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Identification of a method for detecting and determining mixing palm oil with cream and detection of threshold using the chemical and machine test

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Due to the unique composition of milk fat, high prices and the increase in demand has always been the aim of manipulating and fraudulently substitutes. In order to identify a method for the detection of palm olein oil mixed with creamy fat for determining detection threshold, an experiment in a completely randomized design with a mixing ratio of 5, 10, 15, 25 and 50 percent of palm oil and cream was made in 2016. The results showed that the increasing of the amount of palm oil in the cream has significant effect 05/0 p < on the composition of fatty acids; increasing the proportion of mixing results in increasing of unsaturated fatty acids such as oleic acid and their total results in decreasing of fatty butyric acid as an indicator of dairy products and decreasing of the total of saturated fatty acids. The results showed that even with the addition of 50% palm oil to cream, fatty acid profiles is in accordance with the national standard within Iran. Adding the cream of 25 and 50 percent of palm oil fatty acid to cream the ratio of fatty acids C18: 2 / C8: 0 to 3 increased respectively about 3 and 7 times higher, and the ratio of C4: 0 / C16: 0, respectively reduced to about 3/2 and the half.

Keywords: fat of milk, mixing palm oil, chemical tests, fat of cream.

INTRODUCTION

Today a view to promoting nutritional value and proper diet is considered one of the key strategies to improve the health of the community. Meanwhile, milk and other dairies have particular value because milk is the most valuable nutrient and contains almost all the necessary ingredients for growth and survival of human. Milk is the only food that can supply more balanced human food needs [1].

Milk and its products have considerably nutritional value, especially in terms of protein and calcium and also has health benefits such as increasing digestibility of nutrients, improving immune system, anticancer activity and reducing blood serum cholesterol are the reasons why the consumption of milk and milk products in Iran and around the world is increasing [2, 3].

Nutritional quality of dairy products depends on the quality of fat moreover; the composition of milk fat is depends on the process and raw materials and carries the flavor [4]. Milk fat is a complex of natural fats and contains approximately 400 to 500 types of fatty acids [1].

Manipulation and changing the composition of milk and its products cause changes in the physical characteristics and quality of the products if carried out without notification to the consumer in terms of economic and nutrition it will cause in dissatisfaction and fraud [5].

Reducing the quality of the food is done in different ways. Such as mixing food with cheaper materials, replacing and changing the original material with other materials, the dairy industry is one of industries that the possibility of such cases is very high, especially replacing cheap vegetable oil instead of butter because of the price and high nutritional value, therefore the possibility of mixing milk fat with lower quality fats is more [6]. With regard to the special place of milk and dairy products in terms of nutritional value and increasing their consumption, detecting the tampering and fraud in the formulation of these products, especially to replace cheaper fat of vegetables with expensive fat is of considerable importance. According to the reports there was not a comprehensive study so far to provide detection threshold of mixing milk fat with vegetable oil and this project is to review various percentage of detection threshold and evaluate and present. Including as one of the rights of consumers, receiving particularly high quality food and with the expected nutritional value, with the proportional cost of pay, this study by investigating widely used samples on the market, assess the current status of milk, has collected reliable information and if there is a necessity will refer to authorities to be decided in this case [7].

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In this study, we believe that due to the recent crisis in the dairy industry of this country and speculation about mixing plant palm oil with fat milk, cream fat as a dairy product which has a significant amount of fat and the risk of fraud is more effective in terms of mixing with vegetable oil of palm olein, it is chosen for more studies and finally by comparing the results to the appropriate method of identification and detection threshold with the high confidence level the results have been presented.

Since the economic benefits of replacing cheaper vegetable oil with expensive milk fat in dairy product of cream is more likely because of the high percentage of fat content of these products we have used it in this study and with the addition of different amounts of palm oil (RBD) 1 to milk fat in cream and providing certain percentage of mixing we have examined the fatty acid profile, changes of Rascher micelle and Palnesk index, iodine index, the percentage of non-soap materials and, index refract meter index.

Multiple laboratory methods are used to identify fraud in the dairy industry, that the use of the device (GC) 1 is one of the most efficient methods. With this device, type and composition of fatty acids and sterols have been identified. Also by the chemical tests of iodine number, and Palnesk and Rascher micelle index, the percentage of non-soap and other chemical changes caused by the mixture has been reviewed and compared [8].

The overall objective of the research is as follows:

- Recognizing mixing of milk fat with vegetable fat
- threshold detection of vegetable oil mixed with milk fat
- estimating the percentage of mixture of milk fat with vegetable fat
- assessment of macro samples sterile packed light cream in the market

- Checking whether the samples analyzed comply with standard limits of the mixing of milk fat with vegetable oil will be approved or not.

The hypothesis of study is also given below:

- Checking the diagnosis and the percentage of palm oil in the cream instead of organic fat milk
- achieving a comprehensive method to detect fraudulent in use of palm oil in the cream
- insufficient implementation of the results of the analysis of milk fat with standard limits for approval of incorporation

A REVIEW OF EARLIER WORKS

In the study of O.B. Rudalcov et al at 2002 the milk fat were recognized by using chromatographic data. In this study the amount of fatty acids in milk fat, animal fat, vegetable fat and mixed fats were calculated by Capillary GC method. The concentrations of acids such as myristic, palmitic, stearic, oleic, linoleic and linolenic in fat milk were considered as natural markers. For detection, the transformation of additives (modifiers) with variable chemical composition in a product were examined the amount ranging from similar components formulation and the results [9]. In 2005, Celia Domeno et al have proposed a new method to determine sterols in serum samples. This method of extraction by (SPME) 1 in the head space and the presence of more derivative elements such as trimethylsilyl and triflorestamid was used [10].

In a study by B. Canabate- Diaz et al. in 2007 seven-sitosterol in olive oil with HPLC- MS (APCI) 1 positively identified and measured. The samples were soaped and non-soaped part were extracted with diethyl then this part condensed and put under thin layer chromatography (TLC) 2 and was placed on silica gel plates. Then the sterols bar were isolated and extracted with methanol then measured and were identified by LC-MS 3 [11]. In a study by I. Borkoucova et al. in 2009 cholesterol levels in some dairy products and, stigmasterol, sitosterol in butter and margarine with Rp HPLC method examined and analyzed with Capillary GC columns parallel. Before the chromatographic analysis in the stage of soap and extraction of non-soap residue the hexane was used. HPLC and GC methods were compared in this study [12].

Fathi Til et al. in 2012 carried out a study titled as margarine diagnosis in butter to assess the effectiveness of detection methods of purity of butter. Examples of butters as pure bovine, margarine, mix butter and margarine mixtures of 5, 10 and 15% margarine - butter were prepared and subjected to tests to determine purity of butter, including the number of iodine, refractive index, the Rascher micelle and Palnesk index and devices methods include gas chromatography and high efficiency liquid chromatography respectively to determine the fatty acid profile and tökol were used to diagnose the added amount of margarine [13].

Increasing the margarine in butter has significant effect on the refractive index, the index of Rascher micelle and Palnesk. Also it resulted in the increasing of percentage of unsaturated fatty acids, of oleic and linoleic and reducing saturated fatty acids of Myristic, palmitic although these index and

fatty acid profiles, even with the addition of 15% margarine to butter was in the range of consistent with the national standard of Iran. Adding 10 and 15% of margarine to butter has increased fatty acid C18: 2 / C8: 0 to approximately 1.5 and 2.5 and the proportion of C14: 0 / C18: 2 were reduced to about half. Also, with increasing margarine to butter up to 15% in addition to viewing plant Tökөls -β + γ-tocopherol and -β + γ tocotrienols, the amount of these tökөls with -α-tocopherol and total tökөl also significantly has increased, so that fraud detection in 5% of vegetable fats in milk fat by analysis of tökөl can easily be possible [14].

In 2011, Doreta Derewiaka et al have analyzed fraud in animal butter with vegetable fats. The mixture of fatty acids and sterols has been obtained by using GC-MS, Profile triacylglycerol and tocopherol by HPLC method with detectors of DAD 2 and fluorescence and finally, the most efficient methods to the analysis of sterols and tocopherols were introduced [15]. In 2012, Jang-Hyuk Ahn et al have introduced a quick way to determine the amount of cholesterol in emulsion foods containing milk. Quick and easy sample preparation which contains being soaped without heating, separation and cleaning was with method of DSPE 2, in this method the invention and then sample preparation by HPLC with UV detector was used to determine the values (73). In2013, Reyhanselin Uysal et al have studied and analyzed fraud in butter by using margarine with Raman spectroscopy method and chemometrics method. Different samples with percentage of blends 100-0 were tested and this method was introduced as a diagnostic method for testing fraud in butter [16]

RESEARCH METHODS

Summary of sampling and stages of research

In this study, the produced cream was provided from Pegah company cited with repeated visits of inspectors of Food and Drug Department and confirmation of officials of quality control of natural and free from added vegetable fat cream, with similar series of production were purchased, as well as to provide mixings, the palm olein was prepared from a company in Dezful, Iran.

First we extracted fat of cream and mixed with palm olein at a ratio of 25,15,10,5 and 50 percent. In the first stage of research Palnesk and Rascher micelle index was investigated. The second stage by using a refractometer, the refractive index was measured. The third stage involves measuring the iodine number by WIJS method. The fourth step was to measure the percentage of non-soap. The fifth step involves identifying the structure of the fatty acids of the samples by GC with injecting into the device. The sixth step was extracting sterols and identification of sterols collection of samples by GC with injecting into the device.

Sample preparation

To perform the test, creamy fat directly extracted and replaced with vegetable oil of palm olein and integration of operations carried out. Sample preparation for injection into the gas chromatograph was carried out to determine the fatty acids according to national standards 4090 and 4091 [17, 18].

Fat extraction and preparation method of mixing

Fat extraction from cream has been done by using chloroform / methanol. For 30 g cream, 200 ml of chloroform / methanol (2: 1) was added and for one hour placed on a shaker plate, resulting mixture straightened and 25 ml of NaCl saturated to it; chloroform phase (lower) was collected into a sandblaster lid erlen which was completely dry and clean then was passed from filter paper containing sodium sulfate. At the end the straightened filtrate placed under vacuum rotary to evaporate the solvent at 35 ° C. the extracted fat in order to provide the percentage of mixtures were used in this study (Figure 1). In this case, the extracted fat and palm olein is heated to the temperature of melting point by bain marie method then by w/w mixing is done and the palm olein with 50,25,15,10,5 percent ratios was replaced with fat cream. Finally, creamy fat, palm olein oil and the composition of the mixture were used for tests in this study [19].

Method of measuring the refractive index

This test was done in accordance with National Standard No. 5108, the extracted fat samples was called an absolute number by the filter paper and the refractive index in temperatures of 40 and 50 ° C by refractometer heater with approximate 0.0001. [20]

Analysis methodology of fatty acids

Sample preparation for injection into the gas chromatograph to determine the fatty acids was carried out according to national standards 4090 and 4091 [17, 18]. Gas chromatograph conditions for injection of samples for identification and measurement of fatty acids is presented in Tables 1 and 2.

Table 1. Conditions of gas chromatography for the Analysis of fatty acids

device name	GC (Gas chromatography)
The detector	FID
Flow rate	Hydrogen mL/min 350mL/min air : 30:
Injection volume	µL1
Column characteristics	m100 µm×0/25mm × 0/25 ، capillary column،CP-SIL 88
Temperature injection site	ċ260
Temperature of the detector	ċ260
Carrier gas	mL/min1/5:، Nitrogen
Application	Measurement of fatty acids in edible oils and fats

Table 2. the temperature plan for fatty acid analysis

Initial temperature (℃)	First time (Minutes)	Speed of temperature rise (min /Degree)	Secondary temperature (℃)	Speed of temperature rise (min /Degree)	End temperature (℃)	End time (Minutes)
80	5	15	150	3/2	240	10

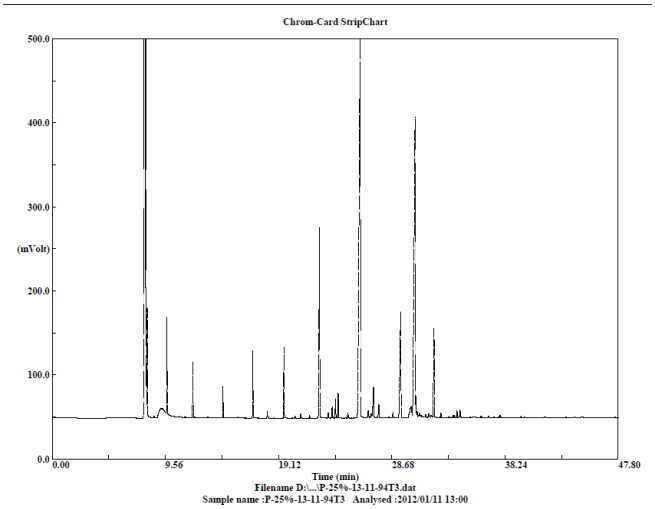


Fig. 1. Analysis of fatty acids in sample mixing 25%

EXPRESSION OF RESULTS AND ANALYSIS

This section states the results of tests obtained from refractive index, profile of fatty acids in palm olein oil, extracted fat of cream, dried and mixed with palm oil and fat cream in ratios (5, 10, 15, 25, 50) Percent Paid.

For all samples the tests were performed three times and the standard deviation were calculated. Numbers obtained from triplicate were close to each other and the resulted standard deviation was very low.

Identification and determination of fatty acid composition

As a result of analysis of samples by gas chromatography, 27 types of fatty acids were separated and their amounts were determined. Peak detection was carried out by comparing the obtained chromatogram with the standard chromatogram and values were expressed in fatty acid percent. The results in the table below is in seven sample preparation are represented with the separation of fatty acids indicator, The represented numbers are the results of triplicate and the resulted mean is obtained from test of determining the fatty acids by gas chromatography.

Butyric acid (C4: 0)

Butyric acid as indicator fatty acids, particularly in milk was investigated and the results are in the table below.

Table 3: The amount of butyric acid (C4: 0) separately in different treatments

sample	(C4)1	(C4)2	(C4)3	Average	Standard deviation
Palm olein	0	0	0	0	0
cream	4/25	4/15	4/17	4/19	0/030
Mixing 5%	4/08	4/10	4/13	4/10	0/025
mixing 10%	3/16	3/22	3/17	3/18	0/018
mixing 15%	2/78	2/71	2/73	2/74	0/020
mixing 25%	2/52	2/68	2/59	2/59	0/08
mixing 50%	1/96	1/89	1/92	1/93	0/020

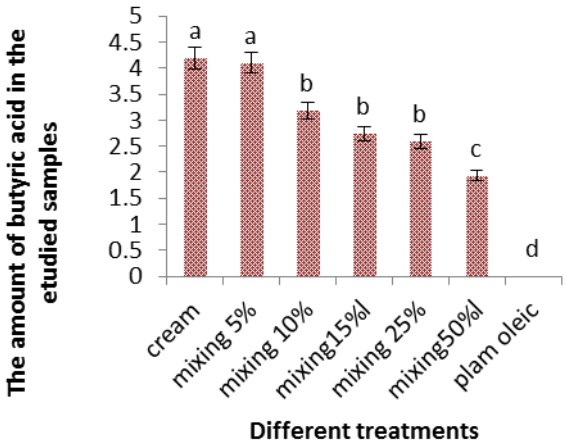


Fig. 2. Comparison of butyric acid in the different treatments

Analysis showed an increase in percentage of vegetable oil in fat of cream, causes in decrease of Butyric acid as, the maximum amount of cream fat and minimum value in mixing of 50% was observed, and as expected, the amount of this fatty acid was zero in palm olein.

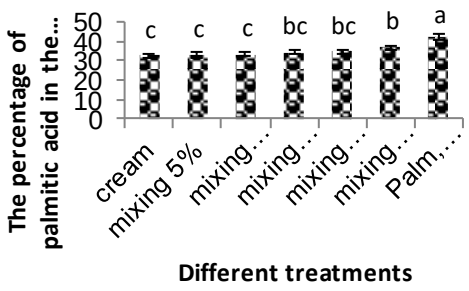
Palmitic acid (C16: 0)

It is including of saturated fatty acids, that has the highest amount in milk fat (% 32.77) and as this fatty acid is one of the most abundant fatty acid in palm oil (% 42.32), fluctuations in prepared mixings was evaluated in the table below:

Table 4: The amount of palmitic fatty acid (C16: 0) separately in the different treatments

samples	(C16:0)1	(C16:0)2	(C16:0)3	avarage	Standard deviation
Palm oleic	42/28	42/34	42/34	42/32	0/034
cream	32/69	32/86	32/78	32/77	0/085
mixing 5 %g	33/23	33/21	33/17	33/20	0/030
mixing 10%	33/63	33/57	33/56	33/58	0/037
mixing 15%	34/42	34/36	34/40	34/39	0/030
mixing 25%	34/88	34/78	34/85	34/83	0/051
mixing 50%	37/03	36/90	36/93	36/95	0/068

Fig. 3. Comparison of palmitic acid in different treatments



Analysis of samples showed by increasing the percentage of mixing vegetable oil with creamy fat, the palmitic acid is increased too, so that the least amount of cream fat and maximum amount of it was observed in the50% mixture.

Oleic acid (C18: 1):

It is the abundant unsaturated fatty acids in dairy products. In this study, the amounts of fat in the cream were measured (%18.69) and in the used vegetable oil in the mixture (% 40.83), changes in amounts of these fatty acids in different mixings is in the table below:

Table 5: The amount of oleic acid (C18: 1) separately in the different treatments

sample	(C18:1)1	(C18:1)2	(C18:1)3	average	Standard deviation
Palm olein	40/91	40/76	40/82	40/83	0/075
cream	18/75	18/63	18/71	18/69	0/061
mixing %5	20/51	20/50	20/45	20/48	0/032
mixing %10	22/43	22/40	22/36	22/39	0/035
mixing %15	24/30	24/26	24/21	24/25	0/045
mixing %25	25/39	25/51	25/45	25/45	0/060
mixing %50	31/12	31/04	31/08	31/08	0/040

Analysis of samples showed by increasing the percentage of vegetable oil with cream fat, oleic acid increased, so that the least amount of it was observed in cream and highest amount of it in the mixing 50% that the used vegetable oil (palm olein) has a rate of two times more than cream fat containing oleic acid. This increase is also expected in terms of theory.

Short-chain fatty acids:

As one of the most important aspects of milk fat is high amount of short-chain fatty acids (C4-C10) the variations of these fatty acids are presented in the table and graphs, it should be noted that the values was in percent and the average is of three replications.

Table 6: Values of short-chain fatty acid separately in different treatments

sample	(C4:0)	(C6:0)	(C8:0)	(C10:0)	(C12:0)
Palm olein	0	0	0/018	0/019	0/2
cream	4/19	2/39	1/40	3/09	3/5
5 %mixing	4/10	2/24	1/24	2/74	3/06
10% mixing	3/18	1/84	1/07	2/35	2/76
15% mixing	2/74	1/69	1	2/24	2/63
25% mixing	2/59	1/57	0/89	1/99	2/31
50% mixing	1/93	1/06	0/60	1/32	1/61

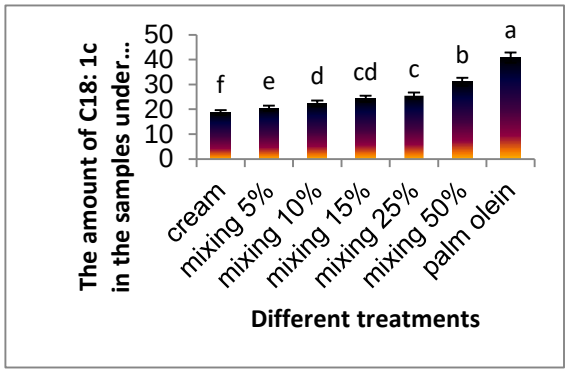


Fig. 4: Comparison of oleic acid in different treatments

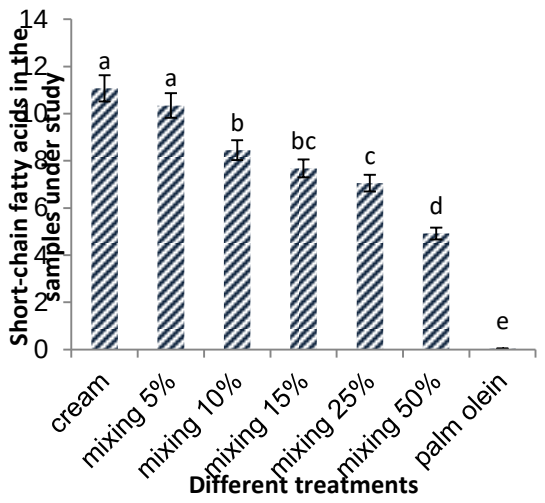


Figure 5: Comparison of the total of short-chain fatty acids in different treatments

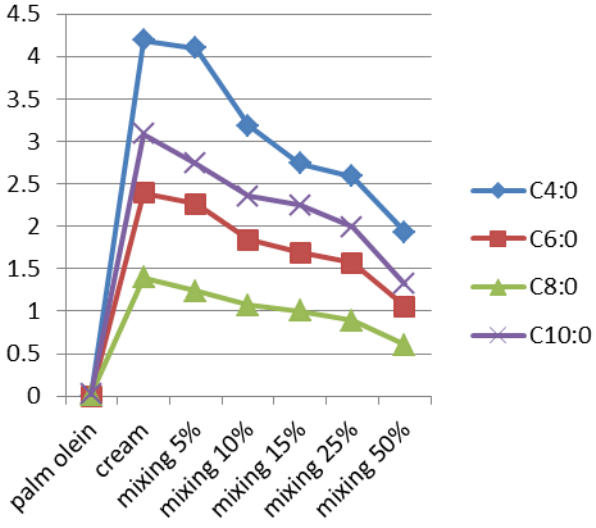


Figure 6: Comparison of changes in the sort-chain fatty acids separately in different treatments

According to figure 8 the results shows that at the same time by increasing the amount of vegetable oil in different treatments, total amounts of polyunsaturated fatty acids increase and the amount of saturated fatty acids fell.

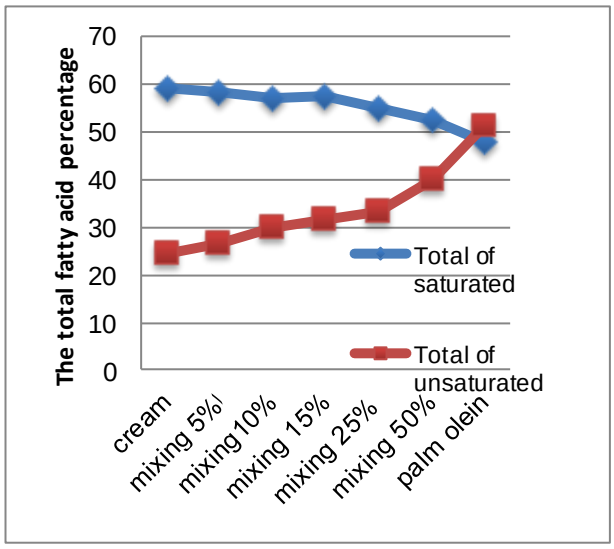


Figure 7: Comparison of saturated and unsaturated fatty acids in different treatments

Measuring the refractive index

The results of measuring the refractive index of the test samples at two temperatures 40 and 50 degrees are in the table (7) below and the chart comparison, for all samples was performed three times and the standard deviation were calculated and the obtained numbers from triplicate are close together and the resulted standard deviation was very low. The results showed an increase in the percentage of mixing in both temperatures and thus reduce the total saturated fatty acids results in increase of the refractive index.

DISCUSSION AND CONCLUSION

The results of the analysis of fatty acids

According to previous discussions the type and amount of fatty acids in the extracted fat of cream, vegetable oil, palm olein and five types of fat cream treated with vegetable oil in amounts of 5, 10, 15, 25 and 50 percent, with three times preparation analyzed. The mean and standard deviation were calculated.

Table 7: Results of refractive index at temperatures of 40 and 50 degrees in the different treatments

Sample	The refractive index at 40 °	Standard deviation	The refractive index at 50 °	Standard deviation
Palm olein	1/45864	0/0001	1/45864	0
Cream	1/45393	0/0002	1/45017	0/0001
5 %mixing	1/45421	0/0003	1/45049	0/0002
10% mixing	1/45454	0/0004	1/45071	0/0004
15% mixing	1/45467	0/0002	1/45101	0
25% mixing	1/45517	0/0003	1/45153	0/0004
50% mixing	1/4563	0/0002	1/45289	0

Short and medium chain fatty acids

These fatty acids can contain short-chain butyric acid (C4: 0) and the fatty acids of 12 carbon, in accordance with national standards, usually in oils with plant origin, usually fatty acids less than 10 carbon does not exist and their values are zero while the most important feature of milk fat, have high levels of short-chain fatty acids (4 to 10 carbons) [21]

In the presence of short-chain fatty acid such as butyric acid (% 4.19) caproic acid (% 2.39), caprylic acid (% 1.4) and capric acid (% 3.09) in cream fat and lack of the palm olein this claiming is sure that the replacing of plant fat instead of milk fat reduces the concentration of these fatty acids in the mixture and this reduction was of the amount of vegetable fat had been added so that the increase of vegetable oils, the concentration of short chain fatty acids decreased as the same proportion.

The results showed (Figure 6) mixing of 5% didn't cause in any significant difference in the total short chain fatty acids while the replacement of 10, 15, 25 and 50 percent caused in a significant difference and it is concluded that the effect of mixing vegetable oil instead of fat cream of replacing 10% and higher is visible. As the amount of butyric acid from 4.19% in the case of blending 5% cream with small change in replacing 10% up to 3.18% and finally in mixing 50 % up to 1.92 is declined. The results show that despite reducing the amount of short-chain fatty acids by increasing the percentage of mixing, the amounts of fatty acids, butyric, caproic and caprylic was not out of the standard limit (No. 191), but the replacement of 50% of the amount of capric and lauric acid was removed out of standard limit.

Other fatty acids

The results showed that despite of reducing the amount of saturated fatty acids by an increase in the percentage of mixtures, saturated fatty acid of palmitic that is the overcome fatty acids in the palm olein and the amount of which is more than fat of cream increased with increasing replacement. Oleic acid in palm olein was significantly higher than other samples and adding palm olein to the cream the amount of this fatty acids increased [9, 14].

As the results show in Section 4 fatty acid composition of cream in the vast majority of cases, even with the addition of 50% of palm oil is not out of the national standard limit. To increase the sensitivity analysis of fatty acids, several different ratios of fatty acids were evaluated as an indicator of purity. It was observed that the addition of 25 and 50 percent of palm oil to the fatty acid C18: 2 / C8: 0 respectively resulted in increase of approximately 3 and 7 fold and the ratio of C4: 0 / C16: 0 to approximately 2/3 and half reduced. Therefore, these ratios may be used as an indicator of fraud detection.

Refractive index

Including physical properties of oils is refractive index and its value is different for different types of oils. In recognition of degree of purity it is useful. Factors such as the degree of saturation, fatty acid composition, chain length, oxidation and behavior of oils in heating are affective therefore the values of the coefficient indicates the presence of larger amounts of unsaturated fatty acids. The results of the study indicate that there is a direct relationship between the amount of mixing percentage and refractive index.

In this study, the refractive index was studied at two temperatures 40 and 50 degrees, in both temperatures the increase of refractive index was in line with increase in the percentage of mixing. The result of analysis of fatty acids in the sample under study indicates that the increase in total unsaturated fatty acids was in line with the increase in vegetable oil with animal fat replacement.

Overall conclusion:

In this study, control samples containing extracted fat of cream and vegetable oil, palm olein as well as samples treated with 25,15, 10,5 and 50% fat of cream in terms of fatty acid profile, profile sterols, iodine number and Palnesk index, the percentage of non-soap materials and refractive index were analyzed and the following results were obtained:

In the study of fatty acids even with 50% mixture, except for fatty acids of capric and lauric none of the others had minor deviations from the standard limit, while essentially a 50% replacement because of adverse effects of quality and rheological seem inapplicable. In order to explore the replacement of animal fats with vegetable oils, fatty acids studied and compared with standard a limit alone which is not enough to confirm the final product. Adding of 25 and 50 percent of palm oil to cream cause in increase of fatty acids C18: 2 / C8: 0 to 3 and 7 times higher, respectively, and the ratio of C4: 0 / C16: 0 reduced to approximately 3.2 and half. Therefore, these ratios may be used as an indicator of fraud detection.

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