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DETERMINATION OF VOLATILE COMPOUND IN FERMENTED CAMEL MILK BY GC-MS

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ABSTRACT

Camel milk is important in sustainable development and in ensuring the nutritional needs of future generations. Thus, this study focuses on camel milk products. Volatile compounds were investigated for fresh camel milk and fermented camel milk using a mixture of the starter bacteria formed of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*. The results showed difference in quality of volatile compounds in fresh camel milk from those found in fermented camel milk, with the presence of compounds such as -:9,12,15 Octa- decatrienoic acid, 2-[(trimethylsilyl) oxy]- 1- [(trimethylsilyl) oxy] methyl] ethyl ester, (Z,Z,Z), cis-13 Eicosenoi cac id, pyridazine2,7- Diphenyl -1,6- dioxopyr idazino [4,5:2',3'] pyrrolo[4',5'-d] ,Octa-decanoic acid,4-hydroxy-, methyl ester, Stearic acid, 3- (octadecyloxy) propyl ester, 1,2-Propanediol, diacetate, Glycine, N-[(3à,5á,7à,12à)-24-oxo-3,7,12-tris [(trimethylsilyl)oxy]cholan-24-yl]-, methyl ester, ethyl allocholate iso1.82, Octasiloxane hexadecamethyl. In fresh camel milk either the compounds that were diagnosed in fermented camel milk were included on, such as tert- Hexadecanethiol, glycerin Desulphosinigrin. The results indicated that many volatile compounds were found in fresh and fermented camel milk, with a clear difference in quality and concentration. In conclusion, the use of the starter bacteria produced an obvious change in the flavor of fermented camel milk.

INTRODUCTION

Number of camels in Iraq increased in the period 1995-2014 from 5,400 to 62,000 camel heads (FAO, 2014). Production of camel milk is one of the most important goals to achieve food and agricultural interdependence in Iraq. Moreover, camel milk substantially contributes in the sustainable development through camel milk products and dairy products. One of these products is fermented camel milk. The fermentation process is one of the most important food industry methods, especially in the field of food preservation technology. It results in desired changes in food making it more popular in terms of taste, and it has a role in preventing the growth of pathogenic microorganisms (Mufandaedza *et. al.*, 2006; Mosha& Vicent, 2004).

Naturally fermented camel milk forms a large part of the diet in the desert and semi-desert regions of East Africa (Farah *et. al.*, 1990). It is also used in the manufacture of a number of dairy products such as fermented milk, yoghurt (Elayan *et. al*., 2008) (Hashim *et. al*., 2008). Furthermore, it has a medical importance as an antidote to many infectious diseases, cancers, and diabetes. On the other hand, camel's milk maybe suitable for drinking and the remainder of it, is used to feed young camels (Yagil *et. al.*, 1984) or may be converted into fermented milk by leaving milk for several hours in pottery or leather containers (Yagil and Etzion, 1980; Ramet, 2001). One of the products of fermented milk is Suusac. It is natural yeast as it is made during self-fermentation and under room temperature for one to two days (Lore *et. al.*, 2005). In general, lactic acid bacteria (LAB), which is a substance that is added to food, is generally recognized as safe (GRAS) for human consumption (Aguirre & Collins, 1993).

Some of the plant or microbial extracts have been found to be highly effective as antimicrobial, anti-inflammatory, anti-oxidant, anti-cholesterol, anticancer, liver protection, anti-inflammatory, anti-histamine. The reason is that these extracts contain natural flavonoids, (Hexadecaonoic acid, ethyl ester and n- Hexadecaonoic acid) as well as containing unsaturated fatty acids and docosatetraenoic acid and octadecatrienoic acid (Kumar *et. al.,* 2010).

Volatile compounds are often used as an indicator of milk quality. Many studies have indicated that the estimation of these compounds gives an impression on the conditions of storage and storage temperature (Urbach, 1990). Thus, in countries with abundant of camel milk the focus has been on studying its composition. In Egypt, El-Agamy (1983) found that camel milk contained 3.7% protein, 2.9% fat, 5.8% lactose, and ash 0.7%. The changes to the flavor of milk and its products are the result of the metabolic effects of micro-organisms where secondary metabolites are introduced during the growth phase (Urbach & Milne 1987). Therefore, the aim of this study is to determine the flavor compounds found in fresh camel milk and estimated in fermented camel milk and changes during fermentation.

MATERIAL AND METHODS

Camel milk samples:

Camel milk samples were collected from central and western Iraq and transferred in sterile and cold conditions to the lab for further processing.

Culture growth and maintenance:

Yogurt starter (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* sp. *bulgaricus*) was obtained from Danisco, Denmark and was used to inoculate skim milk at 42ºC, after being incubated for 24 h and cooled after fermentation to 4ºC for short time use.

Preparation of fermented camel milk:

The fermentation process was prepared as described by Rahman *et al.* (2009). The skim camel milk was pasteurized for 30 min in a water bath in 500 ml screwed bottles at 90 C and cooled immediately to 5±1 C in an ice bath. The milk samples (500 ml) were equilibrated for one hour at the fermentation temperature (42ºC) in a water bath before inoculation with the starter cultures. Each sample was inoculated with 5% (10^6-10^7cfu/ml) of mixed yogurt culture (*S. thermophilus* and *Lactobacillus delbrueckii* sp. *bulgaricus*) at a ratio of 1:1. Samples were thoroughly mixed after inoculation and incubated at 42ºC for 6h.

Water-Soluble Extracts (WSE):

The water-soluble extracts (WSEs) of fresh camel milk and fermented camel milk samples were prepared as described by Kuchroo and Fox (1982).

Extraction and determination of volatile compounds:

The extraction of the volatile compounds in fresh camel milk and fermented camel milk were performed as recommended by the liquid/liquid or liquid/solid extraction (direct extraction techniques) method (Mariaca and Bosset, 1997; Preininger *et. al.,* 1994). This protocol was taken place through acetonitrile solvent for low-fat dairy products. Volatiles

flavored compounds were identified on Gas chromatography –Mass Spectrometry (GCMS) according to Agilent company's instruction manual and according to the following conditions: Column (ZEBRON ZB-FFAP 30meter x 0.25 mm I.D x 0.25 µm). The operating conditions of the device are: sampling time: 1.00min ‹Inj. Initial temp.:250.00.C, Interface temp: 260.00ºC, Column Inlet Pressure: 56.7 kpa, Total flow: 23.0 ml/min. The oven temperature was 70ºC / 3min and the temperature is 260 ºC / 20 min and the pressure inside the column is 56.7 Kpa / 3 min and the pressure is 185.9 Kpa / 20 min.

RESULTS AND DISCUSSION

Volatile compounds, identified in fresh camel milk by using Gas chromatography - Mass Spectrometry (GC/MS), are listed in Table 1. Figure 1 shows {9,12,15-Octadecatrienoic acid, 2[(trimethylsilyl)oxy]1[(trimethylsilyl) oxy]methyl] ethyl ester,(Z,Z,Z)1.78%.cis-13-Eicosenoic acid.17% pyridazine2,7-Diphenyl-1,6 dioxopyr idazino.[4,5:2',3'].Pyrrol.[4',5'-d]pyridazin 2.10% Octadecanoic acid, 4-hydroxy, methyl.ester.2.02%,.Stearicacid 3-(octade-cyloxy) propyl ester 3.67% 1,2-Propane-diol 3, diacetate.6.59% Glycine, N[(3à,5á,7à,12à)-24-oxo 3,7,12-tris [(trimet-hylsilyl)oxy]cholan-24-yl]-, methyl ester 13.24% Ethyl isoallo.cholate 1.82% Octasiloxane hexadecamethyl 30.76%.

The results showed that there is a mixture of volatile flavor compounds and active and bioactive compounds that can be detected using GC-MS as this device has the ability to diagnose long chain hydrocarbons, alcohols, acids, esters, alkaloids, steroids, and amino and nitrogen compounds (Venkatesh *et. al.*, 2014). The process of compounds diagnosis is based on the calculation of retention time (RT), molecular formula, molecular weight, and concentration (Peak area %). Some of the substances that have been diagnosed as volatile compounds have significant efficacy and the importance as antimicrobial agents, as it found that the substance Glycine,N-[(3à,5á,7à,12à)-24-oxo-3,7,12-tris [(trimethylsilyl)oxy] cholan-24-yl]-,ester methyl is important as an anti-bacterialgrowth (Ganesh &Vennil 2011).

The volatile compounds that have been diagnosed in raw milk using solid phase micro-extraction and by using the GC/MS device are belong to different types, which include compounds in the form of aldehydes chains like hexanal, heptanal, octanal, nonanal, or methyl ketones (2-heptanone and 2-nona- none) or ketones (3-octen-2-one and 3,5-octadien-2-one). However, volatile flavored compounds can be used as a guide and indicator of the oxidizing taste of milk as in the case of the use of aldehydes and ketone compounds (Li *et. al.* 2012).It deserves to note that the materials responsible for flavor dairy products include a large number of volatile compounds (Molimard & Spinnler, 1996; Urbach, 1997). These compounds may include large amounts of free carboxylic acids, sulfur compounds and substances containing alkali nitrogen such as amines and pyridines and many neutral compounds such as carbonyl (met-hyl ketones), aldehydes, primary and secondary alcohol, esters, ketones, ethers, aliphatic and aromatic hydrocarbons (Dafflon *et. al.*,1995).

Table 1. Volatile compounds as analyzed by GC-MS in Fresh camel milk

Compound Name	RT	Molecular Weight	Molecular Formula	Area %
9,12,15-Octadecatrienoic acid, 2[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy]methyl]ethyl ester, (Z,Z,Z)	3.81	496	C27H52O4Si2	1.78
cis-13-Eicosenoic acid	4.52	268	C16H28O3	2.14
pyridazine2,7-Diphenyl-1,6-dioxopyr idazino[4,5:2',3']pyrrolo[4', 5'-d]pyridazine	6.19	355	C20H13N5O2	2.10
Octadecanoic acid,4-hydroxy-, methyl ester	7.12	314	C19H38O3	2.02
Stearic acid,3-(octadecyloxy)propyl ester	7.38	394	C39H78O3	3.67
1,2- Propanediol, 3-(hexadecyloxy)-,diacetate	9.54	400	C23H44O5	6.59
Ergosta-5,22-dien-3-ol, acetate, (3á,22E)-	9.87	440	C30H48O2	11.54
Glycine, N-[(3à,5á,7à,12à)-24-oxo-3,7,12-tris[(trimethylsilyl)o xy]cholan-24-yl]-, methyl ester	11.16	695	C36H69NO6Si3	13.24
Ethyl iso-allocholate	15.92	436	C26H44O5	1.82
Octasiloxane hexadecamethyl	22.31	578	C16H50O7Si8	30.76

Table 2. Volatile compounds as analyzed by GC-MS in fermented camel milk

Compound Name	RT	Molecular Weight	Molecular Formula	Area %
tert- Hexadecanethiol	4.38	258	C16H34S	7.60
Glycerin	8.42	92	C3H8O3	82.76
Desulphosinigrin	9.97	279	C10 H17 NO6S	9.64
4-Piperidineacetic acid, 1-acetyl-5-ethyl-2-[3-(2-hydroxyethyl)-1H-indol-2-yl]-à -methyl-, methyl ester	13.81	400	C23H32N2O4	3.81
Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,1 3,13,15,15-hexadecamethy l-	22.17	578	C16H50O7Si8	3.97
Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-	22.54	430	C12H38O5Si6	5.06
Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,1 3,13-tetradecamethyl-	22.74	504	C14H44O6Si7	5.69

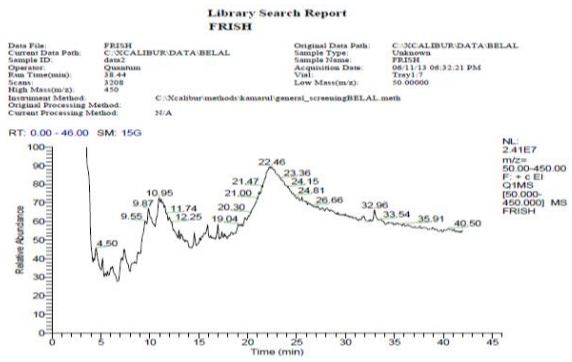

Fig.1.GC-MS analysis of Volatile compounds in Fresh camel milk

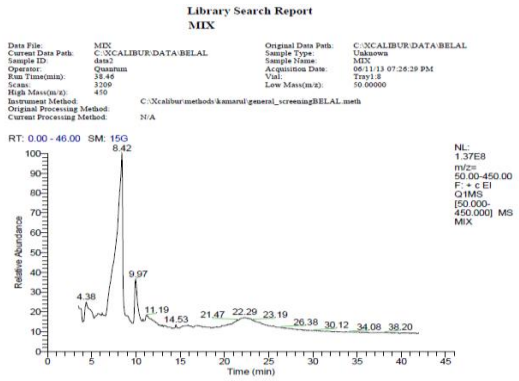

Fig.2.GC-MS analysis of Volatile compounds in Fermented camel milk

Moreover, more than 100 volatile substances, including carbonyl compounds, alcohol, acids, esters, hydrocarbons, aromatic compounds, sulfur-containing compounds, and heterogeneous ring compounds, were found in fermented milk at a low or very low concentration (Cheng, 2010). Finally, the present study showed that the process of fermentation of camel's milk by lactic acid bacteria plays an important role in the flavor of

fermented milk, while the production of most flavor compounds in milk was found to be the degradation of milk fat and microbiological shifts of lactose and citrate.

CONCLUSION

In this study, the volatile components of fresh camel milk and fermented camel milk (Lactobacillus delbrueckii subsp. *bulgaricus* and *Streptococcus thermophilus*) were analyzed by GC MS. Volatile compounds were identified. It was also identified that the major volatile components were alcohols and fatty acids, which played the most important role in imparting the typical aroma and flavor. The results indicated that there were many volatile compounds in fresh and fermented camel milk, with a clear difference in quality and concentration. This study showed that fermentation of camel milk by lactic acid bacteria plays an important role in fermented milk flavor. The use of the starter bacteria produced an obvious change in the flavor of fermented camel milk. There is still a lot of work to be done to get a more complete understanding of the formation of aroma and flavor of milk. Determining the relationship between the main aromatic compounds and the sensory properties of fermented camel milk can provide a better understanding of how milk flavor is affected by the presence of flavor compounds to make it more acceptable to the consumer.

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