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Research Article

Comparison of Amino Acids and Fatty Acids Profiles of Egyptian Kishk: Dried Wheat Based Fermented Milk Mixture as Functional Food

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Abstract

Background and Objective: Kishk is Egyptian traditional sun dried fermented wheat milk mixture produced in upper Egypt (Sa'eed) since 3000 year B.C., up till now. It is highly nutritious home made Egyptian ethnic food. The aim of the present study is an attempt to explore the quality and quantity of free amino acids (FAAs) and fatty acids (FFAs) in Sa'eedi kishk (SK). **Materials and Methods:** Fourty SK samples were procured from local markets in 4 provinces located in Sa'eed region (n = 10 per province). The FAAs were detected using the Pico-Tag technique developed commercially by waters associates. The FFAs were pursued by GC/MS system. **Results:** Overall results revealed that 17 FAAs were found in SK which was grouped as indispensable, dispensable and conditionally indispensable FAAs. Also, SK samples contain different quantities of saturated, monounsaturated and polyunsaturated FFAs from C:10 to C:22 from the samples of the different provinces. **Conclusion:** There are significant variations between the SK samples from the 4 provinces in relation to the quality and quantity of FAAs and FFAs, respectively. The SK samples from one province contain the unique Conjugated Linoleic Acid (CLA) t_{10}, c_{12} C:18 CLA. This is the first report that documented presence of CLA in SK.

Key words: Sa'eedi kishk, wheat dairy fermented food, amino acids, fatty acids

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cereals and cereal-based products are important human food resources and livestock feeds worldwide. The major cereal crops produced worldwide are wheat (*Triticum* spp.), rice (*Oryza* spp.), maize/corn (*Zea mays* L.) and barley (*Hordeum vulgare* L.)¹. Cereals are cheap source of energy and supply a high percentage of calorie and protein to a large segment of the human population, particularly in the developing countries. The nutritional quality of cereals and the sensorial properties of their products are sometimes inferior to or poor in comparison with milk and milk products². The reasons behind this are: (1) Lower protein content, (2) Deficiency of certain essential amino acids (lysine), (3) Low starch availability, (4) Presence of several ant-nutrients (phytic acid, tannins and polyphenols) and (5) Coarse nature of the grains. Therefore, the unique combination of cereals and milk will have a synergistic effect to provide better nutrition, ultimately resulting in value added functional products such as kishk. Milk, though a good source of nutrients, lacks in fiber, whereas cereals are rich in this component. Fiber increases the intestinal transit time of bowels, binds carcinogens and can provide several health beneficial effects. The proteins present in milk have high biological value and good amino acid profiles whereas cereal proteins have low digestibility and are deficient in certain amino acids³. Milk fermentation is one of the oldest most economical method adopted in production and preservation of traditional dairy products wheat based foods worldwide⁴. Fermented milk cereal based dairy products are major dietary constituents in numerous developing countries because of their keeping quality under ambient condition⁵. They are contributing to food security, enhance digestibility and soluble fractions, improving food safety and quality of the proteins (increase in bioavailability of lysine), increase water soluble vitamins and decline ant-nutritional factors.

Kishk is a traditional Egyptian ethnic fermented food with high nutritional quality and long shelf-life at ambient temperature⁶. This product is basically made from a combination of bar boiled wheat with natural local sour milk such as laban khad, laban zeer or laban rayeb⁷. Kishk is rich in nutritive constituents and is a possible source of many vitamins and growth factors associated with the microbial fermentation processes⁸. Kishk has considerable dietary potential as a source of fiber, amino acids, minerals and selenium contents, but is deficient in certain vitamins⁹.

Considering the nutritional value of Sa'eedi kishk, there is lack of information about its amino acids and fatty acids profiles which are important as bioactive

compounds. Therefore, the objectives of this study were to uncover the amino acid and fatty acid profiles of Egyptian Sa'eedi kishk samples collected from four provinces located in Sa'eed region in Upper Egypt. The current study also aimed to compare variations in the amino acids and fatty acids contents between provinces in such ethnic food. This will be useful in future studies to produce novel functional kishk with improved nutritive quality beyond the classic aim of nutrition.

MATERIALS AND METHODS

Kishk sample collection: Forty samples of kishk (~500 g each) were procured from different local markets in four provinces located in Upper Egypt. Those were Assiut (A), Beni-Swif (B), Fayoum (F) and Giza (G). Kishk samples collected from province A were characterized by its round shape (Fig. 1). However, samples obtained from provinces (B, F and G) were of irregular shape as appeared in Fig. 2.

Amino acids concentrations and profile (AAP): The concentrations of amino acids in 40 kishk samples collected from 4 provinces were determined using the Pico-Tag method that was developed commercially by Waters Associates (USA) as described earlier¹⁰. Briefly, 5 g from each sample was dissolved in 5% 5-sulfosalicylic acid dihydrate solution and make up to 25 mL. The sample was filtrated with 0.45 µm membrane filter. Thirty microliters of the filtered sample in 6×50 mm tube was placed into drying vessel and dry in Waters Pico-Tag Workstation in 10-15 min at <50 mL. Aliquot (30 µL) of redry solution (200 µL methanol, 200 µL 0.2 N sodium acetate and 100 µL triethylamine) and dry the



Fig. 1: Kishk round shape⁷



Fig. 2: Kishk irregular shape⁷

sample again in the Workstation Aliquot (30 μ L) freshly prepared of the derivatization reagent (350 μ L methanol). About 50 μ L HPLC grade water, 50 μ L triethylamine and 50 μ L phenylisothiocyanate, PITC to the tube contents and allow to react for 20 min and dry in the Workstation for 15 min. About 30 μ L HPLC grade methanol was added and redry. Add 100 μ L of sample diluent to the tube, vortex and transfer to injection vials. The standard amino acids of solution is treated the same as the sample.

The apparatus used was supplied by Waters, Cooperation, USA with 484 UV/VIS detector and Millenium Software Program as well as Pico-Tag column for amino acids. The analysis was carried out using a gradient of Pico-Tag solvent A and B at 40°C and flow rate 1 mL min⁻¹. Detection of the separated Pico-Tag amino acid at 254 nm wave length. Before injecting of the sample, the illustrated was calibrated by two injections of the standards.

Fatty acids concentrations and profile (FACP): Fatty acids of kishk samples were determined according to Christie¹¹. Firstly, the FFAs in kishk lipid were converted into FAME. Subsequent, samples were injected in GC/MS system (A 6890 Agilent technologies, Santa Clara, CA, USA). The GC system equipped with FID, HP percentage phenyl methyl silicate capillary column (30×0.32 mm, i.d and 0.25 μ m film thickness. Helium was used as a carrier gas at a flow rate of 1.5 min⁻¹. The oven temperature was held at 70°C for 2 min, then increased to 230°C at 8°C min⁻¹ and maintained at this temperature for 20 min. Injector and detector temperatures were 250 and 280°C, respectively. The FAME were identified by comparing the retention times with those of a standard FAME mixture

(Sigma Aldrich, purity <99.0% by GC) using probability merge search software and the National Institute of Standards and Technology MS spectra search program.

RESULTS AND DISCUSSION

Amino acid concentrations and profile (AACP): The AACP of S'aedi kishk samples from four provinces A, B, F and G located in Upper Egypt (S'aed region) are shown in Table 1. Variations within the different amino acids and their concentrations in the 4 provinces are clearly observed from the obtained data. Briefly, in provinces A, B and G, 8 essential amino acids (EAAs) and one conditionally essential amino acid (CEAAs) in kishk samples could be observed. They are His, Ile, Leu, Lys, Met, Phe, Thr, Val and Try as EAAs and CEAAs, respectively. However, in province F only four EAAs and only one CEAAs were found, definitely, His, Met, Thr and Val and Tyr, respectively (Table 1). Noticeably, tryptophan, an important EAA was not detected in kishk samples from all provinces studied. Tryptophan and sulfur containing amino acids are low in kishk, which is not actually a good source for such amino acids¹². This discrepancy was attributed to the loss or decomposition of tryptophan during processing of kishk either during prolonged fermentation of butter milk or subsequently during fermentation of bar boiled wheat fermented milk mixture and sun-drying¹². This explanation could be quite applicable in case of our findings. The Methionine (Met) and cysteine (Cys) may be regarded as principal sulfur containing amino acids because both are members of the canonical twenty amino acids included into proteins¹³. Presence of Met in kishk samples from all provinces under study was confirmed from the results in Table 1. Methionine is one of the most hydrophobic, non polar amino acid and almost is found on the interior of proteins. On the other side, cysteine (Cys) is considered also to be non polar and hydrophobic but does ionize to yield the important product glutathione¹⁴. Methionine and Cys being sulfur containing amino acids plays a crucial role in human nutrition. Each of these acids contributes to the cellular pool of organic sulfur and sulfur homeostasis¹⁴. Cysteine may be converted to such important products as glutathione. Additionally, thiol imbalance in humans has been associated with multiple dysfunctions such as vascular disease, Al zheimer's, HIV and cancer¹⁴. In this study, a question might arise is Cys or thiol derivatives in kishk can restore thiol balance in humans? This question seems very important and only future study *in vitro* and *in vivo* may declare this assumption. Therefore, interested scientists are invited to investigate this point.

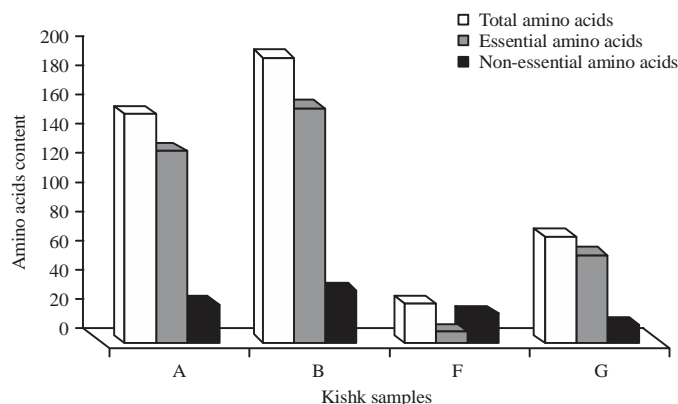


Fig. 3: Concentrations of TAAs, EAAs and NEAAs in kishk samples, A: Assiut, B: Beni-Swif, F: Fayoum, G: Giza provinces

Table 1: Amino acids concentrations and profile of Sa'eedi kishk (mg/100 g sample)

Amino acids (mg/100 g sample)	Sa'eedi kishk samples*			
	A	B	F	G
Indispensable (Essential) amino acids				
Histidine (His)	0.46	0.20	0.31	0.58
Isoleucine (Ile)	15.96	39.37	nd	7.22
Leucine (Leu)	9.03	9.16	nd	6.46
Lysine (Lys)	40.61	29.64	nd	25.61
Methionine (Meth)	11.08	11.44	2.26	2.65
Phenylalanine (Phe)	13.15	26.12	nd	6.94
Threonine (Thr)	2.75	8.85	0.21	0.18
Valine (Val)	21.34	17.96	2.90	7.79
Dispensable (Non essential) amino acids				
Alanine (Ala)	1.21	1.25	0.20	0.43
Aspartic acid (Asp)	0.23	0.34	0.35	0.41
Glutamic acid (Glu)	0.18	0.36	0.78	0.94
Serine (Ser)	2.86	3.06	0.23	0.41
Conditionally indispensable				
Arginine (Arg)	6.69	8.24	12.78	2.64
Cysteine (Cys)	nd	nd	nd	nd
Glycine (Gly)	0.15	0.23	0.62	2.44
Proline (Pro)	14.15	21.10	4.63	4.75
Tyrosine (Tyr)	16.75	17.26	0.92	2.93
Total amino acids	156.60	194.58	26.19	72.38
Essential amino acids	131.13	160.00	6.60	60.36
Non essential amino acids	25.47	34.58	19.59	12.02
Aromatic amino acids	30.36	43.58	1.23	10.45
Sulphur amino acids	11.08	11.44	2.26	2.65

*A: Assiut, B: Beni-Swif, F: Fayoum, G: Giza provinces, nd: Not detected

Both wheat and fermented milk used in kishk preparation are responsible for the quality and quantity of protein and amino acids in such ethnic food. For wheat it was reported that potash fertilizer is crucial to improve its protein quality through increasing the contents of important essential amino acids mainly Lys and Thr. Potash and nitrogen in combination are indispensable for wheat crop to improve both its quality and quantity¹⁵. Therefore, the efforts should be done by the local Egyptian agriculture department in Fayoum province to

urge farmers toward improve their wheat production. Buffaloes prevail in rural villages in Egypt; therefore buffalo's milk (BM) is used to prepare fermented milk traditionally named as laban zeer that subsequently is used to prepare kishk. The quality and quantity of amino acids in BM are affected by the breed of buffaloes¹⁶⁻¹⁸. Therefore, variations in amino acid composition that is observed between kishk from different provinces could be attributed to such factors collectively, quality of wheat and BM respectively (Table 1, Fig. 3). An important conclusion could be stated in this regard that although protein content in Kishk samples from F province is comparatively higher than that of B and G provinces however, protein quality of kishk from province F is apparently of inferior quality. The protein concentrations were reported to be 18.84, 11.60, 11.87 and 11.43% in kishk samples from A, B, F and G provinces, respectively as it is reported in previous study⁷.

Amino acids (AAs) were traditionally classified as nutritionally essential or nonessential for animals and humans based on nitrogen balance or growth. Figure 3 shows the total amino acids (TAAs), essential amino acids (EAAs) and non essential amino acids (NEAAs) concentrations in kishk samples taken from four provinces referred to as A, B, G and F stand for Assiut, Beni-Swif, Fayoum and Giza, respectively. A key element of this classification is that all non-essential amino acids (NEAA) were assumed to be synthesized adequately in the body as substrates to meet the needs for protein synthesis¹⁹. Essential amino acids are crucial for healthy metabolism to maintain healthy life. Even though, estimating the daily requirement for the essential amino acids is difficult, however, recommended daily amounts were set for essential amino acids needed for adult humans²⁰. The requirement for lysine has received most attention given its nutritional importance as the likely limiting amino acid in cereals,

especially wheat. The consultation's estimate of the requirement for lysine amounting ($30 \text{ mg kg}^{-1} \text{ day}^{-1}$) leucine is the most abundant amino acid in tissue and food proteins but specific demands for non-protein functions have not been identified. The consultation's estimate of the requirement for leucine is 39 and $26 \text{ mg kg}^{-1} \text{ day}^{-1}$ for valine. For isoleucine, $20 \text{ mg kg}^{-1} \text{ day}^{-1}$ is recommended. Best estimate of total aromatic amino acid requirement is set at $25 \text{ mg kg}^{-1} \text{ day}^{-1}$. Of the total sulfur amino acids, methionine and cysteine ($15 \text{ mg kg}^{-1} \text{ day}^{-1}$), the former is nutritionally indispensable while the latter, as a metabolic product of methionine catabolism is dependent on sufficient occurrence of methionine to supply the needs for both amino acids, They are important nutritionally and equally abundant in cereal and animal proteins, the main ingredients in kishk.

The nine indispensable amino acids showed in Table 1 are those that have carbon skeletons that cannot be synthesized to meet body needs from simpler molecules in animals and therefore must be provided in the diet. The definition of dispensable amino acids has become blurred as more information on the intermediary metabolism and nutritional characteristics of these compounds has accumulated. Dispensable amino acids were divided into two classes as dispensable amino acids and conditionally indispensable²¹. Dispensable amino acids can be synthesized in the body from either other amino acids or other complex nitrogenous metabolites, while conditionally indispensable are synthesized from other amino acids or their synthesis is limited under special pathological physiology conditions²¹.

Amino acid contents of Egyptian Sa'eedi kishk was earlier reported in the literature^{12,22,23}. However, there is a need to revisit the subject as many factors have been emerged during the past two decades. More sophisticated analytical methods and instruments had emerged along with variation in environmental conditions that may affect the amino acid composition of milk and wheat, the two principal ingredients in kishk. This had necessitated the need to repay attention to update the situation of amino acids in such ethnic food. Worthy mention, the concentrations of amino acids in Lebanese kishk were uncovered by Tamime *et al.*⁹. However, even the Egyptian and Lebanese kishk share the same name and they differ in many aspects. Buffalo's milk is the main ingredients involved in Egyptian kishk while goat or cow milk is used to prepare the Lebanese one hence it is time loss to compare the amino acids in such products because the composition of buffalo milk (BM) differ from cow milk (CM). BM has higher fat, protein, milk sugar, ash and Ca than those in CM²⁴. Buffaloes are most important sources of milk in many parts worldwide¹⁶ and similarly in Egypt.

Fatty acids concentrations and profile (FACP): In the present study, variations in FACP of SK samples from 4 provinces A, B, F and G are observed as appeared in Table 2. With regard to SFAs, there is five, namely, capric, lauric, myristic, palmitic and stearic that were detected in SK samples from province A. However, only three SFAs, myristic, palmitic and stearic were detected in SK samples from the remaining B, F and G provinces respectively. Palmitic acid prevails in SK samples of the B, F and G provinces with appreciable higher concentrations than the situation with A province. Contrary, stearic acid was higher in A province than the other three provinces, respectively. These results showed that SFAs with skeleton less than 10 C atoms were not detected in all SK samples studied. Also, fatty acids of C10 and 12 were absent in SK from B, F and G provinces but were present in the case of A province. Another observation that FA with C14, 16 and 18 are detected in SK from all provinces. On the other side only four MUSFAs in SK from A province are palmitoleic, elaidic, oleic and vaccenic. Comparatively, five MUSFAs strictly, myristoleic, palmitoleic, oleic, vaccenic and erucic were found in SK of B province. In F province 4 MUSFAs myristoleic, oleic, vaccenic and erucic are prominent. The remaining G province samples proved to contain only three MUSFAs namely, palmitoleic, oleic and vaccenic. Oleic acid prevails in all SK samples from all provinces (Table 2). With regard to PUSFAs which include, among others, C18 diene and triene they were detected in SK samples examined with variations. Clear from Table 2, that linoleic acid LA (diene) prevail in all SK samples from all provinces and in appreciable

Table 2: Fatty acid percentage contents of kishk samples from four provinces
Sa'eedi kishk samples*

Fatty acids contents	A	B	F	G
C10:0 capric acid	0.33	ND	ND	ND
C12:0 lauric acid	0.71	ND	ND	ND
C14:0 myristic acid	4.67	1.22	0.92	0.75
C16:0 palmitic acid	1.85	15.96	15.65	16.77
C18:0 stearic acid	6.45	2.75	0.99	1.37
c 9 C14:1 myristoleic acid	ND	0.29	0.22	ND
c 9 C16:1 palmitoleic acid	0.64	0.11	ND	0.29
c 9 C18:1 oleic acid	20.47	21.47	19.35	19.64
t 9 C8:1 elaidic acid	0.29	ND	ND	ND
c 11 C18:1 vaccenic acid	1.68	0.63	0.28	0.59
c 13 C22:1 erucic acid	ND	0.26	0.26	ND
c 9 c 12 C18:2 linoleic acid	9.78	14.92	23.93	22.86
t 10 c 12 C18:2 CLA	0.26	ND	ND	ND
c 6 c 9 c 12 C18:3 γ -linolenic acid	0.96	0.76	0.77	0.96
c 9 c 12 c 15 C18:3 α -linolenic acid	0.55	0.74	1.35	1.57
Σ SFAs ^a	14.01	19.93	17.47	18.89
Σ TUSFAs ^b	34.63	39.18	46.16	45.91
Σ MUSFAs ^c	23.08	22.76	20.11	20.52
Σ PUSFAs ^d	11.55	16.42	26.05	25.39

*A: Assiut, B: Beni-Swif, F: Fayoum, G: Giza provinces, ND: Not detected, a: Saturated fatty acids, b: Total unsaturated fatty acids, c: Monounsaturated fatty acids, d: Polyunsaturated fatty acids

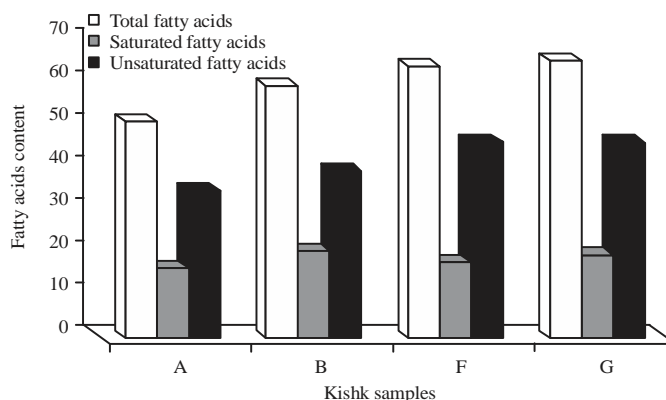


Fig. 4: Concentrations of TFAs, SFAs and USFAs in kishk samples, A: Assiut, B: Beni-Swif, F: Fayoum, G: Giza provinces

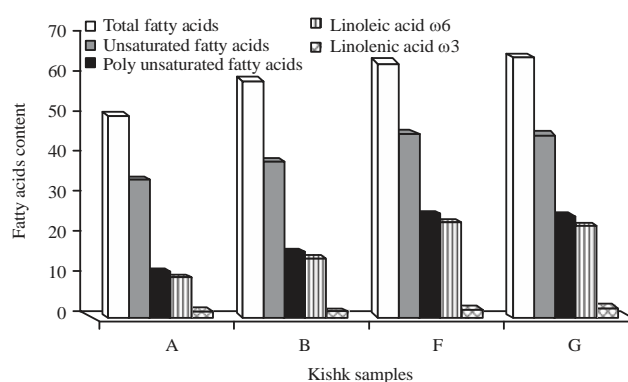


Fig. 5: Ratio of LA and LnA concentrations between TFAs, USFAs and PUFAs in kishk, A: Assiut, B: Beni-Swif, F: Fayoum, G: Giza provinces

concentration compared to CLA isomer. Notably, we detect the occurrence of the unique CLA t_{10}, c_{12} C18:2 isomer in SK from province A. This is the first report in the available literature announced such scientific achievement both nationally and internationally. Two other triene fatty acids namely γ and α LnA were found in SK samples from all provinces with varied concentrations. Figure 4 summarized the concentrations of FAs, SFAs and USFAs in SK samples and Fig. 5 reported the ratio of LA and LnA FAs in SK samples. We found for our best knowledge no references that studied FAs profile of Sa'eedi Kishk. Therefore, this study may consider as the first approach in this trend of research. The variations within the FAsP of SK between provinces may be attributed to more than one factor such as the variation of FAs composition of BF due to diet, species and breeds^{16,18,25}. Variations in method of SK processing prevail in the four provinces such as laban zeer time of fermentation, presence of different non starter lactic acid bacteria (NSTLAB) as natural microbial flora and subsequent SK short time fermentation the final step in processing may contribute and explain such variations. Few

study on the FAsP of kishk-like product are available in the literature. Therefore, data on commercial Lebanese product and Greek trahanas^{26,27} have been used for comparative purposes in this study. The SFAs (% w/w) from C4:0 up to C20:0, MUSFAs C14:1, C16:1 and C18:1 and PUFAs C18:2 and C18:3 were determined in Lebanese commercial kishk (LCK)²⁶. In another study, the mean and the main classes of fatty acids of LCK were 70.07, 23.25 and 7.15% for SFAs, MUSFAs and PUSFAs, respectively⁹. In comparison, we found that the mean values of SFAs, MUSFAs and PUSFAs in Sa'eedi kishk were 17.85, 21.62 and 19.85% w/w, respectively. According to our result the concentrations of SFAs in LCK is higher than those of Sa'eedi kishk contrary to that for PUSFAs. Recently, Georgala *et al.*²⁷ examined forty trahanas samples for FAs composition. They found significant differences in the amount of SFAs compared to USFAs being much higher for the former than for the latter respectively. The results reported by the mentioned researchers take the same trend that SFA% are more higher than that for USFAs contrary to the current results in this study. This may be attributed to more than a reason such as source of milk, method of processing and other

environmental conditions. Worthy mention that SFAs and USFAs contents of buffaloes milk fat (BMF) in Egypt were 71.7 and 28.3%, respectively²⁵. However, in SK the contents of USFAs are higher than that of SFAs as shown in Table 2. This may be attributed to the biochemical reactions during prolonged time of milk fermentation (more than 6 months) to produce laban zeer, traditional sour milk, used to manufacture of SK. Linoleic Acid (LA) and conjugated linoleic acid isomers (CLA) were documented by others to be traced to BMF^{28,29}. So, it is expectedly, to find such bioactive compounds in SK processed using BM. Farmers in Egypt used to feed their buffaloes on pasture grazing which result in buffalo mammary gland to contain high amount of Δ^9 desaturase enzyme activity which can explain the presence of CLA isomers in secreted milk^{24,30}. The CLA is reported to have health benefits including reduction of body fat, trans-10, cis-12 causes reduction of adipogenesis and defective Brown Adipose Tissue (BAT) thermogenesis³¹. Another group of researchers³² have been documented the effect of t_{10} , c_{12} CLA isomer on obesity management and stated that such isomer decreases adiponection assembly by PPAR γ -dependent and PPAR γ -independent mechanisms.

Polyunsaturated fatty acids (PUFA) are bioactive compounds include α -linolenic C18:3 (n-3) and α -linoleic C18:2 (n-6). These essential fatty acids which are not synthesized by the human body should be obtained through the diet. These essential fatty acids are related to the prevention or reduction of incidence of cardiovascular disease and cancer. Because of that, it has been recommended to have a balance between n-3 and n-6 fatty acids in the diet³³.

CONCLUSION

Kishk, the fermented milk wheat mixture is very popular homemade dairy food in Upper Egypt (Sa'eed region) hence it took its name SK. The product is very nutritive with fat, protein, bioactive peptides, free amino and fatty acids, vitamins and minerals. Moreover, SK has an excellent keeping quality under ambient hot temperature that prevails in Sa'eed most of the year. The unique idea behind this marvelous miracle product is that it gather together two in one product (sour milk and wheat), the available ingredients produced by farmers in Upper Egypt. This had achieved since 3000 year BC. It is observed that this art is suspected to extinction during the few coming decades unless sincere efforts have to be paid by scientists to avoid this situation. First of all the method of manufacture should be improved and developed applying modern technologies. Second, there is an urgent need to design a specific lactic starter culture combined with selected suitable yeast species to be applied in fermentation needed in

processing of SK. Thirdly; the method of sun drying should be substituted with a more sophisticated drying method but economical. Lastly, application of extruder technology should be applied to shape SK that will add improved features to the product. We claim with confidence that the subject merit advanced sincere scientific approach. If this is to be done, it will with certainty benefit the dairy food industry for the benefit of school students and other people's anywhere.

SIGNIFICANT STATEMENTS

The obtained findings will help development novel improved SK production with functional health beneficial effects upon modern consumers that are increasingly concerned with their health. Modern technological processes such as extrusion could be applied on an industrial scale to introduce on the market new SK either as soup or Egyptian ethnic meals.

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