MICROBIOLOGICAL AND BIOCHEMICAL ASPECTS OF KISHK FERMENTATION

2- EGYPTIAN SEIDI KISHK

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ABSTRACT:

Functional dairy foods have undergone a major leap in recent years, so emerging evidence on the protective role ascribed to fermented dairy products and specifically the traditional original products as kishk which is a typical popular native food in Egypt and Middle East. It is made from laban zeer and burghul depending on random or spontaneous lactic acid bacteria in its fermentation. It is characterized by richness in nutritive constituents, healthy and has high biological value.

In this research, focus was concentrated on biochemical and microbiological examination on kishk, study was also extended to its hygienic quality through the determination of D (-) and L (+) lactic acid isomers to stand up on the D (-) content which led to disturbance in mammals. Comparison was made between Egyptian Seidi (ES) kishk and Iranian (IR) kishk which was considered as concentrated rayeb milk saturated with salt and formed into marbles then sun dried. Also, the study included the identification of organic acids content in ES-kishk using gas chromatography.

However, results revealed that titratable acidity was 3.03 and 5.45% and the D (-) lactic acid isomer was 5169.71 and 13003.06 mg/kg in ES and IR kishk, respectively. Furthermore, the demonstrated organic acids in ES kishk were lactic, propionic, butyric, succinic and acetic acids. With regards to microbiological examination revealed in 7.37, 7.26 log cfu/gm of *lactobacilli* and *streptococci* respectively, while no *pediococci* was detected in ES-kishk beside of 7.45 and 5.23 log cfu/gm of total yeast and actedione resistant yeast were presented, respectively. In regards to microbiological analysis of ES kishk after 6 months of storage, it was represented 6.00% bacteria corresponding to 94.00% of the microorganisms were yeast. On the other hand no microorganisms were detected in IR-kishk after 6 months of storage due to the high content of salt which led to physiological dryness in IR-kishk.

Key words: Egyptian Seidi Kishk, Iranian Kishk, D (-) & L (+) lactic acid isomers, Lactic acid bacteria. Organic acids

INTRODUCTION

In developing countries, where dairy industry is not well developed, many dairy fermented products are still produced by spontaneous and uncontrolled fermentation of raw milk sources. However, kishk is a very interesting wheat- milk based product with high nutritive, digest ability and biological value **(Benchat, 1983 & Odunfa, 1985 and El-Gendy, 1983 & 2001).** Kishk is still produced at the farm steads using primitive utensils. Fermentation is carried by indigenous micro flora of fermented milk. So that

it is expected to be rather variable. The wide spread nature of LAB suggests that many sources from various origins should be examined in search of effective strains with specific technological properties in relation to their applications **(Zambou, 2004)**. The nutritional impact of fermented foods on nutritional diseases can be direct or indirect. Food fermentation that raise the protein content or improve the balance of essential amino acids or their availability will have a direct curative effect **(Steinkraus, 2002)**. The objective of this research was to provide precise information on the biochemical and microbiological content of kishk including D (-) & L (+) lactic acid isomers content which has not been properly investigated. D (-) lactic acid can accumulate in the blood of patients suffering from short-bowel syndrome and intestinal failure, leading of a manifestation of D (-) lactic acidosis and encephalopathy. Thus, it was determined that D (-) lactate-producing colonizing intestinal lactobacilli were the main factor in the pathogenesis. Furthermore, new born infants may fail to completely metabolize ingested or by intestinal microorganisms produced D (-) lactate because of liver immaturity. Hence, D (-) lactic is not recommended for infants or young children **(Lapierre, *et al*., 1999)**. Preliminary evaluation on native Egyptian Seidi Kishk and Iranian Kishk including determination of D (-) & L (+) lactic acid was the aim of this research.

MATERIALS AND METHODS

Materials:

1- Egyptian Seidi Kishk:

9 samples was made in farmer's houses in Fayoum, Giza and Kena governorates according to the original local method depending on the natural flora in the places.

2-3 Samples of IR Kishk were obtained from Tahran by personal endeavors.

Methods:

1- Burghul:

It was prepared by boiling one unit of wheat till splitting and spread in sun till dryness then milled simply between two big stones called Rahaya.

2- Laban El Zeer:

4 units of buffalos' milk were collected in zeer (earthenware container) fermented randomly and filtered naturally during 5 days.

3- Burghul and Laban El-Zeer:

Produced previously in step 1 and 2 were mixed and 17, 20 and 3gm of Black cumin, Shammer and Chilli were added as Egyptian spices respectively. Mixture was sun dried for 4 days on palm leaves and kneaded daily to encourage "Condioning". During that, it was formed into nuggets.

Iranian Kishk (IR) was prepared in Iranian houses in Iran as follows: Rayeb milk was collected into clothes sacks to discard the whey. The rest was salted till saturation and shaped as marbles which were sun dried and covered continously with soft salt till dryness.

4- Chemical analysis:

Total solids % (T.S), moisture content % (MC) and acidity were determined according to A.O.A.C. (1995).

pH value: 1gm sample/ 25ml distilled water was left half an hour then measured by (Consort D614). Free amino nitrogen: (a FAN) was determined by means of the Ninhydrin method **(**E-B-C analytical, 1975**).**

Total carbohydrate: was determined by using the anthrone reagent method according to **Herbert *et al.*, (1971).** Fiber content: was determined according to **Van soest, (1967)** by using neutral detergent solution (NDS) and Tecalor as fibertec system M1020 hot extractor. D (-) and L (+) lactic acid was determined by enzymatic method according to **Drawert and Hagen, (1970) and** modified by **Mc Murrough and Palmer, (1979).**

5- Microbiological analysis:

Nutrient agar dehydrated (Difco) was used for total count under aerobic condition at 37ºC/48hrs. De Man Rogosa and Sharpe (Oxoid) (MRS) under anaerobic condition. MRS tomato juice, MRS supplemented with 10% v/v tomato juice for *Streptococci, Lactobacilli, pediococci and Leuconostoc* **(Dellaglio and Torriani, 1986)**. MRS PM (MRS+0.5% maltose + 100 ppm pimaricin to prevent yeast growth) according to **Lawerence and Leedham, (1979) & ICMSF, (1996).** Universal beer agar (UBA) (Delft Holland). UBA an P (for Lactobacilli) the UBA+ 100ppm pimaricin (Delft Holland) with pH 4.5 under an aerobic condition. UBAC (for total yeast) UBA+ 25 ppm gentamycin+50 ppm oxytetracyclin+0.5% $CaCo_3$ (under aerobic condition). UBAAC (for actedione resistant yeast). UBA + 15ppm gentamycin + 50ppmoxytetracyclin + 0.5%$CaCo_3$+10ppm actedione (cycloheximide). UBAP for acetic acid bacteria the UBA+100 ppm pimaricin, pH 4.5, aerobic condition at 25 C for 3-5 days. L-S differential media (oxoid) to differentiate between *Lactobacilli* and *Streptococci*.

RESULTS AND DISCUSSIONS

Chemical composition of ES kishk:

Chemical composition of kishk had widly range depending on the cereal and the fermented milk used . However, the mean of the chemical composition in E.S kishk under taken as illustrated in Table (1) were 9.25, 18.10, 6.9, 4.3, 57.84 and 3.03% for MC, TN, fat, ash, total carbohydrate and titratable acidity. The mean of these parameters were partly in agreement with **Morcos, *et al.*, (1973)**, **Shaker, (1979) and Attia& Khattab (1985)** except acidity which looks more less higher than that obtained by the previous authers inspite of its agreement with **Demerdash, (1960) and Shaker, (1979).** With regards to IR kishk, which registered higher values in the most tested chemical components than ES kishk due to the concentrated milk which formed the mainly raw matter in it. However it could be say that I.R kishk as dried milk kept into marbles, saturated with salt as natural preservative material caused physiological dryness in addition to sun drying.

Table (1): some chemical composition of kishk

Parameters%	ES kishk	IR kishk
Moisture content	9.25	3.50
Total nitrogen	23.10	32.4
Fat	6.90	11.90
Ash	4.70	5.50
Total carbohydrate	57.84	42.20
Acidity	3.03	5.45

pH values:

pH values registered 4.71, 4.01 in ES and IR Kishk as detected in Table(2). However the depression of pH was indicated in IR Kishk as detected by titratable acidity. Also reduction of pH was recorded 4.56 after 6 months of storage in ES Kishk.

Titratable acidity% (TA):

Data shown in Table (2) indicated that the titratable acidity in ES was 3.03% (calculated as lactic acid). However, lactic acid as determined enzymatic ally formed 0.96%. On the other side, Gas liquid chromatographic analysis appeared 5 organic acids (acetic, butyric, formic, lactic, propionic, lactic and succinic acids) may be that due to the random fermentation which allowed to different kinds of LAB as hetero fermentative lactic acid bacteria and yeast. In other direction, IR contained 5.4% Titratable acidity(calculated as lactic acid). However, determination of lactic acid enzymatically confirmed that lactic acid content in IR was 2%, this means that 3.4% of acid content in IR was organic acids other than lactic acid.

D (-) and L(+) lactic acid isomers:

D (-) and L (+) lactic acid were determined enzymatic ally in ES and IR Kishk as shown in Table (2). Freshly ES recorded 4431.18 and 5169.7mg/kg of L (+) and D (-) lactic acid, respectively. Whereas were almost as the random LAB produce both isomers with little more of D (-). Furthermore, after 6 months of ES storage little increase in both isomers were detected whereas were 4822.92 and 5223.43 mg/kg in L (+) and D(-) isomers, respectively. This might be due to the partly activities of the LAB viable cells in refrigerator which resulted in over acidification in ES Kishk. Beside that the less more increase of D (-) than L (+) still continued during storage period. Despite of this it could be say that D (-) and L (+) were almost as the same. With regards to IR Kishk D (-) represented the double quantity of L (-) whereas were 13003 and 6714 mg/kg. respectively. This may be attributed to the kind of LAB demonstrated during fermentation. It could be concluded that IR Kishk was less healthy than ES Kishk. However, in human nutrition the two lactic acid isomers are physiologically different in so far as D (-) is less activity metabolized. In the case of an extremely unbalanced diet, this isomer may be increased so that it as physiologically active organic acid may lead to disturbances, it is claimed to cause acidosis, reduces cell metabolism and enhance Ca secretion in the urine **(Rasic and Kurman 1978)** while L (+) isomer is reported to be essential in energy metabolism and in redox reactions in the mammals **(Krusch, 1978)**. Furthermore, strains of thermophilic lactobacilli producing only D (-) lactic acid were in capable of fermenting galactose, while those strains propducing both D (-) and L (+) lactic acid did not ferment galactose. S. thermophilus metabolized the lactose with production of galactose and L (-) lactic acid in the early stage of manufacture. Galactose-positive lactobacilli developed in the latter stages to D (-) and L (+) lactic acid **(Martley and Turner 1983)**. However, in fermented milk *S. thermophilus* produced L (+) and *L. delbrueckii subsp. bulgaricus* produced D (-) isomer **(Elewa, 1992).**

Table (2): Acidity, pH value and lactic acid isomers in ES and IR Kishk

	Acidity %	pH	L (-) lactate mg/kg	D (+) Lactatemg/ kg	Lactic acid mg/kg
ES Kishk *	3.03	4.56	4431.18	5169.71	9600.9
ES Kishk **	3.47	4.23	4822.92	5223.40	10046.3
IR Kishk **	5.45	3.89	6714.20	13003.06	19717.2

*At zero time ** After 6 months of storage.

Organic acids in Egyptian Seidi kishk:

Fig. (1) shows the chromatogram obtained by Gas Chromatography Analysis of the ether extract of standard acids solution on the chromosorb 101 column. The lactic acid measured represented 38.8% of the TA which still categorize the fermentation taking place in kishk among the spontenous lactic fermentation reaction. Propionic, butyric, succinic and acetic acid represented 28.17, 14.15, 11.17 and 7.75% of the TA .

1- Acetic acid 2- Succinic acid
3- Butyric acid 4- Propionic acid
5- Lectic acid.

Fig (1) Elution Profile of several organic acids in Kishk on the chromosorb 101 by temperature programming from 140^{o}C to 22 ^{o}C at 5. min.

Microbiological Analysis:

Data recorded in Table (2) show that ES Kishk contained 6.71 and 6.37 log cfu/g *Streptococci* and *Lactobacilli*, respectively. No growth was detected on UBAPan media, that mean no Pediococci according to **Van dekerckhove (1986)**. However, the total yeast as appeared on UBAC was 6.54 log cfu/g. Actedione resistant yeast enumerated 4.23 log cfu/g as detected on UBAAC. So total yeast represented 75.9% of the total count corresponding to 24% bacteria at zero time. It could be signed to the morphological shape of

bacteria which were in short rods, cocci and gram positive. Furthermore, data registered in Table (2) also reflected that less viable bacterial cells were detected in ES Kishk after 6 months of refrigerator storage, whilst, the microorganisms represented 5.00 log cfu/g. most of them were yeast, as detected on MRS media. However, total yeast and actedione resistant yeast enumeration registered 4.94and 3.57 log cfu/g on UBAC and UBAAC, respectively. Also, neither detection of LAB was on MRSP tomato juice nor L-S differential media. It could be due to dryness and non hygroscopic characteristics of Kishk beside of the partly high acidity content which was 3.47% beside the rest of the LAB antagonisms in Kishk. So most or all of these properties belong the shelf life of the product and did allow the contamination of microorganisms to develop except some yeast could be able to tolerate that condition. With regards to IR Kishk, no growth of any microorganisms was observed on the used media. It could be due to the high acidity content (5.45%) and high content of sodium chloride which was added to IR Kishk till saturation and led to high osmosic pressure, that beside of dryness.

Table(3): Lactic acid bacteria and yeast in ES and IR Kishk

Media	LAB	Lactobacillii	Streptococci	Pediococci	Total yeast	Act. R. yeast	%
	MRS	MRS P M	L-S differential	UBA an P	UBAC	UBAAC	
ES Kishk *	7.1x106	3.7x106	2.6x106	ND	5.4x106	2.3x104	24.10%`B 75.90% Y
ES Kishk **	1.0x105	ND	ND	ND	9.4x104	5.7x103	06.00% B 94.00% Y
IR Kishk**	ND	ND	ND	ND	ND	ND	

* AT zero time ** After six month of storage

MRSPM: MRS media+ Pemiricin +Maltose

Act. R. yeast: Actedione resistant yeast.

LAB: Lactic acid bacteria. B: Bacteria. Y: yeast

ACKNOWLEDGMENT:

The auther would like to express her deepest thanks to Herbert Verachtert, Prof. K.U. Leuven for his personal interest, stimulation and support through the different stages of the work.

REFERENCES:

A. O. A. C. (1995). Official Methods of Analysis, 16th Edition Association of Official Analytical Chemists Inc., Arlington, Virginia. USA.

Attia, I. A. & Khattab, A.A. (1985). Microbiological studies on kishk. Alex. Sci. Exch.6.(1):63-71.

Beuchat, L. R. (1983). Indigenous fermented food and feed production with microorganisms. Rehn H. Jand Reed G.(ed.), Verlog chemie, Weinhein,W. Germany/ 477-529.

Dellaglio, F. and Torrian, S. (1986). DNA-DNA homology. Physiological characteristics and distribution of lactic acid bacteria isolated from silage. Journal of Applied Bacteriology (6o): 83-92.

Demerdash, M. A; (1960). Studies in microbiology of fermented milk common in Egypt. M. Sc. Thesis. Faculty of Agriculture, Cairo University PP. 92.

Drawert, F. and Hagen, DI. (1971). Enzymatische Analysenmethoden zur Bestimmung von wurze- und Bier in haltsstoffen 1-Bestimmung von L (+) und D (-) Milchsaurein Bier Brauwissenschaft 23.1

E.B.C. -analytical (1975). Free alpha amino nitrogen in worts and beer. E 61-E 62 .

Elewa, N.A.H. (1992). Studies in some fermented dairy products. Ph.D.Thesis in Dairying. Cairo University.

El-Gendy, Sh. M. (1983). Fermented foods in Egypt and middle east. Journal of food protection vol.45, No 4,358-367.

El-Gendy, Sh. M.,(2001). Some traditional fermented dairy products in Egypt. Proc. 8th Egyptian Conf. Dairy Sci. & Techn. 465-479.

Herbert, D., Phillips, PJ and Strange, RE.(1971). The chemical analysis of microbial cells In (Methods in microbiology) vol.5B, P. 209 Ed. Norris, JR and Ribbons, DW., Academic press, London.

ICMSF International Commmittee on Microbiological Specification for Foodc (1996). Microogenisms in foods: Their significance and Method of Enumeration. 2nd ed. University of Toronto Press, Tronto, Canada, P. 564- 790.

Krusch, U. (1978). Physiological aspects of L(+) and D(-) lactic acid. Kieler Milchwirtschaftliche Forschungsbericht 30 (3) 341-346.

Lapierre L., Germond J., Ott A., Deelley M., and Mollet B. (1999). D (-) Lactate Dehydrogenase Gene inactivation and metabolic effect in the Lactobacilli Johnsonii strains La1 and N312. Applied and Environmental Microbiology vol 65,9:4002-4007.

Lawrence, D.R. and Leedham, P.A. (1979). The detection of lactic acid bacteria. J. Inst. Brew vol 85, pp. 119-121.

Martley, F.G. and Turner, K.W. (1983). "Galactose fermentation by thermophilic lactobacilli". Significancy in classification and cheese manufacture. cf: Lactic acid bacteria in food genetics, metabolism and applications. Washington, The Netherlands symposium 7-9 September (1983).

Mc Murrough, I. and Palmer, V. (1979) Lactic acid production in sweet worts. Journal of institute of Brewing 85:11-14.

Morcos, S.R., and Hegazi, S.M., and El-Damhougy, S.T., (1973). Fermented foods in common use in Egypt.1. The nutritive value of Kishk. J. Sci. Food Agric. 24:1153-1156.

Odunfa S.A. (1988). Review: African fermented foods: from art to science. Mircen J 4:255-273.

Rasic, J.L. and Kurmann, J.A. (1978). Yoghurt-scientific grounds, technology, manufacture and preparation. Technical Dairy publishing House Copenhagen. Denmark.

Shaker, R.R. (1979). Chemical and microbiological studies on kishk and Tarkhina in Northern Iraq.M. Sc. Thesis. College of Agric., University of Alexandria Egypt.

Steinkraus, K. (2002). Fermentations in world food processing. Comprehensive Reviews in food science and food safety vol. 1, 23-30

Van dekerckHove, A., (1986). Melkzuurbacterien en wortfermentaties M.D thesis, Faculty of Agricultural sciences, Catholic University of Leuven, Belgium.

Van soest, P.J., and Wine, R.H., (1967). Use of detergents in the analysis of fibrous foods.

Zambou N. F.; El-Dousouky Z; Abd El-Arazek S.; Mbiapo T.F. and El-Soda M. (2004). Important technological properties of lactic acid bacteria isolated from milk and traditional dairy products. Egyptian J. Dairy Sci., 32:201-220.

سمات ميكروبيولوجية وبيوكيميائية في تخمر الكشك
٢- الكشك المصري الصعيدي

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وثبت الأغذية اللبنية الوظيفية وثبه كبيره في السنوات الأخيرة، وهذا دليل متجدد على الدور الوقائي لمنتجات الألبان المتَخمرة وبشكل محدد المنتَجات التقليدية مثل الكشك الذي يعتبر غذاء محلي شعبي مثالي في مصر والشرق الأوسط. يصَنعَ من لبن الزير laban zeer والبرغلِ وبكتيريا حمض اللاكتيك الطبيعية. وهو يتميز بأنه غني في المكونات الغذائية والصحّية وله قيمة حيويةُ عاليةُ.

في هذا البحث، اهتمت الدراسة بالفحص الكيمياوي الحيويِ والفحصِ الميكروبيولوجى للكشك، وامتدت الدراسة أيضاً إلى الناحية الصحية من خلال تقديرِ صورتى حمض اللاكتيك L (+) & D (-) isomers للوقوف على المحتوى من الصورة D(-) الذي يؤدى إلى الإضطرابِ في الثدييات. تمت المقارنةُ بين الكشك الصعيدى والإيراني الذي يعتبر وكأنه مركز لبن رايب مشبع بالملحِ ومشكل فى صوره كرات صغيرهِ ثم جفف شمسيا. كذلك تضمنتُ الدراسةَ التعرفِ على الأحمـاضِ العضوية فى الكشك الصعيدى باستخـدام.Gas liquid chromatography. أظهرت النَتائِج أن الحموضـةَ titratable acidity كانـت ٣.٠٣، ٥.٤٥ %. والصورة (-) D من حامض اللاكتيك كانت ٥١٦٩.٧١ ١٣٠٠٣.٠٦ملج/كيلوغرام في الكشك الصعيدى والإيرانى على التوالي. كما أوضحت النتائج احتواء الكشك الصعيدى على كل من الاحماض العضوية الاتيه البروبيونيك والبيوتريك والسكسينيك وحامض الخليك كما سجل الفحصِ الميكروبيولوجى ٧.٣٧ و٧.٢٦ cfu/gmلكل من Streptococci & Lactobacilli على التوالي، بينما لم تظهر Pediococci في الكشك الصعيدى هذا بجانب ٧.٤٥ و ٥.٢٣ cfu/gm مِن الخميرة الكلية والخميرة المقاومة للـ actedione على التوالي. كذلك وضح التحليلِ الميكروبيولوجى للكشك الصعيدى بعد ٦ شهورِ من التخزينِ سيادة للخمائر وصلت ٩٤% بينما كانت البكتيريا ٦% من العدد الكلى. بينما لم تَظهر التحليلات الميكروبية وجود اى كائنات حيّة مجهريةْ في الكشك الايرانى بعد ٦ شهور من التخزينِ، ربما يرجع السبب لارتفاع الحموضة والمحتوى العاليِ للملحِ الذي أدى إلى الجفاف الفسيولوجيِ في هذا النوع من الكشك.

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