Application of Oil Fractionation in Butter Blend Making

Nadia A. Abou-zeid

Dairy Science and Technology Department, Faculty of Agriculture, Menoufia University, Shebin El-kom, Egypt
nadia_abou_zeid@yahoo.com

Abstract: Cotton seed oil (S) was fractionated into high (F1) and low freezing *point* fractions (F2) by the dry fraction procedure at 0°C. Cream was combined with S, F1 or F2 at 50, 40 and 30% of the fat amount in cream. Butter blends were evaluated when they have just been taken out of the refrigerator (refrigerator temp.) or one hour after they have been taken out of the refrigerator. Results illustrated that high freezing *point* oil fraction (F1) at the ratio 40% is the best chosen for making butter oil blend, it taste and perform like butter but are spreadable and soft right from the refrigerator and at ambient temperature. However butter blended with whole cotton seed oil (S) or with low freezing point fraction (F2) had undesirable excesses softness texture at ambient temperature. Blending butter with cotton seed oil or one of its fractions resulted in significant increase ($P \leq 0.01$) in total unsaturated, polyunsaturated, omega (3 & 6) fatty acids and decrease in $C_{16:0}$ and $C_{18:1}$ trans-fatty acid. Furthermore F1B contain the lowest content of saturated fatty acids such as Palmitic and stearic acids,and had lower atherogenic index(AI) than F2B. Also, it had more omega -6 and omega -3 than F2B. Whereas texture profile analysis shows SB, F1B and F2B were softer and less springiness, adhesive, gummy, resistant to chew than control. In conclusion, it is feasible to produce healthy butter blends with the best sensorial, instrumental textural and spread properties,easier to use at both refrigerator and ambient temperatures and more convenient than ever, by blending cream with F1 in a ratio 40% of fat in cream.

[Nadia A. Abou-zeid. **Application of Oil Fractionation in Butter Blend Making.** *Life Sci J* 2014;11(11s):738-745]. (ISSN:1097-8135). http://www.lifesciencesite.com. 163

Key Words: butter, nutritional properties, blends, cotton seed oil fractions

Abbreviation key: CB =control butter – SB, F1B, F2B butter blends of whole, high and low freezing point fraction cotton seed oil, respectively.

1.Introduction

Coronary heart disease is a leading cause of death in America (American Heart Association, 2000) and most industrialized countries (Gurr, 1995). In 1997, cardiovascular diseases were responsible for 41.2% of all deaths in the US. Dairy products, in particular butter, have been considered to increase the risk for cardiovascular diseases in humans because, in comparison to other lipid sources, they contain a higher proportion of lauric, myristic, and palmitic acids and a lower proportion of unsaturated fatty acids (Ulbricht and Southgate (1991), Noakes *et al.* (1996), Roche *et al.* (2001) Khosla and Fungwe (2001), Sacks and Katan (2002), Terpstra (2004) and Fox and Mc Sweeney (2006).Therefore recent trends have been shifted away from saturated animal fat toward more unsaturated vegetable fat and oil. Concerns have also encouraged food manufacturers to reformulate products to replace saturated fat. (Anette *et al.*, 2010).

In addition to the previous nutritional limitations of butter, it also has several undesirable functional attributes like hardness and poor spread ability at refrigeration temperature that due to the high ratio of saturated fatty acids (Edmondson *et al.*,1974; Taylor and Norris, 1977).

Blending milk fat with vegetable oils is used to modify the nutritional properties of the butter products Furthermore, it enhances the physical properties of the blended systems. (Rousseau 1996 and Mohammed *et al.* (2011). Butter blends carry a flavour similar to that of butter and the product is softer at low temperatures, which results in easy spreadability directly from the refrigerator, but texture of butter oil blend is often critical as time advanced little after it have been taken out of the refrigerator, hens it showed undesirable excess soften as time advanced little after it have been taken out of the refrigerator.

Therefore, the objective of the current study was to test whether the use of oil thermal fractions instead of whole oil in manufacture butter oil blends is capable to produce butter oil blends without texture defects after some time it have taken from refrigerator.

2. Material and methods

Material:

Cotton seed oil 100 % fully refined, (Produced by Extracted oils & derivatives Co., Alexandria, Egypt), was thermally fractionated into two fractions using a process of dry fractionation described by Fox and Mc Sweeney (2006), using freezing process with agitation at 0°C.

Buffalo's Cream was standardized to 40% fat, pasteurized, and cooled to13 º C and left in the refrigerator to next day. The cream was then divided into ten batches. The first batch was kept without adding oil to used as control, the other batches were put in three groups each of three batches. The appropriate amount of oil or one of its fractions was mixed with each batch at a ratio of 60, 40, or 30% of the fat in cream. However the first group included blends of cotton seed oil (S) the second group included blends of high freezing point cotton seed oil fraction (BF1) and the third group included blends of low freezing point cotton seed oil fraction (BF2). Then each patch was churned to butter with electric churns as described by Nadia (2009).

Initial sensory evaluation:

Fifteen panelists used Star charts/diagrams to carry out the initial sensory evaluation. Butter were analyzed when they have just been taken out of the refrigerator (24 hours after production) to select the best ratios of cotton seed oil/ or its fractions to make desirables blend butters. Six attributes that describe the characteristics of the butter were chosen, which are: melting, spread ability, smoothness, softness, richness of taste and creamy flavors. After the blends samples had been tasted, the panelists decided on the quality for each attribute, using the following point quality scores as described by Rady and Badr (2003):

9=excellent, 7=good, 5=fair, 3=poor and 1=extremely poor.

Control Butter and blends were made again with the ratio which have been selected in the Initial sensory evaluation and Butter were analyzed when they have just been taken out of the refrigerator (24 hours after production) these butters were analyzed when they have just been taken out of the refrigerator as well as after one hour they have just been taken out of the refrigerator The analysis performed on the following:

Fatty acid composition:

We employed HPLC using a modified method of Liu *et al.* (1993) to separate fat. Fatty acids were methylated as described by Park and Goins (1994). Fatty acid methyl esters were injected by an auto sampler into a Hewlett-Packard 5890A gas chromatograph with a flame ionization detector (Hewlett-Packard, Sunnyvale, CA). The column was a fused silica capillary SE30 length 25 meters, diameter 0.25 m. Helium was the carrier gas. The column temperature program was: initially isotherm at 140 ^{0}C for 10 min, an initial programmed rate of 1^{0}C/min up to 160 ^{0}C, then a second rate of 2^{0}C/min up to 220 ^{0}C and a final isotherm for 15 min. Samples were injected into the split mode.

Texture profile analysis:

Texture analyzer CNS – (The Farnell, England) was used. The probe was TA_{14} (45^O angle and 25 mm diameter) at speed of 1 mm/ second and 10 mm distance in butter. Samples (50g) of 15.5 ^{0}C were placed into glass Petri dishes (1.3cm×.1.5cm deep). Probe was set to penetrate the samples to a depth of o.4 cm.

Sensory evaluation:

Butter blends were evaluated when they have just been taken out of the refrigerator (samples of refrigerator temp) or one hour after they have been taken out of the refrigerator (samples of ambient temp).

The panelists consisted of ten expert members using the following 9-point quality scores as described by Rady and Badr (2003):

9=excellent, 7=good, 5=fair, 3=poor and 1=extremely poor.

Statistical analysis:

Factorial designed of three replicates and the completely Randomized Design were used to analyze all the data. Newman Keuls Test was followed to make the multiple comparisons (Steel and Torrie, 1980) using Costat program. The evaluation was based on 1 % significance levels.

3. Results and Discussion

Initial sensory evaluation:

Because of the documented beneficial health effects, there is a potential niche market for dairy products with higher unsaturated fatty acids (Ulbricht and Southgate 1991; Noakes *et al.,* 1996; Sacks and Katan, 2002). The aim of our study was to incorporate the highest amount of oil in butter in order to obtain the highest nutritional benefits, but unfortunately, the increase in the proportion of oil in butter caused the butter to be excess softer and loose its texture when it kept in room temperature for a little time, Therefore initial sensory evaluation were performed on various blends to select the highest ratios of oil or its fractions that can be used for making butter blends without causing any objectionable softness which may compromise the acceptability of blends.

Preliminary sensory evaluation for butter blends at refrigerator temperature,showed that as the level of cottonseed oil or its fractions increased to greater 2:3 ratio w /w (oil: fat in cream) objectionable softness happened. The use of 40% ratio w /w (oil: fat in cream) resulted in a great improvement in butter blend quality, as well as overcoming all control butter defects such as crumbly texture, high resistance to spread & and moisture migration to the surface of butter. in the other word 40% ratio w /w is the highest possible combination which could be used to improve the textural and sensorial properties of butter.

Fatty acids profile:

Fatty acids profile Table 1 clearly supports that blend oil or one of its fraction with butter improving the nutritional quality of the resulted blends. The following observations explain that:

All butter blends showed a great increase in polyunsaturated, and a slight decrease in monounsaturated fatty acids compared with the control butter.

Control butter had the highest percentage of saturated fatty acids (67.38) and lowest one for unsaturated (32.62) Table 1: F1B had significance lower saturated fatty acid values than F2B. Palmitic acid was the major SFA in control (30.6% of total fatty acids) was decreased to 26.9, 24.6 and 28.1 % in SB, F1B and F2B, respectively; Stearic acid was 15.22 % in control decreased to 11.59, 10.71 and 12.68% in SB, F1B and F2B, respectively. Modification of the fatty acid profile in milk fat to yield lower saturated fatty acid content and greater polyunsaturated fatty acid content has been a major research focus for the dairy industry. Fatty acid profile can be modified through physial blends of milk fat with vegetable oils, (Rousseau 1996$_a$ and Mohammed *et al.* (2010).

The reduced saturated fatty acid content could act in concert with reducing the ratio of LDL: HDL and of total cholesterol: HDL.

Blending oil with milk fat would not only simply increase unsaturated fatty acids but also alter a type of geometric isomerism in unsaturated fatty acids ($C_{18:2n6c}$ fatty acids). The increased levels of cis-fatty acids can have a detrimental influence on human health (Zock and Katan, 1992). Additionally, SB, F1B and F2B contained approximately 6.3, 5.9 and 4.9- folds higher concentrations of ω−6 fatty acids (omega-6 fatty acids) compared with CB. It was observed that F1B had more amount omega -6 and omega-3 than F2B. On the other hand ω−3 fatty acids were absence in CB and were0.35, 0.69 and 0.40 in SB, F1B and F2B, respectively.

Fox and Mc Sweeney 2006 mentioned that the increase in concentration of omega -6 and omega -3 fatty acids is an important factor in human health issues, because these are essential fatty acids which cannot be synthesized by mammals and present at low concentrations in milk fat.

Inclusion of high melting point fraction F1 resulted in marked increase in short chain fatty acids content. C4:0 to C8:0 which were approximately ^{two} fold higher in (F1) B compared with the control. Earlier studies showed that fatty acid chain length affect the melting properties of fats (Vasic and McMan, 1968; Walstra, 1987).

The blends samples had lower atherogenic index (AI) than control butter, they were 2.3, 1.2, 1.3 and 1.5 for CB, SB, S(F-1)B and S(F-2)B, respectively. It was obvious that F1B samples had lower AI than F2B. Consumption of dairy products with lower (atherogenic index) decreases the concentrations of total and low-density lipoprotein cholesterol in blood plasma in comparison to consumption of commercial dairy products (Noakes *et al.,* 1996; Poppitt *et al.,* 2002).

(CB) contain the highest content of $C_{16:0}$ (30.6. %), the corresponding values for F1B, F2B, SB were 24.59, 28.10, 26.93. %, respectively. The decrease in concentration of $C16_{16:0}$ is an important factor in human health issues, because high $C_{16:0}$ content of dietary related to increase in low –density lipoprotein (LDL) cholesterol in plasma, Denke and Grundy, 1992.

Accounting for differences in *trans* C18:1 of control and experimental blends (Table 1) indicated that CB contained 1.3, 1.3 and 7.8 times more than that in SB, F1B and F2B, respectively. Obviously blends have been considered to decrease the risk for cardiovascular diseases in butter consumers, because (Mensink and Katan, 1993) mentioned that clinical trials have shown that *trans* C18:1 increases LDL-cholesterol and decreases the HDL-cholesterol, thus, producing an unfavorable affect on LDL –HDL ratio.

Texture profile analysis:

Significant differences ($p < 0.01$) of butter texture profile analysis at both room and refrigerator temperatures are shown in Table (2) and Figure (1). Texture properties significantly improved at room and refrigerator temperatures ($P \leq 0.01$) by blending either whole oil or oil fractions. At room temperature samples of r by 47, 36 and 52 %, by 50,16 and 12 %, more spreadable (less spring) by 12, 7.0 and 26 %, less cohesive by 35, 20 and 47 %, less gummy by 77, 64 and 85 % and less resistant to chew by 74, 62 and 80 %, respectively than CB samples. Reddy *et al.* (1996) reported that in blending the harder fat dissolves in the softer fat, resulting in eutectic interactions. Rousseau *et al.*(1999) mentioned that when milk fat was blended with canola oil, changing in hardness index was observed.

At refrigerator temperature Table (2) and Figure (1), samples of SB, F1B and F2B were softer by 44, 31, and 48%, less adhesive by 30, 5 and 2%, more spreadable (less spring) by 20, 14 and 24 %, more cohesive by 43,15, 59%, less gummy by 37, 41 and 31 % and less resistance to chew by 36, 31and 43 %, respectively than were butter samples of the control.

Sensory evaluation (Sensory properties):

Sensory evaluation at refrigerator temperature Table(3) and Figure (2) showed a narrow variability of texture attribute of various blends, however there were no significant differences for other sensory attributes (appearance and flavor),but there were significant differences between control and all experimental butter blends. CB had the lowest scores for appearance and texture attributes,it was grainy, lumpy, brittle behaves like solid, has poor spread ability, slow melting in the mouth, and had faded or dull colour, other wise all blends tasted and performed like butter but were more spreadable and softer right from the refrigerator, they were also smoother, more homogeneous,. melted quickly in the mouth and were shiny products. Blend F1B received the highest texture rating. Unexpectedly, intensity and sharpness of fresh cream aroma and flavor were the same in control CB and all experimental butter blends but flavor appeared quicker in the later.

At room temperature the results of sensory evaluation Table (3) and figure 2 showed that blend S(F-1)B was significantly ($p < 0.01$) better than CB in attributes of color, texture, spread ability and smoothness. However, Blend F1B was highly accepted by panelists in terms of the acceptance level of appearance, texture and overall acceptability, it has the highest organoleptic scores. There were no significant differences between texture of SB and F2B but there were significant differences between both of them and F1B in attributes of softening where the formers showed undesirable excessive softening.

Panelists reported that neither cottonseed oil nor its fractions changed the aromatic profile of butter. On the contrary, the absence of them kept the undesirable specific pale colour of buffalo's butter, however high differences in colour between all experimental butter blends and the control were visually observed. The control butter was dirty pale white, while all experimental butter blends seemed shiny slightly bright yellow;

Finely, it was obvious that F1 B was easier to use and more convenient than ever at both refrigerator and ambient temperatures.

Table (1): Fatty acids profile of control butter and butter oil blends

Fatty acids	CB	SB	F1B	F2B
$C_{4:0}$	ND	ND	4.8	ND
$C_{6:0}$	2.6	1.8	3.1	2.4
$C_{8:0}$	2.4	1.5	2.4	2.0
$C_{10:0}$	2.0	1.4	0.54	0.62
$C_{12:0}$	2.3	0.20	1.7	1.0
$C_{13:0}$	10.5	ND	ND	ND
$C_{14:0}$	ND	7.8	7.4	8.8
$C_{14:1\,n9c}$	1.4	1.6	0.90	1.5
$C_{15:0}$	1.3	0.91	0.83	0.89
C 16: 0	30.6	26.9	24.6	28.1
$C_{16:1\,n9c}$	1.6	1.4	1.4	2.1
$C_{17:0}$	0.64	0.79	0.48	0.54
$C_{18:0}$	15.2	11.6	10.7	12.7
$C_{18:1\,n9t}$	2.1	1.7	1.7	0.27
$C_{18:1\,n9c}$	23.7	23.0	21.0	23.2
$C_{18:2\,n6c}$	2.9	18.3	17.1	14.3
$C_{20:1}$	0.88	0.36	ND	0.37
$C_{18:3\,n3}$	ND	0.35	0.69	0.40
$C_{20:0}$	ND	0.69	0.83	0.90
$C_{22:0}$	ND	ND	ND	ND
$C_{22:1\,n9}$	ND	0.37	ND	ND
Summation	100.00	100.00	100.00	100.00
Short –chain C_4-C_8	4.9^a	3.3^a	10.2^c	4.5^a
Medium chain C_{10}-C_{12}	4.4^a	1.6^b	2.3^c	1.6^b
Long-chain C_{14}-C_{18}	61.7^a	40.0^b	27.1^c	42.2^b
Saturated	67.4^a	53.3^b	57.3^b	58.0^b
Unsaturated	32.6^a	46.7^b	42.7^b	42.5^b
Omega 6	2.9^a	18.3^b	17.1^b	14.3^c
Omega 3	ND^a	0.35^b	0.69^c	0.40^b

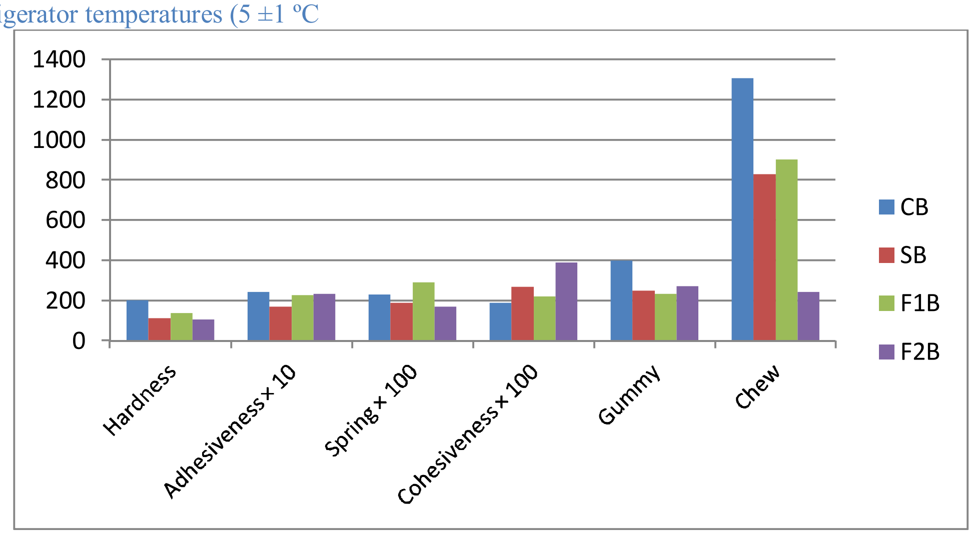
Replicate of samples (n) =3, Statistical evaluation was based on a 1 % significance levels.
Abbreviation key: C =control butter – SB, F1B, F2B butter oil blends of whole cotton seed oil, high, low melting point fractions cotton seed oil.

Table (2): Instrumental texture profile of control butter and butter oil blends samples at refrigerator (5 ±1 ºC) and ambient (25 ± 1ºC) temperatures

Samples	Hardness	Adhesiveness	Spring	Cohesiveness	Gummy	Chew
(g/sec)	(g/sec)			(g/sec)	(g/sec)	
At refrigerator temperatures (5 ±1 ºC)						
CB	202.7[a]	24.2[a]	2.3[a]	1.9[a]	398.1[a]	1306.5[a]
SB	113.9[b]	16.9[b]	1.9[b]	2.7[b]	250.6[b]	829.2[b]
F 1B	139.4[c]	22.8[c]	2.9[c]	2.2[c]	234.9[c]	900.9[c]
F 2B	105.4[d]	23.5[a]	1.7[b]	3.9[a]	273.0[d]	743.3[d]
At ambient temperatures (25 ± 1ºC)						
CB	184.2[a]	24.5[a]	0.8[a]	3.0[a]	584.3[a]	417.2[a]
SB	96.3[b]	22.5[b]	0.7[a]	1.9[b]	132.5[b]	108.5[b]
F 1B	116.3[c]	20.2[c]	0.8[a]	2.4[c]	210.5[c]	155.8[c]
F 2B	87.4[d]	21.8[c]	0.6[a]	1.5[b]	86.4[d]	82.2[d]

- Abbreviation key: C =control butter – SB, F1B, F2B butter oil blends blend butters of whole cotton seed oil, high melting, low melting fractions cotton seed oil, respectively
- Replicates of samples (n)=3,evaluation was based on a 1 %significance levels.
- a, b, c and d means with the same letter among treatments are not significantly different (*P*≤0.01).

At refrigerator temperatures (5 ±1 ºC



At ambient temperatures (25 ± 1ºC)

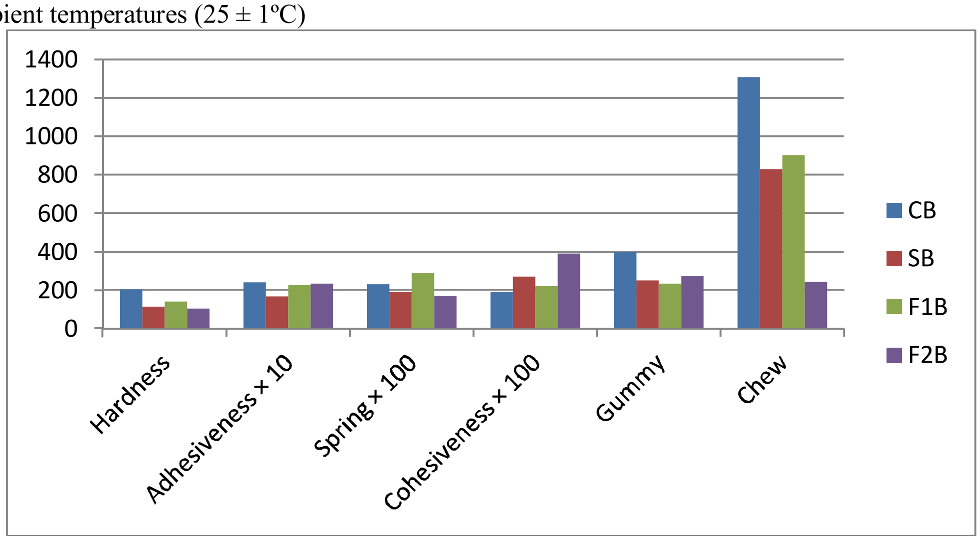


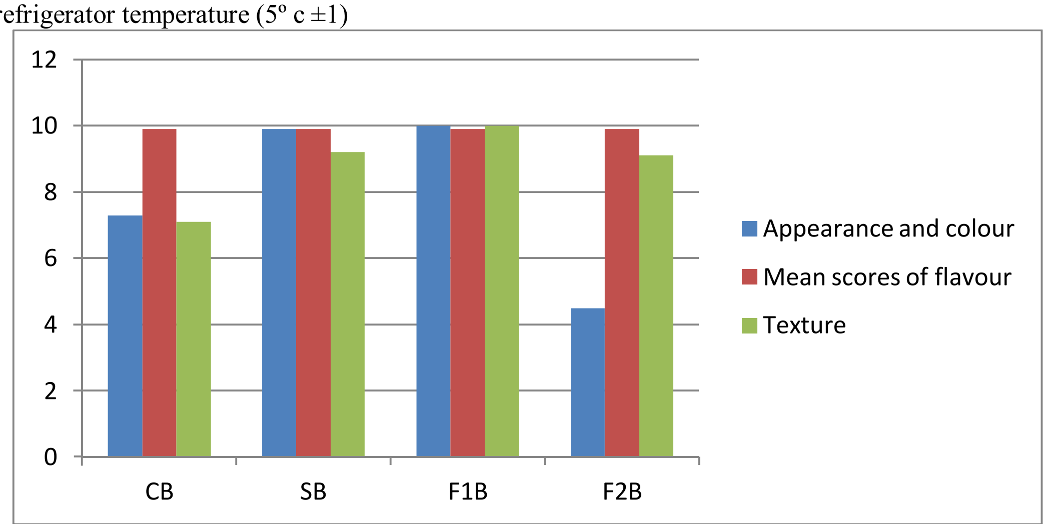
Figure (1) Instrumental texture profile of control butter and butter oil blends at refrigerator (5 ±1 ºC) and ambient (25 ± 1ºC) temperatures

Table (3): Organoleptic evaluation for butter oil blends samples at refrigerator (5 ±1 °C) and ambient (25 ± 1°C) temperatures

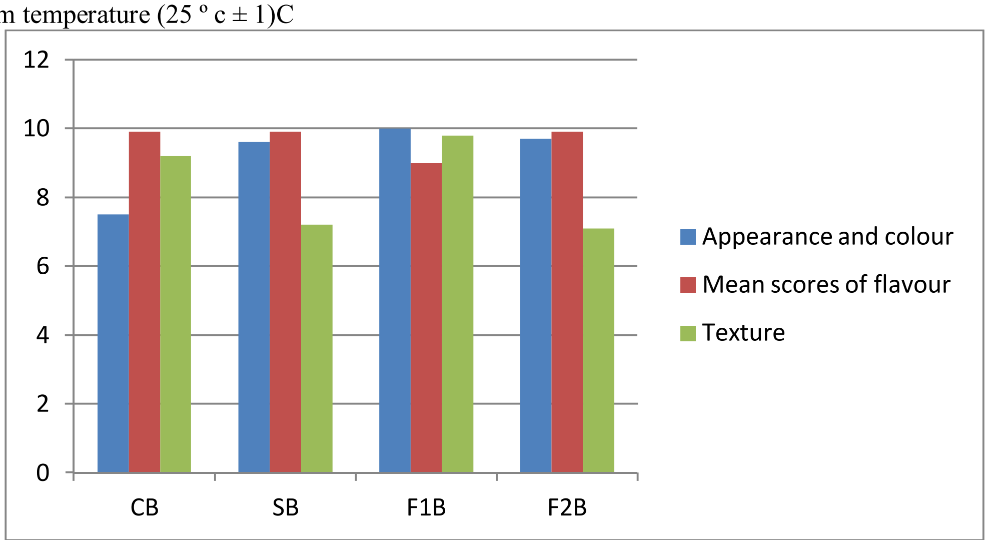
Butters	Appearance	Mean scores of	Texture
and colour	flavour		
At refrigerator temperature (5° c ±1).			
CB	7.3 ^{a}	9.9^{a}	7.1^{a}
SB	9.9 ^{b}	9.9^{a}	9.2^{b}
F1B	10.0 ^{b}	9.9^{a}	10.0^{c}
F2B	9.9 ^{b}	9.9^{a}	9.1^{b}
At room temperature (25 ° c ± 1)			
CB	7.5^{a}	9.9^{a}	9.2^{a}
SB	9.6^{b}	9.9^{a}	7.2^{b}
F1B	10.0^{c}	9.9^{a}	9.8^{c}
F2B	9.7^{b}	9.9^{a}	7.1^{b}

- Abbreviation key: C =control butter – SB, SF-1B, SF-2B blend butters of whole cotton seed oil, high melting, low melting fractions cotton seed oil, respectively
- Replicates of samples (n)=3, evaluation was based on a 1 and 5 % significance levels.
- a, b, c and d means with the same letter among treatments are not significantly different (*P*≤0.01).

At refrigerator temperature (5° c ±1)



At room temperature (25 ° c ± 1)C



Figure(2): Organoleptic evaluation for control butter butter oil blends at refrigerator (5 ±1 °C) and ambient (25 ± 1°C) temperatures

Conclusions

Blending of cream with either cottonseed oil or one of its fractions in 40% ratio w /w (oil: fat in cream) resulted in production butter blends with a modified quality compared with control butter, however F1B is the most beneficial for the elimination of textural defects generally seen in butter or butter oil blend From nutritional point of view F1B. offers added advantages because it had the highest percentage of unsaturated fatty acids, polyunsaturated fatty acids, ω−6 fatty acids and lowest monounsaturated fatty acids, AI (therogenic index) making it more desirable than both control butter and other experimental butter blends from nutritional point of view. F1B also, offers added advantages in textural, instrumental and sensory properties of butter that consumers desire at ambient and refrigerator temperatures because it taste and perform like butter but are spreadable and soft right from the refrigerator and at ambient temperature, but SB and F-2 B offer these advantage at the refrigerator temperature only and as time advanced after samples have been taken out from refrigerator by an hour only they exhibited excess softness and lost their texture.

Therefore, our study concluded that the use of high freezing point cotton seed oil fraction F1 in 40% ratio w /w (oil: fat in cream) to produce modified butter blend suitable for use at both ambient and refrigerator temperatures.

Butter samples from cows with a more unsaturated milk fatty acid composition had a lower atherogenic index, and the butter samples were more spreadable, softer, and less adhesive. Thus, phenotypic variation in milk fatty acid composition among cows fed the same diet is sufficient to produce butter with different textural properties.

References

1. American Heart Association. Cardiovascular disease statistics. Heart and Stroke Guide. 2000. http://www.americanheart.org/. Accessed April 17, 2001.
2. Anette E, Victoria F, Elena R *et al.* (2010) Modifications in Dietary Fat Quality Are Associated with Changes in Serum Lipids of Older Adults Independently of Lipid Medication Journal Nutrition. Vol. 140, No. 1, 88-94.
3. Denke, M A, Grundy S M (.1992) Comparison of effects of lauric acid and palimitic acid on plasma lipids and lipoproteins. Am. J. Clin. Nutr. 56:895–898.
4. Edmondson, L F, Yoncoskie RA, Rainey NH *et al.* (1974). Feeding encapsulated oils to increase the polyunsaturation in milk and meat fat. J. Am. Oil Chem. Soc. 51:72–76.
5. Fox P F, Mcsweeney PLH (2006) Advanced dairy chemistry. Volume 2 Lipids. Third Edition. Springer.com. U. S. A.
6. Gurr M I(1995) Nutritional significance of lipids. Pages 349–402 in Advanced Dairy Chemistry: Lipids, P. F. Fox, ed. Chapman & Hall, Great Britain.
7. Illingworth, DR, Ullmann D(1990) Effects of omega-3 fatty acids on risk factors for cardiovascular disease. Pages 39–69 *in* Omega-3 Fatty Acids in Health and Disease. R. S. Lees and M. Karel, ed. Marcel Dekker, Inc., New York, NY.
8. Liu, J., T. Lee, E. Bobik, M. Guzman-Harty, and C. Hastilow. 199 3.Quantitative determination of monoglycerides and diglyceridesby high-performance liquid chromatography and evaporative light-scattering detection. J. Am. Oil Chem. Soc. 70:343–347.
9. Khosla, P, Fungwe TV(2001) Conjugated linoleic acid: Effects on plasma lipids and cardiovascular function. Curr. Opin. Lipidol. 12:31–34.
10. Mensink R. P, Katan, MB (1993) Trans monounsaturated fatty acid in nutrition and their impact on serum lipoprotein levels. Prog. LIPID Res., 32,111-122.
11. Mohammad I. Khan, M. R. Asha, K. K. Bhat, Sakina Khatoon (2011). Studies on chemical and sensory parameters of coconut oil and its olein blends with sesame oil and palmolein during wheat flour-based product frying. Journal Food Science and Technology Volume 48, Issue 2, pp 175-182.
12. Nadia A. Abou -Zeid I (2009) Basic of dairy industry. Pages 254–265, Modern Knowledge Library, Egypt.
13. Noakes M, Nestel P J, Clifton PM (1996) Modifying the fatty acid profile of dairy products through feedlot technology lowers plasma cholesterol of humans consuming the products. Am. J. Clin. Nutr. 63:42–46.
14. ParkP W, Goins R E(1994) *In situ* preparation of fatty acid methyl esters for analysis of fatty acid composition in foods. J. Food Sci. 59:1262–1266).
15. Poppitt S D, Keogh G F, Mulvey TB, *et al.* (2002) Lipid-lowering effects of a modified butter-fat: a controlled intervention trial in healthy man. Eur. J. Clin. Nutr. 56:64–71.
16. Rady AH., Badr HM. 2003. Keeping the quality of cows' butter by Y–irradiation. *Grasas y Aceites* 54, 410-418.
17. Reddy SY, Full N,DimickPS, Ziegler GR(1996) Tempering method for chocolate containing milk –fat fractins. J. Am. oil Chem. Soc. 73,723-727.

18. Roche H M, Noone E, Nugent A, Gibney M J (2001) Conjugated linoleic acid: A novel therapeutic nutrient? Nutr. Res. Rev. 14:173–187.
19. Rousseau D, Forestiere Hill AR., Marangoni AG (1996a) Restructuring butterfat through blending and chemical interesterification. I. Melting behavior modifications. J. Am. Oil. Chem. Soc. 73,963-972.
20. Rousseau, D, Forestiere Hill AR, Marangoni AG (1999). The effects of interesterification on physical and sensory attributes of butterfat and butterfat-canolaoil spreads. Food Res. Int. 31,381-388.
21. Sacks, F M, Katan M (2002) Randomized clinical trials on the effects of dietary fat and carbohydrate on plasma lipoproteins and cardiovascular disease. Am. J. Med. 113:13S–24S.
22. Same-Bali O, Ayadi M.A (2009). Traditional Tunisian butter: Physicochemical and microbial characteristics and storage stability of the oil fraction LwL- Food science and technology, volume 42 issue 4 pp 899-905.
23. Steel, R.G.D. and J.H. Torrie. 1980. Principle and Procedures of Statistics. A biometrical approach. 2nd Edit. McGraw-Hill Book Co., NY.
24. Taylor, M W, Norris R (1977) The physical properties of dairy spreads. N.Z. J. Dairy Sci. Technol. 12:166–170.
25. Terpstra, A H M (2004) Effect of conjugated linoleic acid on body composition and plasma lipids in humans: An overview of the literature. Am. J. Clin. Nutr. 79:352–361.
26. Ulbricht TLV, Southgate DAT (1991) Coronary heart disease: seven dietary factors. Lancet 338:985–992.
27. Vasic I, De Man TM (1968) Rheology and texture of food stuffs.Soc.Chem Ind.Monoger.27:251.
28. Walstra, P (1987) Fat crystallization, page 67, Food Structure and Behavior. J.M.V. Blanshard and P. Lillford, ed, Academic press, New York NY.
29. Zock, P L, Katan M B (1992) Hydrogenation alternatives: Effects of *trans* fatty acids and stearic acid versus linoleic acid on serum lipids and lipoproteins in humans. J. Lipid Res. 33:399–410.

12/1/2014