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SPECTROSCOPIC AND CHEMICAL STUDIES ON THE COMPOSITION OF MILK FROM DIFFERENT ANIMALS

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ABSTRACT

Milk samples from two sources; namely Egyptian cow, buffalo and camel as well Saudi camel and goat were lyophilized. The freeze-dried samples were analyzed for amino acids using HPLC and fat/protein content using the FT-IR spectroscopy technique. The recorded spectra of samples revealed that the characteristic band of protein appeared at the frequencies 3290,2950,2800,1650 and 1540 cm-1, while the diagnostic bands of lipid are located at 2960,1740 and 1460cm-1. The analysis of the spectra showed also that the intensities of the absorption bands of the lipids in the samples of Saudi camel and goat as well as Egyptian camel are comparatively strong, which means that the lipid contents of these samples are relatively higher.

On the other hand, the characteristic absorption bands of proteins in the spectra of cow milk appeared stronger than their characteristic bands of lipid. Based on the obtained data, it was concluded that the milk samples from cow contain a relatively higher content of carotenoid pigments compared to the others. Moreover, the milk from Egyptian cow contain the highest value of protein in comparison to its lipid content, while the milk samples from Saudi animals contained the lowest values of protein. The semiquantitative atomic emission spectroscopy was applied for the determination of the metallic elements in the samples under study. In addition, values of histidine, therionine, aspartic acid, methionine and arginine amino acids present in Egyptian camel milk were higher than that of Saudi camel milk proteins.

Keywords: Milk, Amino acids, FT-IR and HPLC.

INTRODUCTION

The Egyptian consumers consider Buffalo's milk as the most popular and appreciated kind of milk. However, buffaloes are found in many parts of Asia, in Egypt and also in South Italy.

Buffalo's milk is consumed raw and used in production of milk products like cheese. Goats are more suitable for improvement because of their high productivity, grazing ability and the suitability of their milk for human consumption (Sirry and Hassan, 1954; Mehaia, 1974).

The Egyptian camel produces 1068 to 1373 Kg milk per lactation period (Knoess, 1977), but Egyptian goats were found to produce about 51.14 Kg of milk in 146.3 days (Fares, 1964). Such level of production is much lower than that of improved breeds, and this level was reported in the USA as about 470 Kg of milk in 247 days (Grossman and Wiggans, 1980).

The camel milk contains all essential nutrients and has a composition similar to that of cow's milk (Yagil, 1982).

Production of the milk in Saudi Arabia is divided into three sectors. Firstly, goats and camels raised by traditional farmers and nomads, Secondly, the modern dairy cattle farms, thirdly, the recombining dairy plants which use imported by milk powder (Anonymous, 1990). However, some dairy plants are currently used to package, pasteurized camel and goat milks, which are being sold in some parts of the Saudi Kingdom.

The composition of the goat milk varies considerably from that of the camel milk produced in the Saudi Kingdom (Sawaya *et al*., 1984 a&b and Mehaia et al., 1992). Also, the goat milk produced in the other countries has a wide range of milk components (Parakash and Jenness 1968, Jenness, 1980). Variation in the milk composition is mainly due to feeding type, environmental temperature, the available amounts of water and feed, age of the animal and stage of lactation.

Recent development has been directed to introduce infrared technology into dairy factories. Several workers used the Fourier Transform-Infrared (FT-IR) spectroscopy for quantitative analysis of milk fat, milk protein and lactose content (IDF 1990, Rathfelder et al., 1990, Gelderman et al., 1996, Kjaer and Ridder 1997 and Pabst 1997).

In this paper, the FTIR spectroscopy and amino acid analysis are applied for differentiation between the Egyptian and Saudi milks from different species namely; camel, goat, cow and buffalo. The milk fat and protein are also determined quantitatively by IR spectra.

MATERIALS AND METHODS

Milk Samples

The Egyptian camel milk was obtained from Assiut City and Fresh bulk buffalo and cow milks were obtained from the herd of the Faculty of Agriculture, Cairo University. The Saudi goat and camel milks were obtained from healthy flocks in the desert. The all milk samples were lyophilized and analysis.

Gross Composition Analysis

Samples were analyzed for total solids, fat, titeratable acidity, pH and ash contents according to methods mentioned by AOAC (1990). The total protein was determined by the semimicro Kjeldahl method according to Ling (1963).

Amino Acid Analysis

Amino acids composition of milk from different animals species were determined as described by method of Millipore Co-operative (1987) using high pressure liquid chromatographic analysis (HPLC) of amino acids in foods using a modification of the PICO-TAG methods.

FT-IR Spectroscopy Measurements

FT-IR [Fourier Transform Infra Red Spectroscopy] spectra were recorded for the milk samples in the dry powder by using the potassium bromide (KBr) pellet technique: In this technique, 2 mg of the sample were thoroughly mixed with 198 mg of specpure KBr to get 1% sample

concentration suitable for obtaining a good spectrum. In this work, Jasco FT/IR-300 E spectrometer was used for recording the spectra of the samples under investigation. For quantitative analysis, the baseline method was applied to calculate the precise absorbency values of the absorption bands.

RESULTS AND DISCUSSION

Gross Composition

The chemical composition of Egyptian camel, cow, buffalo and Saudi camel and goat milks are shown in Table (1). It is clear that the total solids and fat contents of Egyptian and Saudi camel milk were almost of the same values, being 11.28 and 11.24 for total solids as well as 3.5 and 3.5% for fat respectively. On the other hand, the total protein content of Saudi camel milk was higher than that of Egyptian one.

The protein fat ratio of cow milk is the highest value among all the other milks, values were 1.539 for cow milk but the protein fat ratio of the other milks were 0.797, 0.774, 0.926 and 0.636 for Egyptian camel,buffalo, Saudi goat, respectively.

The buffalo milk contains almost the same value of fat content as in Saudi goat milk, values were 5.3 and 5.5% for Egyptian buffalo and Saudi goat respectively.

The total solids of goat milk (Saudi) were the highest one (13.88%). Also, it could be seen from table (1) that total protein concentration of buffalo and cow milks were significantly higher than those of camel (Egypt and Saudi) and goat milk.

Table (1): Chemical composition from different animal species

	Egyptian		Saudi		
	Camel	**Cow**	**Buffalo**	**Camel**	**Goat**
pH	6.70	6.55	6.50	6.53	6.42
Acidity	1.3	1.7	1.9	1.4	2.00
Total Solids %	11.28	10.24	13.97	11.24	13.88
Total protein %	2.79	4.0	4.10	3.24	3.5
Fat %	3.5	2.6	5.3	3.5	5.5
Prot/Fat ratio	0.797	1.539	0.774	0.926	0.636

Amino acids composition of freeze dried milk from different animal species (Saudi camel and goat as well as Egyptian camel, cow and buffalo) were determined using HPLC method [Millipore Co-operative, 1987].

The results are given in table (2). It is apparent that values of histidine, therionine, aspartic methionine and arginine amino acids present in Egyptian camel milk, were higher than that Saudi camel milk proteins . Also, the milk of the Egyptian cows contains higher content of arginine, isoleucine and therionine amino acids than those found in the Egyptian camel and buffalo milk proteins (Table 2). Also, this table shows that milk proteins of the Egyptian buffalo and camel have lower content of isoleucine, phenylalanne, therionine, aspartic and tyrosine than that of the cow's milk protein, which agreed with the results reported by Williams et al., (1976). From the obtained data, it could be seen that the histidine, isoleucine,

phenylalanine, therionine, tyrosine and aspartic acids content of Egyptian cow's milk proteins were more higher than those presented in Egyptian buffalo milk proteins.

The ratios of essential to non-essential amino acids obtained by Farah (1993) was (1.04) This value was lower than that resulted in our study (1.09) for Saudi, as well as (2.27) for Egyptian camel milk samples respectively. On the hand, this the ratio of essential to non- essential amino acides obtained by Larsson (1986) (1.27) was higher than that found on the present study of Saudi camel milk samples .

Moreover, it appears from the table (2) that the ratios of aromatic amino acids; phenylalanine: tyrosine (Ph: Tyr) in Saudi camel milk (1.40) was higher than that of the corresponding Egyptian milk (1.03) and Saudi camel (1.07) given by Farah (1993).

On the other hand, the ratios of the sulpher-containing amino acids; (Methionine: Cystine) are 0.18 and 0.81 for Saudi and Egyptian camels respectively. These values are lower than that of corresponding one (2.5) reported by Farah (1993). The ratio of essential to non-essential amino acids for Saudi goat milk proteins (1.37) is lower than that of the Egyptian goat milk (1.56) as reported by Mahran et al., (1988).

Table (2) : The amino acids composition of milk from different species (g/100g protein)

Amino Acids Essential	Egyptian Camel	Species		Saudi Species	
		Cow	Buffalo	Camel	Goat
Arg	5.87	6.49	4.62	2.29	4.44
His	8.16	5.79	0.66	1.41	2.27
Ile	2.01	6.08	3.3	6.11	3.14
Leu	3.78	4.47	7.59	6.13	1.50
Lys	10.55	7.11	15.35	3.1	1.76
Met	6.02	2.09	2.8	1.09	2.39
Phe	4.95	4.95	1.82	3.47	4.16
Thr	13.95	17.8	5.12	6.70	7.17
Val	6.98	0.79	3.63	6.54	1.17
Cys	7.40	1.96	1.98	5.97	6.56
Non-essential					
Ala	1.08	0.36	5.45	3.65	0.78
Asp	18.41	22.04	10.40	6.52	5.03
Glu	3.12	7.96	13.53	13.95	12.63
Gly	1.13	3.65	3.3	1.65	0.45
Ser	2.16	1.32	2.15	5.24	0.87
Tyr	4.82	5.23	1.82	2.48	5.39

The above mentioned results hold true for the aromatic amino acids; the Egyptian goat milk assumes higher values (1.77) as mentioned by Mahran et al., (1988) than that of Saudi goat milk (0.77). Furthermore, the ratio of sulpher-containing amino acids Meth: Cys is lower in saudi goat milk (0.36) than in the Egyptian goat milk protein (0.89) as determined by Mahran et al., (1988). In the present study it is clear that the ratio of essential to non-essential amino acids for the Egyptian cow's and buffalo's [1.63][1.27] are higher than those for cow's and buffalo , (1.11) determined by Farah,(1993).

Comparison between the obtained amount (3.8 and 3.1) of amino acids of the Egyptian cow's and buffalo's milks and the amount determined by Taha and Kielwein, (1989), revealed that the former amount is lower than the latter one. It was observed that the Ph : Tyr, exhibited the same behavior. The values of this ratio for the Egyptian milk from camel's buffalo's and cow's (1.03 , 1.0 and 0.95 respectively) are lower than their content determined by Farah, (1993) (1.07). It was also noticed that the ratio of sulpher-containing amino acids in the milk protein of the Egyptian camels, buffalo and cows were 0.81 , 1.42 and 1.06, respectively, whereas the ratio reported by Farah, (1993) was 2.5.

Freez dried milk samples from two sources namely: Egyptian cow, buffalo and camel and Saudi camel and goat were analyzed using the FT-IR speetroscopy technique.

The recorded spectra of these samples are shown in fig. (1). Careful analysis of the spectra revealed that the characteristic bands of protein appeared at the frequencies 3290, 2950, 2800, 1650 and 1540 Cm^{-1} ,while the diagnostic bands of lipid are located at 2960, 1740 and 1460 $Cm^{-1,}$ .The frequencies of the absorption bands and their assignments are given in table (3). It is apparent from the spectra that the intensities of the absorption bands vary from sample to sample. The intensities of the absorption bands of the lipids in the samples of Saudi camel and goat and Egyptian camel are comparatively strong, which means that the lipid contents of these samples are relatively high.

On the other hand, the characteristic absorption bands of proteins in the spectra of cow milk appeared more intense then the characteristic bands of lipid the spectra of the cow samples exhibited stronger absorption bands characteristic to the protein than those of the lipid.

Over the spectral region 1000-1100 Cm^{-1} several striking differences in the spectral features are visible. The differences in the spectral features of the bands in the 1000-1100 Cm^{-1} region imply that the percentages of the biochemical constituents of the different samples of milk from the different animals species are different.

The spectrum of the milk sample from cow shows two strong absorption bands at 1040 and 1080 Cm^{-1}, while the spectrum of the milk sample from Egyptian camel shows bands of medium intensities at the same frequencies. These two bands appear very weak in the spectra of the other samples.

Figure (1): The infra red absorption spectra of milks from different animals (A) Saudi goat, (B) Saudi camel, (C) Egyptian camel, (D), Egyptian cow and (E) Egyptian buffalo

Table (3): The frequencies of the most evident absorption bands of the milk and their assignments

V Cm-1 (Wave number)	Intensity	Abs-Assignment
3290	S	N-H Stretching vibration (Amid-A)
2960	V.S	C-H Stretching vibration
2950	V.S	C-H Stretching vibration (Amid-A)
2800	V.S	C-H Stretchy vibration
1740	V.S	C=O Sretching vibration
1650	M	C=O Stretching vibration of Lipid (Amid II).
1550	S	N-H bending
1470	M	C-H bending
1420	Sh	C-H bending
1380	S	
1310	Sh	C-N Str., C=O Str., N-H bending
1240	S	Po2 Stretchy
1170	M	CO-O-C Str.
1120	Sd.	C-O Str.
1040	Sh	C-O-P Str.
890	V.S	
780	S	
720	S	

S=Strong M=Medium V=very d= doublet Sh= Shoulder

It is well know that cis-trans isomerism in carotennoid pigments, and the stereochemically sensitive regions are found to be ~ 1380, ~1000-940, and 770 Cm^{-1}. The cis-configuration, of methylated and unmethylated double bands shows differences in the infra red spectra.

The trans-C-CH=CH-C group shows its characteristic absorption band at frequency about 970 Cm^{-1}. This grouping occurs five times in all-trans-B carotene, neo-U, and neo_B molecules, but only four times in the central-mono cis-isomer.

Based on the above mentioned data one can came to the conclusion that the milk samples from cows contains a relatively higher content of carotenoid pigments compared with the other milk samples. The milk samples from Saudi animals contain only very trace amounts of the carotenoid pigments.

The absorbances ratio A 1650 Cm-1/A 1740 Cm-1 was taken as a measure for the protein/lipid content ratio. The determined values of this ratio for all samples under investigation are listed in table (4). This table indicates that the milk from Egyptian cow contain the highest value of protein in comparison to its lipid content, while the milk samples from Saudi animals contain the lowest values of protein.

Table (4): The absorbances ratio A 1650 Cm-1 /A1740 Cm-1 and the chemically measured protein/fat ratio for the milk of the different animals

Source of milk	A 1650 Cm^{-1}/A1740 Cm^{-1}	Chemically measured protein/fat
Saudi goat	0.389	0.636
Saudi camel	0.394	0.926
Egyptian camel	0.914	0.797
Egyptian cow	1.307	1.539
Egyptian buffalo	0.959	0.774

In the present study the semiquentitative atomic emission spectroscopy was applied for the determination of the metalic elements in the samples under study. The detected elements with their concentration are given in Table (5). The contents of trace elements from different animal species reported in the literature differ only marginally (Sawaya et al., 1984; and Alkanhal, 1993) but are always subjected to variations depending on the conditions prevailing at the time the animal is milked. This statement holds true for the concentrations listed in table (5).

Table (5): Semiquantitative spectrographic analysis of different milk samples (Conc. %)

Elements	E. Camel	E. Cow	E. Buff.	S. Camel	S. Goat
Ca	**Mj**	**Mj**	**Mj**	**Mj**	**Mj**
Na	5	5	7	7	5
Mg	3	3	5	3	3
P	1	3	4	3	0.8
K	1	0.8	0.6	1	0.8
Al	0.3	0.1	0.3	0.1	0.3
Cu	0.03	0.01	0.01	0.1	0.1
B	0.03	0.03	0.01	0.03	0.03
Si	0.03	0.01	0.05	0.01	0.015
Fe	0.003	0.001	0.002	0.001	0.001
Mn	0.001	0.0003	0.001	0.001	0.001
V	0.001	0.001	0.001	0.001	0.001
Pb	0.0003	0.0003	0.0003	0.0003	0.0003

E= Egypt. S= Saudi.

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دراسات طيفية وكيماوية على مكونات اللبن من الحيوانات المختلفة

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أستخدم في هذه الدراسة ألبان الجمال –الجاموس- البقر المصري وتم مقارنتها بالبان الجمال والماعز في المملكة العربية السعودية- تم تجفيف العينات بطريقة التجفيد . تم تحليل كل العينات لمحتوياتها من الاحماض الأمينية باستعمال جهاز ال HPLC وكذلك مكوناتها من البروتينات والليبيدات باستعمال الطرق الطيفية في منطقة الأشعة تحت الحمراء FT-IR وتم أيضا تحليل مكوناتها من العناصر المعدنية باستعمال جهاز atomic absorption وقد دلت النتائج على مايلى :

1- ظهرت البروتينات من خلال الطرق الطيفية في منطقة الأشعة تحت الحمراء على أرقام موجة تكرارية 3290- 2950 – 2800 – 1650 – 1540 سم-1 أما بالنسبة للليبيدات فهي تقع تحت أرقام موجة 2960- 1740 – 1460 سم-1 . ومن النتائج المتحصل عليها هو احتواء الألبان البقري على صبغات كاروتينيه خلاف الألبان الأخرى.

2- تصل نسبة الكالسيوم إلى درجة عالية في جميع الألبان تحت الدراسة أما الصوديوم فتصل نسبته إلى 7% في ألبان الجمال السعودية والجاموس المصري أما ألبان الحيوانات الأخرى تحت الدراسة فتصل إلى 5% .

3- توجد الأحماض الأمينية الهستدين – الثريونين- الاسبارتيك – الميثيونين- والارجنين في ألبان الجمال المصرية أكثر من مثيلتها في ألبان الجمال السعودية التي تحتوى على أقل كمية من الأحماض الأمينية الليوسين- أيزوليوسين- الالانين-التيروزين-السيرين- والجليسين عنها في بقية الألبان الأخرى.

4- يوصى البحث على أنه يمكن استخدام طريقة الأشعة تحت الحمراء لدراسة مكونات الألبان المختلفة خاصة البروتينات والدهون دون استهلاك مواد كيماوية كثيرة وكذلك كمية كبيرة من العينة حيث يكفى لهذه الطريقة ملليجرامات قليلة منها ودقة عالية .

Figure (1): The infra red absorption spectra of milks from different animals (A) Saudi goat, (B) Saudi camel, (C) Egyptian camel, (D), Egyptian cow and (E) Egyptian buffalo

Absorbance

Wave number (cm^{-1})