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Fourier Transform Infrared (FTIR) Spectra in Relation to the Composition of White Soft Cheese

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Abstract: White cheese is one of the most important dairy products manufactured, marketed and consumed in Egypt. To study differences in the composition and sensory properties of local white cheese, forty two cheese samples were collected from different markets in Cairo such as small producers, vendors, supermarkets and well-known cheese factories and compared with experimentally prepared white soft cheese (control sample). All cheese samples were analyzed for gross chemical composition, sensory properties. Also, Fourier Transform Infrared (FTIR) spectroscopy was used to monitor the differences between cheese samples. Obtained results showed significant differences in the gross composition between market cheeses and with the prepared white cheese (control sample), most market samples contained high fat and low protein contents probably due to the addition of different types and concentrations of vegetable oils during cheese manufacture. Tthe differences in the chemical composition between white cheese reflected on the FTIR spectra..

Key words: Cheese market · Chemical composition · FTIR spectroscopy · Organoleptic properties · White cheese

INTRODUCTION

Cheese is a major dairy product that provides consumers with important and essential nutrients. Technological progress has led to a multitude of different types of cheese on the market, varying in texture and flavor. Nowadays, a shift can be observed from the objective of optimum product quality to the more economic product for the consumer. Research is no longer directed to the production of high quality cheeses but more towards the commercialization of cheese as a functional food [1]. Several factors have been reported to influence the cheese composition and flavor, including raw materials, manufacturing process, nonstarter or starter lactic acid bacteria, cheese type and the biochemical reactions such as proteolysis, lipolysis [2]. Simultaneous measurements of flavor-related volatile compounds such as short chain fatty acids and nonvolatile compounds such as amino acids and organic acids are needed to evaluate the quality of cheese. Current methods for cheese analysis, including chromatography and sensory analysis are laborious, time consuming, expensive and complete characterization of cheese quality may require multiple methods. Hence, there is a need for rapid and reliable instrumental method for simultaneous determination of composition and flavor quality of cheese [3].

Fourier transform infrared (FTIR) spectroscopy is a simple and rapid technique that monitors the molecular changes in the various compounds and provides an overall chemical profile of the analyzed sample. This technique could be a rapid tool for quality control in the cheese industry to provide more consistency the cheese analysis and grading and improving cheese quality [4]. FTIR spectroscopy has been recommended for application in dairy products. The AOAC international has approved in FTIR method for determination of fat, protein, lactose and total solid content in some dairy products [5] and to monitor the flavor related minor compounds in Swiss cheese [6]. Also, Infrared spectroscopy is an important technique in the quality control of milk products at the manufacturer's levels,

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especially regarding the control of intermediate products throughout all production steps [7]. Subramanian *et al.* [8] suggested that Cheddar cheese samples could be classified on the basis of their flavor quality using FTIR spectroscopy. The objective of this work was to study the relation between the Fourier Transform Infrared (FTIR) spectra and composition of market and experimentally made white cheese.

MATERIALS AND METHODS

Cheese Collection: Forty two samples of white cheese were collected from Cairo markets and categorized into four groups according to their source as follow: Group-1, from small producers (15 samples), Group-2, from vendors (15 samples), Group-3, from supermarkets (6 samples), Group-4, from well-known cheese factories (6 samples). Buffalo's milk retentate was obtained from Dairy Industry Unit, Animal Production Research Institute, Ministry of Agriculture, Cairo, Egypt. The composition of milk retentate was 29.2% total solids, fat 15.5 %, total protein 12 % and pH was 6.7. Microbial rennet powder, *Mucor mehiei,* was obtained from Novo, Denmark.

Preparation of Control Cheese: milk retentate was salted to a concentration of 3% NaCl, pasteurized at 73°C for 15 sec, the curd was hold at 40°C for 30 min in plastic containers after adding the rennet (9 g/100 kg). Three replicates were prepared and all cheese samples were analyzed for the gross chemical composition, sensory properties and by FTIR spectroscopy.

Chemical Analysis: All cheese samples were analyzed for total solids according to the A.O.A.C. [9]. Fat and total nitrogen contents were determined according to the method describe by Ling [10]. The crude protein content was obtained by multiplying the percentage of total nitrogen by 6.38. The pH values were recorded using a digital pH-meter with a glass electrode, Model GC, Germany. Water-soluble nitrogen (WSN) of cheese samples was extracted and determined as described by Coskun and Tuncturk [11] and the ripening index (%) of cheese sample was calculated according to the equation:

$$\text{Ripening index (\%)} = \frac{\text{Water Soluble Nitrogen (WSN)}}{\text{Total Nitrogen (TN)}} \times 100$$

All samples were analyzed in triplicate.

Sensory Evaluation of Cheese: Cheese samples were assessed by a panel consisted of fifteen panelists (9 female and 6 male aged between 25-40 years old) who have experience and regularly used for assessing white cheese. They scored the cheese for taste (10 points), color (5 points), body and texture (5 points) and mouth feel (5 points). The panelists were also asked to record any unexpected or unpleasant flavor defects.

FTIR Spectroscopy: Thin section of selected white soft cheese samples collected from different producers was prepared and analyzed using Fourier Transform Infrared (FTIR) spectrometer (Nexus 670 Fourier Transform Infra-Red spectrometer, Thermo Nicolet, USA). The FTIR spectra were analyzed using "Omnic 5.2a" software.

Statistical Analysis: Statistical analysis of experimental data was performed by analysis of variance (ANOVA) producers using SAS PROC GLM/STAT [12]. Differences among means were identified using Duncan multiple range test.

RESULTS AND DISCUSSION

Overlaid FTIR spectra of white cheese markets collected from different producers in Cairo compared with control cheese are shown in Figs 1-4. It can be noticed that all samples have the same basic peaks representing the different chemical groups constituting in white soft cheese with differences between samples. The FTIR spectra of cheese (4000-400 cm^{-1}) present series bands with different intensities and forms and could be divided into 6 regions as mentioned by Subramanian *et al.* [13] as follows:

Region A: corresponds to the 3600-3100 cm^{-1} where represent O-H stretching in the hydroxyl groups. This region in our results was in the range of 3770 to 3048 cm^{-1}s, different broad bands could be attributed to the moisture contents of the different white soft cheese collected from different producers. Tables 1-4, which present the gross composition of that cheese samples, indicate the significant differences ($P \leq 0.05$) in the moisture contents related to cheese producers and the control. The OH band in all samples masked the N-H band, which occur in the 3330-3060 cm^{-1} region. These results are in agreement with Woodcock *et al.* [14], who mentioned the region from 3800 to 3100 cm^{-1} consists of absorbance from O–H and N–H stretching vibrations of hydroxyl groups and Amide A of polypeptides and amino

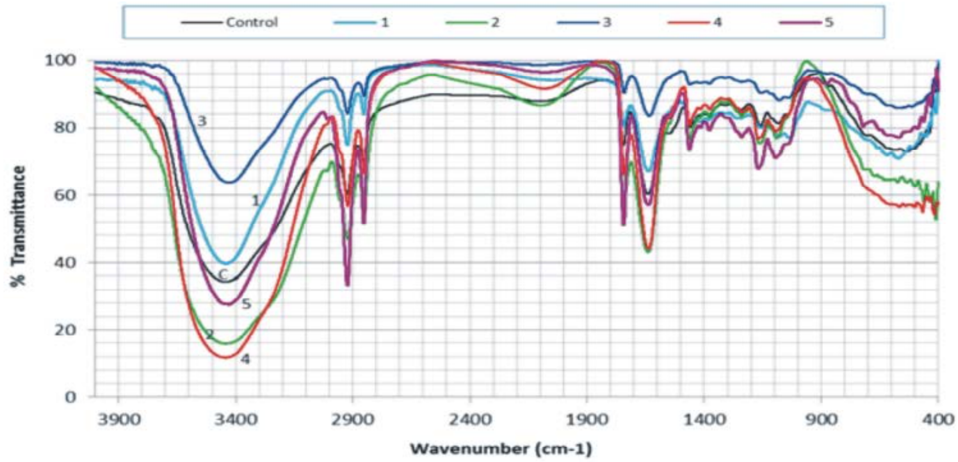

Fig. 1: Overlaid FTIR spectra of white cheese from small producers (Group-1) compared with the control

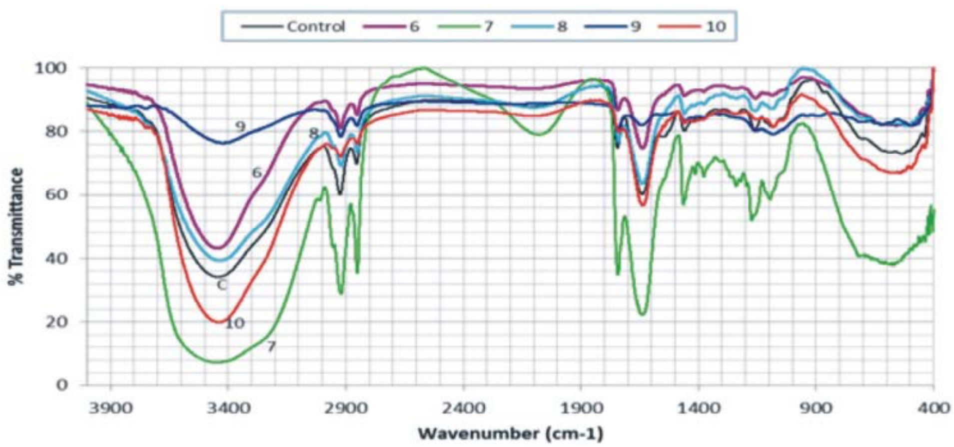

Fig. 2: Overlaid FTIR spectra of white cheese from vendors (Group-2) compared with the control.

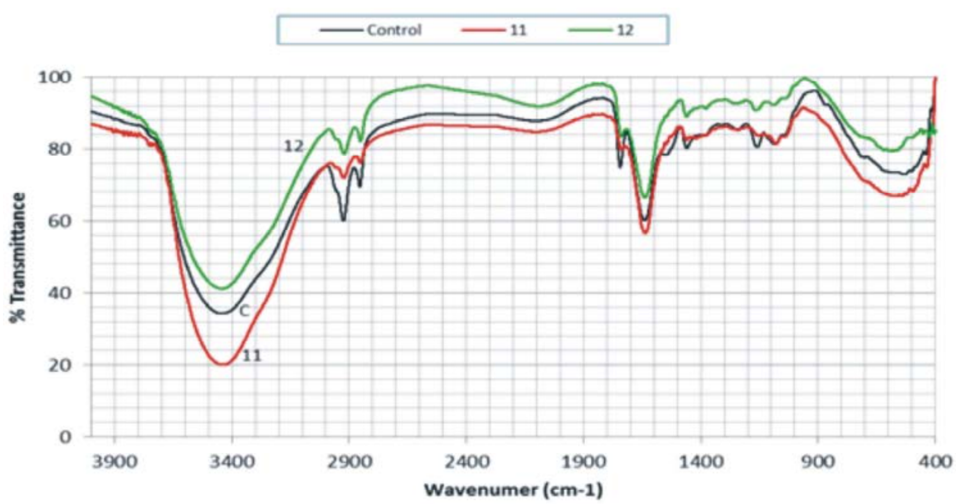

Fig. 3: Overlaid FTIR spectra of white cheese samples from supermarkets (Group-3) compared with the control

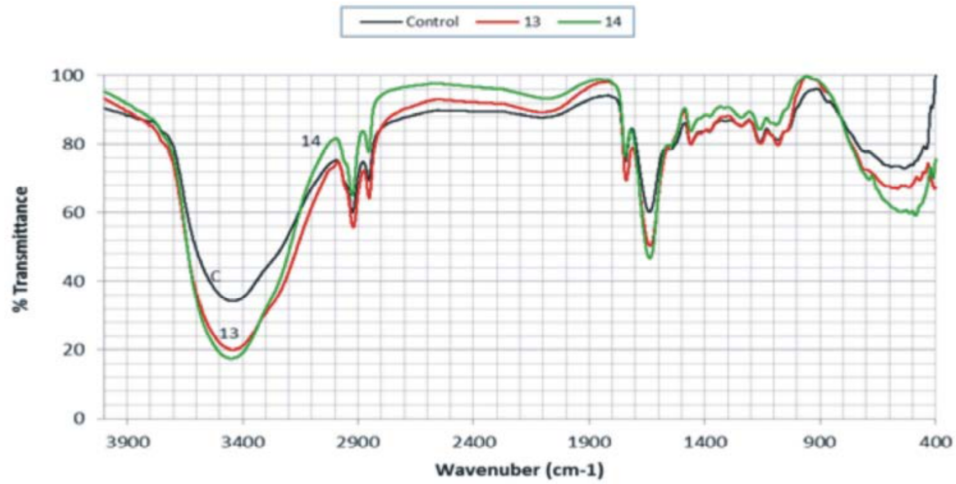

Fig. 4: Overlaid FTIR spectra of white cheese from well-known cheese factories (Group-4) compared with the control.

Table 1: Gross composition of white cheese collected from small producers (Group-1) compared with the control

	Parameters					
Cheese Sample	Moisture(%)	Fat (%)	pH	Protein(%)	Water-Soluble Nitrogen (%)	Ripening Index (%)
1	51.33^{b} ± 0.21	41.0^{b} ± 0.0	4.14^{e} ± 0.06	4.07^{d} ± 0.79	0.183^{b} ± 0.01	28.76^{ab} ± 7.63
2	49.26^{c} ± 2.21	43.0^{a} ± 2.0	4.26^{d} ± 0.01	5.05^{c} ± 0.84	0.196^{b} ± 0.01	24.85^{bc} ± 2.14
3	51.63^{b} ± 0.56	41.5^{b} ± 0.5	4.21^{d} ± 0.01	3.41^{e} ± 0.37	0.193^{b} ± 0.05	36.10^{a} ± 4.11
4	59.33^{a} ± 1.09	32.25^{c} ± 1.75	4.37^{c} ± 0.02	5.58^{b} ± 0.79	0.126^{d} ± 0.05	14.41^{de} ± 1.52
5	60.45^{a} ± 1.11	30.25^{d} ± 0.75	4.46^{b} ± 0.05	5.43^{b} ± 0.35	0.156^{c} ± 2.04	18.41^{cd} ± 1.41
Control	60.39^{a} ± 0.41	14.27^{e} ± 0.38	6.7^{a} ± 0.02	11.22^{a} ± 0.42	0.26^{a} ± 0.01	10.22^{e} ± 0.63

Data are mean values ± Standard Deviation, data obtained from three independent measurements.

Different letters within the same column are significantly different ($P \leq 0.05$).

Table 2: Gross composition of white cheese collected from vendors (Group-2) compared with the control

	Parameters					
Cheese Samples	Moisture %	Fat %	pH	Protein %	Water-Soluble Nitrogen %	Ripening Index %
6	45.53^{c} ± 1.11	41.00^{c} ± 1.0	6.75^{a} ± 0.07	9.23^{b} ± 0.29	0.267^{a} ± 0.01	18.44^{c} ±0.73
7	55.77^{b} ± 0.54	36.00^{d} ± 0.0	4.40^{b} ± 0.02	4.25^{d} ± 0.36	0.19^{b} ± 0.01	28.73^{a} ± 4.03
8	56.50^{b} ± 0.93	35.25^{d} ± 1.25	4.48^{b} ± 0.01	4.39^{d} ± 0.39	0.156^{d} ± 0.01	22.67^{b} ± 2.79
9	39.76^{d} ± 0.73	47.50^{b} ± 0.50	6.58^{a} ± 0.07	10.51^{a} ± 0.35	0.176^{c} ± 0.01	10.73^{d} ± 0.67
10	41.10^{d} ± 0.91	51.50^{a} ± 0.50	4.43^{b} ± 0.18	5.36^{c} ± 1.23	0.15^{d} ± 0.02	17. 86^{c} ± 4.01
Control	60.39^{a} ± 0.41	14.27^{c} ± 0.38	6.7^{a} ± 0.02	11.22^{a} ± 0.42	0.26^{a} ± 0.01	10.22^{d} ± 0.63

Data are mean values ± Standard Deviation, data obtained from three independent measurements.

Different letters within the same column are significantly different ($P \leq 0.05$).

Table 3: Gross composition of white cheese collected from supermarkets (Group-3) compared with the control

	Parameters					
Cheese Samples	Moisture %	Fat %	pH	Protein %	Water-Soluble Nitrogen %	Ripening Index %
11	58.13^{b} ± 0.75	18.75^{b} ± 0.25	4.46^{b} ± 0.04	14.93^{b} ± 0.56	0.156^{c} ± 0.01	6.67^{c} ± 0.01
12	40.92^{c} ± 2.13	38.33^{a} ± 1.15	4.26^{c} ± 0.02	15.12^{a} ± 1.11	0.196^{b} ± 0.01	8.27^{b} ± 0.01
Control	60.39^{a} ± 0.41	14.27^{c} ± 0.38	6.7^{a} ± 0.02	11.22^{c} ± 0.42	0.26^{a} ± 0.01	10.22^{a} ± 0.63

Data are mean values ± Standard Deviation, data obtained from three independent measurements.

Different letters within the same column are significantly different ($P \leq 0.05$).

Table 4: Gross composition of white cheese collected from well-known cheese factories (Group-4) compared with the control

	Parameters					
Cheese Samples	Moisture %	Fat %	pH	Protein %	Water-Soluble Nitrogen %	Ripening Index %
13	59.81^{b} ± 0.33	30.50^{a} ±0.35	4.49^{b} ± 0.01	6.27^{c} ± 0.21	0.28^{a} ± 0.01	28.48^{a} ± 0.55
14	64.98^{a} ± 0.0	14.75^{b} ± 0.0	4.23^{b} ± 0.0	9.37^{b} ± 0.0	0.253^{b} ± 0.0	17.24^{b} ± 0.0
Control	60.39^{b} ± 0.41	14.27^{b} ± 0.38	6.7^{a} ± 0.02	11.22^{a} ± 0.42	0.26^{b} ± 0.01	10.22^{c} ± 0.63

Data are mean values ± Standard Deviation, data obtained from three independent measurements.

Different letters within the same column are significantly different ($P \leq 0.05$).

acids and the moisture band can cause masking of the band of N-H which must be presented in the 3330-3060 cm^{-1} region. Another weak band representing water at 2065 cm^{-1} was observed clearly in some white cheese samples, same result was obtained by Kong and Yu [15].

Region B: in the interval of 3100-2800 cm^{-1} from the IR spectra presents the absorption bands which characteristic to the symmetrical and asymmetrical vibrations C-H stretching in fatty acids [13]. Figs 1-4 show sharp bands at 2900 cm^{-1} with different intensities for the different cheese producers, this could be attributed to the fat contents. Fat portion in cheese samples is represented by strong bands in the interval of 2873 – 2833 cm^{-1}. Similar results were obtained by Belton *et al.* [16] and Mendenhall [17], who reported the interval of 2950-2800 cm^{-1} present C-H bending and the intensity of these peaks differ from sample to other according to fat content. Tables 1-4 show significant differences ($P \leq 0.05$) in fat contents between the white cheese collected from different producers and the control cheese.

Region C: corresponds to the interval 1800-1600 cm^{-1} represents C=O of acids and esters and exhibited variations in the wave number range (1750-1650 cm^{-1}) consisting of absorbance from esters of fatty acids. This can be attributed to differences in the level rate of lipolysis at different stages of ripening [13]. This region in our results was in the range of 1740–1669 cm^{-1} as shown in Figs 1-4. Also, Tables 1-4 show significant differences ($P \leq 0.05$) in water soluble nitrogen (WSN) and the ripening index of white cheese related to the different producers and compared with the fresh control cheese.

Region D: corresponds to the interval 1600-1390 cm^{-1} represents Amide I and Amide II of proteins. This region in our results was in the range of 1770-1479 cm^{-1} as shown in Figs (1-4). Beltone *et al*. [16] and Wilson *et al.* [18] found peaks at ≈ 1650-1540 cm^{-1} which could be corresponding to the protein. While, Garland [19] found strong bands of H_2O in the range between 1650-1640 overlapped with protein amide É band at 1690-1620 cm^{-1}. Susi and Byler [20] reported that the secondary structure of protein was reflected in the IR spectrum due to absorbance in amide É region between 1620 and 1690 cm^{-1} primarily due to stretching vibrations of the carbonyl groups. The absorbance bands around 1635 cm^{-1} could be associated with beta-structure, while the bands close to 1653 or 1646 cm^{-1} were associated with the helical portions and random portions of the protein respectively. The gross composition of white soft cheese collected from different producers (Table 1-4) shows significant differences in the protein contents between the cheese samples and with the control.

Region E: corresponds to the interval 1390-1200 cm^{-1} represents esters and aliphatic chains of fatty acids. The bands between 1450-1410 cm^{-1} contain absorbance from acidic amino acids such as glutamic acid and the aliphatic chains of fatty acids [13]. This region in our results was in the range of 1361–1199 cm^{-1} with different intensities related to the white cheese collected from different producers.

Region F: corresponds to the interval 1200-800 cm^{-1} represents bands characteristic to the C-C links and to the vibration links C=O [13]. Subramanian *et al*. [3] mentioned that the spectral range 1800-900 cm^{-1} contains signals from polypeptides, amino acid, carbonyl groups of fatty acids, hydroxyl groups, carboxylic acid groups and fatty acid esters (typically short chain) and representing organic acids, alcohols, short chain fatty acids and their esters, amino acids and small water soluble peptides, this lead to visual comparison of the raw spectra showed numerous differences between organoleptic cheese sample especially in that region. Rodriquez-Saona *et al.* [21] reported that the range 1800-900 cm^{-1} was found to be important in the analysis of cheese flavor by FTIR and this region consists of signals from C-O and C=O (≈1175 cm^{-1}), C-H bending (≈1450 cm^{-1}), esters (1750-1700 cm^{-1}), C-O (≈1240 and 1170-1115 cm^{-1}). This region in our results was in the range of 1185-1153 cm^{-1} and

Table 5: Organoleptic properties of local white soft cheese collected from different markets in Cairo compared with the control sample

	Parameters			
Cheese Samples	Taste (10 points)	Color (5 points)	Texture (5 points)	Mouth feel (5 points)
Small producers	$5.3^{d} \pm 1.52$	$5.0^{a} \pm 0.57$	$4.0^{b} \pm 1.0$	$4.3^{b} \pm 1.15$
	$5.0^{d} \pm 2.00$	$5.0^{a} \pm 0.0$	$3.7^{b} \pm 0.57$	$4.7^{a} \pm 0.52$
	$4.0^{e} \pm 1.73$	$4.7^{a} \pm 0.0$	$3.7^{b} \pm 1.0$	$4.3^{b} \pm 0.15$
	$5.7^{d} \pm 0.30$	$5.0^{a} \pm 0.0$	$4.0^{b} \pm 0.57$	$4.3^{b} \pm 0.52$
	$6.3^{c} \pm 1.52$	$5.0^{a} \pm 0.0$	$4.7^{a} \pm 0.57$	$4.3^{b} \pm 0.57$
Vendors	$4.7^{de} \pm 3.05$	$4.7^{a} \pm 0.0$	$3.7^{b} \pm 0.15$	$4.3^{b} \pm 0.51$
	$6.3^{c} \pm 2.08$	$5.0^{a} \pm 0.45$	$4.0^{b} \pm 1.00$	$4.7^{a} \pm 0.57$
	$6.3^{c} \pm 1.52$	$4.7^{a} \pm 0.57$	$4.0^{b} \pm 1.00$	$4.3^{b} \pm 0.52$
	$8.3^{a} \pm 0.57$	$4.7^{a} \pm 0.95$	$4.3^{a} \pm 0.57$	$4.7^{a} \pm 0.85$
	$8.3^{a} \pm 0.57$	$4.0^{ab} \pm 0.0$	$3.7^{b} \pm 0.15$	$4.3^{b} \pm 0.15$
Supermarket	$5.7^{d} \pm 1.52$	$3.7^{b} \pm 1.15$	$3.0^{c} \pm 1.73$	$3.7^{c} \pm 1.52$
	$5.3^{d} \pm 1.52$	$3.3^{c} \pm 1.52$	$3.0^{c} \pm 1.78$	$4.3^{b} \pm 1.15$
Well-known Cheese factories	$7.7^{ab} \pm 1.15$	$4.7^{a} \pm 0.57$	$4.3^{a} \pm 0.57$	$4.3^{b} \pm 0.5$
	$7.3^{b} \pm 1.15$	$4.7^{a} \pm 0.57$	$4.3^{a} \pm 0.57$	$4.7^{a} \pm 0.37$
Control	$8.3^{a} \pm 1.52$	$5.0^{a} \pm 1.52$	$4.3^{a} \pm 1.73$	$4.7^{a} \pm 0.0$

Data are mean values ± Standard Deviation, data obtained from three independent measurements.

Different letters within the same column are significantly different ($P \leq 0.05$).

1030-631 cm^{-1} and contained many small peaks with different intensities which related to the flavor components in the white cheese samples. Table 5 shows significant differences in the taste and mouth feel scores of white soft cheese samples related to the producers.

Regarding to, the variations among chemical composition of white cheese samples collected from different markets and the control as shown in Tables 1-4. It could be noticed that the differences in the moisture contents could be due to the difference on protein contents, adding additives such as calcium chloride and the kind of vegetable oil in such cheeses and its ability of holding moisture. The differences in fat, protein and moisture contents might be attributed to the variations in initial total solids in milk and other raw mixture used for manufacturing the cheese. The high level of fat content in the cheese market could be related to the use of extra vegetable oils instead of milk fat to reduce the cost of cheese making because it is the cheapest fat in the market and such fat gives better properties of cheese texture. In addition to, the control cheese was dependent on fat percent in the initial milk used to manufacture the cheese. Regarding to pH values, there was a wide range of the pH values of all cheese samples which is related to the age of marketed cheese, increasing the level of milk fat substitution led to decrease in the pH value. Also, it may be attributed to the addition of Glucono Delta Lactone (GDL) as acidic substance during making these cheeses according to the manufacturer methodology. Water Soluble Nitrogen (WSN) fraction is commonly used in cheese as an index of ripening. The variation in ripening index in Market cheese samples and which was higher than the control, was possibly due to cheese age and the variation in manufacturing conditions [22].

CONCLUSION

- Fourier transform infrared spectroscopy the chemical composition of white cheese analyzed. However, further studies are need in order to predict the relation between the composition of white cheese and the FTIR absorption curves to determine the quantitative analysis between samples based on the FTIR spectra.
- Wide differences were found in the gross composition of white cheese collected from different sources mainly due different milk and other raw mixture used for cheese manufacture such as calcium chloride, vegetable oil, addition of some acidulants, and cheese maturation
- White cheese collected from the vendors (the cheapest cheese markets) obtained the highest taste scores due to adding cheese flavor or some additives to mask the possible cheese adulteration.

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