

APPLICATION OF FAT FRACTIONATION IN TRADITIONAL EGYPTIAN RAS CHEESE

Nadia A. Abou-zeid^{1*} and Awad S²

¹Dairy Science and Technology Department, Faculty of Agriculture, Menoufia University, Shebin El-Kom, EGYPT

²Dairy Science and Technology Department, Faculty of Agriculture El -Shatby, Alexandria University, EGYPT

ABSTRACT

Ras cheese was manufactured from a mixture of skim cows and buffaloes milk of equal amounts, standardized at 5 % fat by anhydrous milk fat (A cheese), anhydrous milk fat fraction of a low or a high melting point (H cheese) and (L cheese), respectively. Cheeses were analyzed for sensory evaluation, texture profile, yield, chemical composition, ripening indices and fatty acid profile. Samples varied in hardness between hard (L cheese), less hard (A cheese), firm /typical (H cheese). H cheese received the highest sensory scores, yield, as well as the best texture profile and nutritional properties.

Received on: 27th-July2015

Revised on: 27th-Sep-2015

Accepted on: 7th-October-2015

Published on: 2nd-Nov-2015

KEY WORDS

Ras cheese; Oiling off defect; Fat fractionation

*Corresponding author: Email: nadia_abou_zeid@yahoo.com

INTRODUCTION

Ras cheese is known as the most popular Egyptian hard cheese. It is similar to the Greek variety "Kefalotyri" [1]. Cheese is considered a soft solid material consisting of a network composed mainly of protein, water, and lipid [2]. The mechanical properties of cheese are related to network composition, structure, and interactions among molecules within the network [3-5]. Although the utilization of milk fat fractions provides potential benefits in a variety of products; and unique properties are imparted by fractions optimized for their use [6-7] no work has been done yet in this field in Ras cheese. The oiling-off at room temperature in summer, excess hardness, dryness and the lack of springy, flexible firm body and smooth texture are the most common defects that may be found in Ras cheese and may be contributed to fat. Fat functionally modification of the properties of milk fat can be performed by fractionation. Thus, modification of Ras cheese properties by incorporating milk fat fractions was the main purpose of this investigation. Fractionation of milk fat can be done by several methods that separate the triglycerides of milk fat [8].

MATERIALS AND METHODS

Butter was melted at 60° c and filtered through Whatman number 1 filter paper under vacuum to remove protein and other materials. The obtained anhydrous milk fat (AMF) was separated and then fractionated using a dry fractionation process using the method described by Grall and Hartel [9]. This method included a two-step process: during the first step, the anhydrous milk fat was fractionated at 25.5 °c to produce a high melting point (solid) fraction, during the second step, the solid fraction obtained from the 25.5°c fractionation was refractionated at 35° c to produce a higher melting point (more solid) fraction which is referred as high melting point fraction. Liquid fractions obtained at 25.5° c and 35° c were combined together to be referred as low melting point fraction. Part of the same lot of anhydrous milk fat (AMF) was used as it is.

Cheese making

Ras cheese was manufactured from a mixture of fresh cows and buffaloes milk of equal amounts obtained from the herds of the Faculty of Agriculture, Menoufia University. Four batches of milk were prepared for cheese making. The first batch of milk without any addition standardized at 5% fat C milk, the other three batches were skimmed and standardized at 5% fat (A, H and L milk) using one of the following:

- 1) Anhydrous milk fat, (A milk)
- 2) High melting point (solid) anhydrous milk fat fraction, (H milk)
- 3) Low melting point (soft) anhydrous milk fat fraction, (L milk).

Cheese making was carried out by the method described by Nadia [10] using the previously prepared milk to manufacture control cheese (C), anhydrous milk fat cheese (A), high melting point fat fraction cheese (H) and low melting point fat cheese (L) with the same respect mentioned above in milk preparing.

Methods of cheese analysis

Cheese was analyzed after 5 months of storage as following:

Sensory evaluation: Taste panel of 20 persons was chosen from the staff and assistants at the department of Dairy Science, Faculty of Agriculture, Menofiyia University. The panelists scored the cheese according to the description of Foegeding and Drake [11]. The texture was classified into soft, firm/typical, hard and very hard.

Reological analysis: The texture of Ras cheese was qualified by texture analyzer CNS – (The Farnell, England). The probe was TA17 (with angle 30° and 25 mm in diameter) using a speed of 1 mm/ second and a distance 10 mm in cheese. Cheese samples were cut into cubes 3cm³ and kept at 12° c for 1 hour before analysis.

Cheese yield: The cheese yield was calculated as kg cheese /kg milk. Cheese that obtained the highest organoleptic scores was subjected to the rest of analysis

Chemical composition and ripening indices determination: The cheese samples were analyzed for moisture, acidity, fat, total nitrogen and soluble nitrogen according to Ling [12], total free amino acids nitrogen was assessed according to the method of Chebotarev and Veltsova [13] with slight modification as adopted by Nasr [14]. Soluble tyrosine and soluble tryptophane were determined as outlined by Vakaleris and Price [15]. Total volatile fatty acids were determined by the method described by Kosikowski [16]

Fatty acids profile analysis: Fat was extracted from cheese samples according to the method of Bligh and Dyer [17]. 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) Looor [18].

Statistical analysis: Factorial design of three replicates and the completely randomized design were used to analyze all the data. Newman Keuls test was then used to make the multiple comparisons using Costat program. Evaluation was based on a 1% and 5% significance levels.

RESULTS AND DISCUSSION

Sensory evaluation

Supplementary Table –1 shows the sensory evaluation. The texture of the samples was described by panelists and classified into hard cheese (samples C and L), semi hard cheese (sample A) and semi soft & typical /firm cheese (sample H). The data obtained demonstrated that cheese of 5 months age gained equal or higher scores compared with cheese of 6 months age, therefore we shall discuss only the organoleptic data for cheese at 5 months age only. Samples (C and L) were characterized by the highest hardness and crumbling under compression forces. When they were cut with a knife into layers and left at summer room temperature (35°c) for 12 hours, they exhibited oiling-off defects. These samples also showed the least flavor piquancy. They received total organoleptic score of 75 and 73, respectively.

Sample (A) also showed oiling-off defects when left for 12 hours at summer room temperature (35°c) after being cut into layers. It showed moderate flavor piquancy. It received total organoleptic score of 81. Sample (H) exhibited spring flexible firm body, smooth texture and piquant flavor. It was pliable with no fractures observed when compressed in hand. It possessed high degree of elasticity. It did not exhibit oiling-off defects when left at summer room temperature 35°c for 12 hours after being cut into layers. It received the highest total organoleptic score 98.

Organoleptic scores significantly increased by the replacing milk fat with anhydrous milk fat (A cheese) or high melting point anhydrous milk fat fraction (H cheese), while it significantly decreased by the replacing milk fat with low melting point anhydrous milk fat fraction (L cheese).

Cheese yield

Supplementary Table –2 shows the yield of cheese at both fresh and ripe states. Cheese yield significantly increased by replacing milk fat with anhydrous milk fat (A cheese) or high melting point anhydrous milk fat fraction (H cheese), while it significantly decreased by the replacing milk fat with low melting point anhydrous milk fat fraction (L cheese). This may be due to the applied of higher melting point fraction in Ras cheese making could be a tool to modulate the oiling –off which causing an increase in cheese yield.

Reological characteristics

Supplementary Table –3 shows the reological characteristics of the Ras cheese samples as affected by modified milk fat. The average of hardness for (C) and (L) Ras cheese samples was 982 and 932 g respectively. Sample (A) was hard but less than (C) and (L) samples, its average hardness value was 812. Sample (H) recorded the lowest hardness value with average of 621 g. It is clear from **Supplementary Table–4** that there is a great difference between C and H cheese in consistency. H cheese had the lowest consistency. Therefore, there is a positive relationship between hardness and consistency. The difference in springiness between C and L cheese was insignificant, but it was significant between C, A and H cheese ($P \leq 0.01$). The H cheese had the highest springiness value because the higher melting point fraction yielded a higher viscosity which causing an increase in springiness and the lower melting point fraction yielded a larger amount of free oil which causing a decrease in springiness. Significant differences were noticed between control and treatment samples in chewiness. H cheese had the lowest value. C cheese had chewiness value about one and a half times more than H cheese. A positive relationship was found between hardness and chewiness, these results agree with that reviewed by Katsiari et al, Bryant et al. and Fox et al. [7, 22, 23]. There is a positive relationship between either fracturability or Cohesiveness and hardness of Ras cheese. H cheese had the lowest fracturability value.

Very significant differences were noticed in Adhesiveness between H cheese and all other cheese samples including C cheese. There were also significant differences between C cheese and other treatments. L cheese had the highest value, so there is a negative relationship between hardness and adhesiveness.

Chemical composition

Moisture, acidity, salt/water, fat/D.M. values were higher for H cheese ($P \leq 0.01$) than for the corresponding control cheese (Table 4). This may be attributed to the ability of the cheese curd to maintain the high melting point fraction of anhydrous milk fat to a greater extent than milk fat, this is because milk fat contains low melting point fat fraction which was completely melted during the process of cheese manufacturing and thus allowing a lot of it to be lost in whey. Results also showed that pH and T.N./ D.M. had opposite trend to moisture, acidity, salt/water, fat/D.M. values. Lower N/D.M. % in H cheese is attributed to its higher fat content compared with C cheese.

Ripening indices

The ripening indices of the cheese at both fresh and 5 months age are shown in **Supplementary Table –5** for S.N./T.N.%, T.F.A.A.N %, T.V.F.A, S.tyr and,S.trp. H cheese had higher values than C cheese. This may be due to higher moisture content in H cheese which stimulates microbial and enzymatic activities leading to more protein decomposition and fat hydrolysis [24].

Fatty acid profiles

Supplementary Table –6 shows the fatty acid profiles for H and C cheese. Distinct differences were found between them: C cheese contained more saturated fatty acids than H cheese. C cheese contained one and a half times more short chain (C4:0 to C8:0) and medium-chain (C10:0 to C12:0) fatty acids than H cheese. The proportion of lauric acid (C12:0) in H cheese is 63.96% of its proportion in C cheese. Ulbricht and Southgate [25] proposed an atherogenic index (AI) for lipids as a dietary risk indicator for cardiovascular disease. The AI is the sum of the proportion in the fat of lauric and palmitic acids and four times myristic acid divided by the proportion of the total unsaturated fatty acids. H cheese had more long-chain saturated fatty acids (C16:0 to C18:0). The most distinct difference between the two cheese samples in the long chain fatty acid content was the amount of

C18:0, which increased from 13.24 in C cheese to 16.39 in H cheese. Cholesterol showed great decrease in H cheese compared with C cheese. Shukla et al. [19] reported that HMT (high melting triglyceride) butter offers the advantages of lower cholesterol and saturated fat contents. However, the contents of C8 and C10 fatty acids were also reduced.

CONCLUSION

Modified cheese (H) exhibited modifications in the nutritional and functional properties compared with the control one (C). Lauric acid (C12:0) content in H cheese is 63.96% of its content in the control cheese. H cheese had more unsaturated fatty acids than C cheese. The high solid fat content of H cheese has led to greater emulsion stability at elevated temperatures. This increased stability was reflected in higher texture scores for H cheese. The improved texture and emulsion stability eliminated the oiling-off to cheese surface at 35°C.

Therefore, cheese made from high melting point fat fraction shows good potential for use at ambient and higher temperatures at which the control cheese normally exhibits problems such as oiling-off, hardness and crumbling. Additionally, storage cost of this modified cheese would be lower because the product is stable at room temperature and does not require refrigeration to overcome the problem of oiling-off at the cheese surface. A lower cholesterol and saturated fat content of the product offer added advantages that consumer's desire.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

ACKNOWLEDGEMENT

None.

FINANCIAL DISCLOSURE

No financial support was received to carry out this project.

REFERENCES

- [1] Phelan JA, Renaud L and Fox PF. [1993] Some non European cheese varieties .In Cheese: Chemistry, Physics and Microbiology.2.Major cheese groups.Chapman and Hall ,London, p 421-465.
- [2] Walstra P. [2003] Physical Chemistry of Foods. Marcel Dekker, Inc., New York, NY.
- [3] Lucey JA, ME Johnson, aDS Horne. [2003] Perspectives on the basis of the rheology and texture properties of cheese. *J Dairy Sci* 86:2725–2743.
- [4] Siew Kim Lee, Henning Klostermeyer, Skelte G Anema. [2015] Effect of fat and protein-in-water concentrations on the properties of model processed cheese ,*International Dairy Journal* 50: 15–23.
- [5] Stocco G, Cicolat-Gotet C, Cecchinato A, Calamari L, Bittante G. [2015] Milk skimming, heating, acidification, lysozyme, and rennet affect the pattern, repeatability, and predictability of milk coagulation properties and of curd-firming model parameters: A case study of Grana Padano . *J Dairy Sci* 98:5052–5067.
- [6] Hartel RW. [1996] Applications of milk fat fractions in confections.*J Am Oil Chem Soc* 73:945.
- [7] Katsiari,MC, Voutsinas LP, Kondyli E.[2002].Improvement of sensory quality of low-fat Kefalogra-viera-type cheesewith commercial adjunct cultues. *Inter dairy J* 12:757.
- [8] Fox PF and PLH Mcsweeney. [2006] *Advanced dairy chemistry. Volume 2 Lipids*.Third Edition.Springer.com. U. S. A.
- [9] Grall D, and RW Hartel. [1992] Kinetics of butterfat crystallization. *J Am Oil Chem Soc* 69:741.
- [10] Nadia A Abou Zeid. [2008] Cheese and its fractions. first Edition Alzahraa Publishers, Inc.,El reyad, Sodja Arabic kingdom pages 102–106.
- [11] Foegeding EA and MA Drake. [2006] Invited Review: Sensory and Mechanical Properties of Cheese *Texture1 J Dairy Sci* 90:1611-1624.
- [12] Ling,ER.[1963] A Text Book of Dairy chemistry .Vol 2 Third edition.,Champan and Hall ,London.
- [13] Chebotarev A I.and Veltsova,s Maxia. [1959] Composition and changes in the amino acid complex in the process of cheese ripening .*Inter Dairy Congr Xv* : 691.
- [14] Nasr WI.[2001] .Studies on acceleration manufacture steps of hard cheese .M. Sci.Thesis, Moshtohor, Zagazig University.
- [15] Vakaleris DG.and Price WV. [1959].A rapid spectrophotometric method for measuring cheese ripening *J Dairy Sci* 47: 264-276.
- [16] Kosikowski,FV.[1978]Cheese and fermented milk foods 2 nd Ed .Cornell Univ.Ithaca,New York.
- [17] Bligh EG, and WJ Dyer. [1959] A rapid method for total lipid extraction and purification. *Can J Biochem. Physiol* 37:911–917.
- [18] Loor JJ. [2001] Alterations in mammary gland synthesis and secretion of fatty acids in response to trans isomers of octadecenoic acid or conjugated linoleic acid isomers. Ph.D. Diss., Virginia Tech, Blacksburg, Virginia.
- [19] Shukla A, Bhaskar AR, Rizvi SSH, Mulvaney SJ. [1994] Physicochemical and Rheological Properties of Butter Made from Supercritically Fractionated Milk Fat . *Journal of Dairy Science*,77: 1.

- [20] Awad SEI –Atter,A, Ahmed,N.and El-Soda, M.[2002].Proteolysis and texture changes during the ripening of white soft cheese (Talaga) .1st Arab Mansoura Conference for Food and Dairy Science &Technology,1-3 October 2002.305–316.
- [21] Awad SEI –Atter,A, Ayad EHE and El-Soda M.[2003] Characteristic of Egyptian market Ras cheese .sensory evaluation ,rheological,physico-chemical properties and microbiological analysis .*Egyptian J Dairy Science* 31:289–303.
- [22] Bryant A, Z Ustunol, and J Steffe. [1995] Texture of Cheddar cheese as influenced by fat reduction. *J Food Sci* 60:1216–1219, 1236.
- [23] Fox PF, TP Guinee, TM Cogan, and PLH McSweeney. [2000] Cheese Rheology and texture. Pages 305–333 in *Fundamentals of Cheese Science*. Aspen Publishers, Inc., Gaithersburg, MD.
- [24] Law BA.[1997]Microbiological and Biochemistry of cheese and fermented milk 2ndEd.T.J.Podstow.Cornwall,Great Britain.
- [25] Ulbricht TLV, Southgate DAT.[1991] Coronary heart disease: seven dietary factors. *Lancet* 338:985–992.

SUPPLEMENTARY TABLES (as provided by authors)

Supplementary Table: 1. Effect of modified milk fat on sensory evaluation of Ras cheese after ripening for five and six months

Scoring points	Ras cheese age(months)					
	Five			Six		
	Taste & Flavour 60	Body & Texture 40	Total 100	Flavor 60	Body & texture 40	Total 100
C	51.4 ^a	31.1 ^a	84.5 ^a	52.2 ^a	33.8 ^a	85.0 ^a
A	50.8 ^a	31.2 ^a	82.0 ^a	51.3 ^a	32.3 ^a	83.6 ^a
L	47.4 ^b	26.3 ^b	73.7 ^b	48.2 ^b	26.5 ^b	74.7 ^b
H	59.3 ^c	39.5 ^c	98.8	58.9 ^c	39.6 ^c	98.5 ^c

a–c Values for each parameter with the same letter in the same column do not differ ($\alpha = 0.01$)

Supplementary Table: 2. Effect of modified milk fat on yield % of fresh and ripe Ras cheese

Cheese samples	Ripening periods	
	Fresh	5 months
C	14.8 ^a	13.7 ^a
A	13.5 ^a	12.5 ^a
L	11.6 ^b	10.5 ^b
H	18.2 ^c	17.1 ^c

a–c Values for each parameter with the same letter in the same column do not differ ($\alpha = 0.01$)

Supplementary Table: 3. Effect of modified milk fat on rheological properties of Ras cheese after ripening for five months.

Samples	Hardness (g)	Consistency g/sec	Springiness Mm	Factorability (g)	Shewiness G mm	Cohesiveness ratio	Adhesiveness g/Sec
C	812.3a	4062.3a	4.9a	787.4a	3952.3a	0.86a	42.6a
A	832.4b	4342.5b	4.7a	812.3b	4192.4b	0.99a	40.3a
L	982.7c	4522.6c	4.0b	853.3c	4316.8c	1.3b	46.4b
H	621.6d	3663.2d	5.9c	675.9d	2756.1d	0.67c	33.5C

a–d Values for each parameter with the same letter in the same column do not differ ($\alpha = 0.01$)

Supplementary Table: 4. The chemical composition of H and C cheese at fresh state and after ripening for five months

Chemical composition%	Ras cheese samples			
	Fresh		5 month age	
	C	H	C	H
Moisture	40.2 ^a	43.7 ^b	29.8 ^a	33.5 ^b
Acidity	1.3 ^a	1.5 ^a	2.3 ^a	2.6 ^a
pH	5.7 ^a	5.5 ^a	5.4 ^a	5.2 ^a
Salt/water	6.9 ^a	6.7 ^a	13.0 ^a	11.5 ^b
Fat/D.M.	51.4 ^a	60.5 ^b	42.2 ^a	51.9 ^b
T.N./D.M.	7.5 ^a	6.1 ^b	6.3 ^a	5.2 ^b

a–b Values for each parameter with the same letter in the same row do not differ ($\alpha = 0.01$)

Supplementary Table: 5. The ripening indices for H and C cheese at fresh state and after ripening for five months

Ripening indices	Ras cheese samples			
	Fresh		5 months age	
	C	H	C	H
S.N./T.N.%	7.4 ^a	6.9 ^a	15.5 ^a	18.3 ^b
T.F.A.A.N %	0.06 ^a	0.04 ^a	0.18 ^a	0.64 ^b
T.V.F.F.A*	6.8 ^a	7.9 ^a	13.6 ^a	21.6 ^b
S.tyr.**	60.2 ^a	74.6 ^a	178.5 ^a	223.9 ^b
S. trp ***	47.2 ^a	50.7 ^a	143.1 ^a	175.5 ^b

a–c Values for each parameter with the same letter in the same row of the first and the second columns or the third and the fourth columns do not differ ($\alpha = 0.01$); * ml 0.1 N NaOH /100g of cheese; ** mg/100g of cheese; ***mg/100g of cheese

Supplementary Table: 6. Fatty acid profiles for H and C cheese ripened for five months

Fatty acids	Cheese	
	C	H
Fatty acids concentration %		
C _{4:0}	3.00 ^a	1.25 ^b
C _{6:0}	0.38 ^a	0.25 ^a
C _{8:0}	1.28 ^a	0.43 ^b
C _{10:0}	1.49 ^a	0.31 ^a
C _{12:0}	3.08 ^a	1.97 ^b
C _{13:0}	0.19 ^a	0.14 ^a
C _{14:0}	10.88 ^a	9.49 ^b
C _{14:1 n9c}	1.51 ^a	1.53 ^a
C _{15:0}	1.59 ^a	1.96 ^b
C _{16:0}	29.38 ^a	29.14 ^a
C _{16:1 n9c}	2.03 ^a	0.95 ^b
C _{17:0}	0.88 ^a	1.49 ^b
C _{18:0}	13.24 ^a	16.39 ^b
C _{18:1 n9t}	26.72 ^a	29.34 ^b
C _{18:1 n9c}	0.00	0.00
C _{18:2 n6c}	1.63 ^a	1.34 ^b
C _{20:1}	0.25 ^a	1.44 ^a
C _{18:3 n3}	0.72 ^a	0.45 ^b
C _{20:0}	1.40 ^a	1.03 ^b
C _{22:0}	0.15 ^a	0.23 ^b
C _{22:1 n9}	0.20 ^a	0.37 ^a

Cholesterol, mg/100g		112.92 ^a	53.69 ^b
Short –chain C ₄ -C ₈		4.66 ^a	1.93 ^b
Medium chain	C ₁₀ -C ₁₂	4.57 ^a	2.28 ^b
Long-chain	C ₁₄ -C ₂₂	90.58 ^a	95.75 ^b
Unsaturated	\	33.06	35.42

a-b Values for each parameter with the same letter in the same row do not differ ($\alpha = 0.01$)