm 0.01\%$

decreased in Robe to 2.18±0.06%. The NPN content of Robe was greater than that of fresh milk.

Very few data have been previously published on the chemical composition of Robe. The value of 3.3% protein content of Robe samples reported by (Dirar, 1993) and the value 3.6% reported by (El-Mardi, 1988) indicates small differences from that of the current work. There are no previous reports concerning the protein fractions and amino acid composition of Robe, therefore it is difficult to compare. In all Robe samples there was a relative increase in whey protein (1.86±0.12%) when compared to that of fresh milk (0.6 ±0.1%). This could be attributed to the fact that *Robe* after few hours of its processing is separated to whey (safwa) and curd (Abdel-Gadir *et al*.1996). In contrast, the casein contents of Robe samples decreased when compared to that of fresh milk because of precipitation of part of casein as a result of lactic acid bacterial action resulting in formation of curd. The NPN content was slightly increased from (0.22±0.02%) in fresh milk to 0.25±0.01% in Robe.

Table (1): Protein content and protein fractions of fresh milk (FM) and Robe samples

Protein Freactions	**Barakat**		**Darweesh**		**El-Mekki**	
	FM	Robe	FM	Robe	FM	Robe
Total Protein	3.4	4.08	3.33	4.18	3.7	4.0
True Protein	3.19	3.85	3.14	3.9	3.5	3.75
Whey Protein	0.60	1.79	0.50	1.90	0.70	1.83
Casein	2.81	2.29	2.83	2.28	3.00	2.17
NPN	0.21	0.23	0.19	0.28	0.20	0.25
Protein Fractions	**Atra**			**Neshishiba**		**Mean + Sd**
	FM	Robe	FM	Robe	FM	Robe
Total Protein	3.8	3.9	3.6	4.1	3.5±0.14	4.04±0.2
True Protein	3.58	3.66	3.35	3.83	3.35±0.14	3.79±0.2
Whey Protein	0.60	1.07	0.60	1.85	0.60±0.1	1.86±0.12
Casein	3.20	1.93	3.00	2.25	2.86±0.01	2.18±0.06
NPN	0.22	0.24	0.25	0.27	0.21±0.02	0.25±0.1

The free amino acids expressed as mg amino acid/100gram sample is shown in (Table 2). Comparison of the free amino acids profiles of fresh milk and Robe samples indicated that fermentation resulted in an increased accumulation of free amino acids; however, the level of increase was different depending on the amino acid. In addition, fermentation increased the availability of certain amino acids particularly histidine, isoleucine and methionine. The free amino acid contents of Robe, with exception to certain amino acids, were about twice as high as those of fresh milk. The basic amino acids (lysine, histidine and arginine), the sulphur-containing amino acids (methionine and cystine) and isoleucine represented much lower proportions of the free amino acids. The amino acid profile of fresh milk was in close agreement to the values of literature. The data in (Table 5) indicated that Robe product is rich in most of the amino acids. These amino acids may have a role in the development of Robe flavour.

The ash content of fresh milk (Table 3) ranged from 0.77-0.85% with an average of 0.81±0.02%. This range is in close agreement to that of Robe samples, which had a range of 0.68-0.8% with an average 0.76±0.05% ash. The latter value is comparable to the literature values.

Table (2): Amino acid composition of fresh milk (FM) and Robe samples

Amino acid	**Barakat**		***Darweesh***		**El-Mekki**		**Mean ± Sd**	
	FM	**Robe**	FM	Robe	FM	Robe	FM	Robe
Alanine	Bql	Bql	Bql	Bql	Bql	Bql	Bql	Bql
Arginine	o.18	0.24	0.16	0.26	0.21	0.26	0.18±0.02	0.2±0.01
Asp acid	0.22	0.5	0.18	0.5	0.17	0.7	0.19±0.02	0.56±0.1
Cysteine	0.08	0.1	0.1	0.14	0.13	0.24	0.1±0.02	0.16±0.05
Glutamic acid	1.4	3.4	1.2	2.8	1.6	2.8	1.4±0.16	3.0±0.2
Glycine	0.05	0.3	0.06	0.3	0.08	0.36	0.06±0.01	0.32±0.02
Histidine	Bql	Bql	Bql	Bql	Bql	Bql	Bql	Bql
Isoleucine	Bql	0.07	Bql	0.09	Bql	0.12	Bql	0.1±0.03
Leucine	1.5	3.1	1.3	1.8	1.5	2.1	1.4±0.09	2.3±0.5
Lysine	0.4	0.5	0.4	0.6	0.3	0.7	0.36±0.04	0.6±0.08
Methionine	Bql	0.12	Bql	0.13	Bql	0.12	Bql	0.12±0.004
Phenylalanine	0.3	0.5	0.4	0.8	0.4	0.9	0.36±0.1	0.73±0.17
Proline	0.5	0.9	0.3	0.8	0.3	0.7	0.36±0.1	0.8±0.08
Serine	1.1	4.0	0.8	3.	0.9	4.1	0.9±0.12	2.3±1.4
Threonine	0.14	0.17	0.15	0.18	0.16	0.2	1.5±0.008	1.8±0.01
Treptophan	ND	ND	ND	ND	ND	ND	ND	ND
Tyrosine	0.4	0.6	0.2	0.3	0.3	0.5	0.3±0.1	4.6±0.1
Valine	0.33	1.1	0.16	0.93	0.42	1.2	0.3±0.1	1.07±0.1

Bql: Below quantifiable limit of 0.11 m mole amino acid/100g, ND: Not detected with the procedure utilized

Data in (Table 3) shows also the macro-and microelements of fresh milk and Robe samples. It is clearly seen that the fermentation of fresh milk into Robe resulted in increasing most of the macro-and micro-elements expressed as mg/100gram/However, the contents of sodium and manganese were not increased with fermentation, in contrast, their contents decreased in most samples. The concentration of macro-elements calcium, magnesium and phosphorous of fresh milk averaged 263 $\pm$ 6.2mg/100g, 28 $\pm$ 2.1mg/100g and 166.8 $\pm$ 3.3mg/100g, respectively. Macro-elements contribute to the buffering capacity of milk, the maintenance of milk pH, the ionic strength of milk, and milk's osmotic pressure On the other hand, the concentration of microelements Fe, Cu, Mn and Zn of fresh milk investigated in this study were generally low. Generally, micro-elements may enter the milk during milk synthesis or by contamination after the milk is removed from the cow; for example from metal containers.

The analysis of Robe samples for minerals revealed that the concentration of most macro- and microelements increased as a result of fermentation; in addition, most of the results were comparable to those of yoghurt as determined by (McDonogh *et al*., 1983). The calcium concentration varied between 260-297mg/100g with an average of 279 $\pm$ 0.15mg/100g. The concentration of magnesium ranged between 36-39mg/100g with an average of 36.8 $\pm$ 0.2mg/100g. The concentration of sodium and potassium was slightly lower than those of yoghurt. Generally, the concentrations of microelements were very low and hardly detectable in Robe. However, most results were in close agreement to those of yoghurt. From a nutritional point of view, some of microelements have significant importance. For the dairy manufacturer, cupper is of great importance due to its catalytic action on fat autoxidation, natural copper in milk does not promote oxidation.

The variability of most of macro- and micro-elements could be attributed to the fact that concentrations of some of these elements in milk can be increased by an increase in their levels in feeding, also some elements can enter the milk by contamination following milking. The low sodium content of Robe is a particularly desirable attribute for a dietary component for the cardiovascular and hypertension.

Table (3): Ash % and Mineral contents (mg/100g) of fresh milk (FM) and *Robe* samples.

Source	**Barakat**		**Darweesh**		**El-Mekki**	
Parameter	FM	***Robe***	FM	Robe	FM	Rob*e*
Ash %	0.81	0.8	0.83	0.72	0.77	0.68
Ca	263	282	269	293	271	297
Mg	29	36	27	37	28	39
Na	54	50	52	48	60	62
K	174	216	178	212	178	210
P	95	118	90	116	112	121
Mn	.002	.002	.002	.001	003	.004
Fe	0.06	0.14	0.04	0.23	0.09	0.14
Zn	0.83	1.01	0.78	0.96	0.84	0.93
Source	**Atra**		**Neshesh.**		**Mean ± Sd**	
Parameter	FM	Robe	FM	Robe	FM	Robe
Ash %	0.85	0.80	0.81	0.80	0.81±0.2	0.76±0.05
Ca	254	263	258	260	263± 6.2	279±0.5
Mg	26	36	32	36	55.6±3.4	36.8±1.1
Na	56	44	56	59	55.6±3.4	52.6±6.8
K	168	211	160	207	171.6±6.8	211.2±2.9
P	98	111	89	118	96.8±8.2	166.8±3.3
Mn	.002	.002	.003	.002	0.002±.001	0.16±0.03
Fe	0.07	0.12	0.05	0.18	0.006±.001	0.16±0.03
Zn	0.93	0.98	0.82	1.12	0.84±.004	1.0±0.01

Results are reported as means of triplicate determination

Documented literature on the Sudanese traditionally fermented sour milk (Robe) is limited although it is a common food in the rural areas of Middle and Eastern Sudan where it is drunk on its own or consumed with other food products. Robe is a pleasantly sour product with a characteristic buttery flavour. There is only rudimentary information concerning the technology of Robe, both from economic and health aspects. It is concluded in this study that Robe is a nutritious food product contains good amounts of high quality proteins with all essential amino acids although the amounts of some of them were low. It also contains good amounts of macro-and-microelements and other chemical components.

Upgrading traditional Robe production should be geared towards the modification of some unit operations in the manufacturing process with respect to Robe production. These modifications should focus on the shortening of the traditional laborious and time-consuming method. It is highly recommended to apply the industrial processes for Robe manufacturing. This could be accomplished by using of controlled starter cultures developed from lactic acid bacteria and yeasts with known metabolic profiles, aiming primarily at production of Robe of standard uniform quality. In addition, the sanitary processing techniques coupled with pasteurization of fresh milk that will be used for making Robe, are necessary for increasing the scale of production.

Further research on Robe and other Sudanese traditionally fermented dairy products should include; studies of the possible probiotic effects of the fermenting organisms, studies on the mechanisms of toxin degradations and the detection of plasmids by microbes.

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