

International Journal of Food Properties

ISSN: 1094-2912 (Print) 1532-2386 (Online) Journal homepage: www.tandfonline.com/journals/ljfp20

Effect of cholesterol removal on compositional and the physicochemical characteristics of anhydrous cow milk fat (cow ghee)

Piyush Bhatia, Vivek Sharma, Sumit Arora & Priyanka Singh Rao

To cite this article: Piyush Bhatia, Vivek Sharma, Sumit Arora & Priyanka Singh Rao (2019) Effect of cholesterol removal on compositional and the physicochemical characteristics of anhydrous cow milk fat (cow ghee), International Journal of Food Properties, 22:1, 1-8, DOI: 10.1080/10942912.2018.1564762

To link to this article: https://doi.org/10.1080/10942912.2018.1564762

© 2019 Piyush Bhatia, Vivek Sharma, Sumit Arora, Priyanka Singh Rao. Published with license by Taylor & Francis Group, LLC.

Published online: 16 Jan 2019.

Submit your article to this journal

Article views: 11383

View related articles

View Crossmark data

Citing articles: 7 View citing articles

Full Terms & Conditions of access and use can be found at
https://www.tandfonline.com/action/journalInformation?journalCode=ljfp20

INTERNATIONAL JOURNAL OF FOOD PROPERTIES
2019, VOL. 22, NO. 1, 1–8
https://doi.org/10.1080/10942912.2018.1564762

Taylor & Francis
Taylor & Francis Group

OPEN ACCESS Check for updates

Effect of cholesterol removal on compositional and the physicochemical characteristics of anhydrous cow milk fat (cow ghee)

Piyush Bhatia, Vivek Sharma, Sumit Arora, and Priyanka Singh Rao

Dairy Chemistry Division, National Dairy Research Institute, Karnal, India

ABSTRACT

Low-cholesterol anhydrous milk fat (ghee) with 90% less cholesterol was prepared using butter as a base material and β-cyclodextrin as an adsorbent. Various physicochemical properties of low-cholesterol ghee such as Reichert–Meissl value, Polenske value, and colour value were found to be almost unaltered by the process of cholesterol removal. However, vitamin-D content reduced by 75% in low-cholesterol ghee as compared to control ghee. Triglyceride profile of low-cholesterol ghee was comparable to control ghee. However, total polyunsaturated fatty acids showed a slight decrease in absolute terms, i.e., 5.48% in control ghee, whereas 4.61% in low-cholesterol ghee.

ARTICLE HISTORY
Received 25 July 2018
Revised 26 November 2018
Accepted 14 December 2018

KEYWORDS
Low-cholesterol ghee; Composition; Butter; β-Cyclodextrin; Physicochemical characteristics; Fatty acid; Triglyceride

Introduction

Heat clarified anhydrous butterfat, generally known as "ghee" in India, is prepared from the milk of cow or buffalo or a combination thereof. Ghee is also rich in many nutritious and health-promoting components. It contains a significant level of butyric acid, which is anti-carcinogenic short-chain fatty acid along with essential fatty acids. Ghee contains a good amount of fat-soluble vitamins (A, D, E, and K), particularly vitamins A and D. As a human food, ghee has been considered immensely superior to other fats.[1] Cow ghee contains about 0.32% cholesterol while that of buffalo has around 0.27%.[2] Around 10% of the cholesterol in milk fat is esterified, remaining is in free form.[3] Many researchers in the past have shown that people who have elevated level of blood cholesterol are more prone to suffer from coronary heart disease, also called coronary artery disease. A correlation of increased risk of cardiovascular disease with an increased consumption of cholesterol-rich foods has been reported in the past.[4,5] Animal studies have supported the fact that dietary intake of cholesterol induces atherosclerosis.[6] Moreover, upon processing and storage of cholesterol-rich foods, its oxidation products may be formed. Few of them have further deleterious effects such as mutagenicity, cytotoxicity, andcarcinogenicity.[7] A number of physical, chemical, and biological processes have been developed for the reduction of cholesterol content of dairy products. However, only the methods involving the use of β-cyclodextrin have found success. Method for preparation of low-cholesterol ghee using the cream as a base material has already been standardized. However, not much information is available on the preparation of low-cholesterol ghee using butter as a base material. Since butter is the most common base material used by dairy industry to prepare anhydrous milk fat (ghee), an attempt was made in the present investigation to prepare low-cholesterol ghee using butter as a base material. The prepared product was then evaluated for its physicochemical properties and the compositional parameters like vitamin-D and fatty acid profile. Since triglyceride (TG) profile is considered by the International Organization for Standardization (ISO)[8] to ascertain the purity of milk fat, hence it has also been reported.

CONTACT Vivek Sharma vishk12000@yahoo.com Dairy Chemistry Division, ICAR-NDRI, Room No. – 236, Karnal, Haryana 132001, India

Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/ljfp.

© 2019 Piyush Bhatia, Vivek Sharma, Sumit Arora, Priyanka Singh Rao. Published with license by Taylor & Francis Group, LLC.
This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Materials and methods

Preparation of low-cholesterol ghee

Cow butter that was used for the preparation of low-cholesterol ghee was procured from the experimental dairy of ICAR- National Dairy Research Institute, Karnal. The extraction of cholesterol from butter was carried out on lab scale by using the conditions essentially based on the methodology.[9] β-cyclodextrin was used as an adsorbent to remove cholesterol from butter.

Beta (β)- cyclodextrin, Supelco 37 Component FAME Mix (Sigma–Aldrich, USA), TG mixture (C24, C30, C36, C42, C48; Sigma–Aldrich, St. Louis, MO, USA), gas chromatography system, and accessories (Shimadzu, Japan).

Determination of cholesterol content in ghee

Cholesterol content of ghee was determined using enzymatic diagnostic kit.[10]

Iodine value

Iodine value was determined by the Wij's method.[11]

Butyro refractometer (BR) reading at 40°C

BR reading of ghee samples was determined by using Butyro Refractometer.[11]

Reichert–Meissl (RM) and Polenske values

RM and Polenske values of ghee samples were determined by the standard method.[11]

Free fatty acid (FFA) value

FFA content of ghee samples was determined by the standard method for estimation of %FFA in terms of oleic acid.[11]

Peroxide value

Peroxide value of ghee samples was determined by the standard method.[11]

Moisture content

The moisture content of ghee samples was determined by the standard gravimetric method.[11]

Vitamin D

Vitamin D content of the ghee sample was estimated by the method.[12]

Color value

Colour value of ghee samples was measured using color intensity desk[13]

Fatty acid profiling

For determination of fatty acid profile of low-cholesterol ghee, chromatography technique was used. SLB-1 L 100 capillary column of dimensions 30 m X 0.25 mm X 0.25 μm was used in a gas chromatograph (Shimadzu GC 2010 PLUS, Kyoto, Japan, with GC solution software), equipped with flame ionization detector and temperature control module. Nitrogen was used as carrier gas with a column flow rate of 1.93 mL/min and a split ratio of 1:50. For the purpose of injection, fatty acid methyl esters (FAME) of the samples were prepared using a standard method.[14] One micro-liter of sample was injected into the gas chromatography machine. Injector and detector temperature used was 240°C. Oven was initially maintained at 50°C and then the temperature was raised to 220°C at 3/min.

Triglyceride profiling

Triacylglycerol profile of control and low-cholesterol ghee was analyzed by gas chromatography using MXT-5 capillary column of 5 m length (cut from 15 m X 0.25 mm X 0.25 μm) in gas chromatograph (Shimadzu GC 2010 PLUS, Kyoto, Japan, with GC solution software), equipped with auto-injector, flame ionization detector, and temperature control module. The methodology of ISO)[8] was used for the analysis of the samples. Nitrogen was used as carrier gas with a column flow rate of 2.81 mL/min and a split ratio of 1:20.

For the preparation of the sample, 100 μL of molten ghee was transferred to a 10 mL volumetric flask, and the volume was made up using n-hexane. The injection volume used for analysis was 0.5 μL, and the temperature of both injector and detector was set to 370°C. The initial column temperature was 80°C which was held for 0.5 min, then raised to 190°C at 50°C/min for no hold, and finally raised to 350°C at 6°C/min and held for 7 min.

Statistical analysis

All measurements were performed in triplicate and reported as the mean ± standard deviation. All statistical analyses were performed with GraphPad Prism software (version 5.01 for Windows), San Diego, CA, USA.

Results and discussion

Effect of cholesterol removal on composition

Cholesterol

Table 1 depicts the various physicochemical properties of low-cholesterol and control ghee. As shown in Table 1, around 90% reduction in cholesterol content was observed when low-cholesterol ghee was prepared using butter as a base material. The cholesterol content of control ghee was 250 ± 3.79 mg/100g of ghee while that of low-cholesterol ghee was 24.00 ± 3.00 mg/100g of ghee.

Vitamin-D

It is evident from the data presented in Table 1 that there was about 75% reduction in vitamin-D content of ghee as a result of cholesterol removal. This may be due to similarities in the structures of cholesterol and vitamin-D, which allows the β-cyclodextrin to bind both vitamin-D along with cholesterol. The findings of the present investigation are in consonance with the reports available for low-cholesterol ghee,[2] wherein 65–70% reduction in vitamin-D content in low-cholesterol ghee was reported.

Fatty acids

Fatty acid profile of milk fat has a profound impact on its physicochemical properties. Hence, the knowledge of fatty acid profile of milk fat is of considerable significance. FAMEs of milk fat were

Table 1. Comparison of physicochemical and compositional characteristics of control and low-cholesterol ghee.

S.no.	Physicochemical property	Low-cholesterol ghee	Control ghee
1	Cholesterol content (mg/100g)	24.00 ± 3.00	250 ± 3.79
2	Iodine value	36.04 ± 0.14	35.38 ± 0.16
3	Butyro refractometer (BR) reading at 40°C	42.03 ± 0.06	42.03 ± 0.06
4	Reichert–Meissl (RM) value	28.16 ± 0.06	28.21 ± 0.05
5	Polenske value	1.77 ± 0.06	1.86 ± 0.06
6	Peroxide value	0.00 ± 0.00	0.00 ± 0.00
7	Free fatty acid (FFA) value	0.22 ± 0.00	0.25 ± 0.01
8	%Moisture content	0.09 ± 0.01	0.11 ± 0.01
9	Vitamin D (IU/g)	3.27 ± 0.14	13.05 ± 0.72
10	Color value	L* 47.95 ± 1.24	L* 50.08 ± 1.53
		a* −14.73 ± 0.81	a* −13.46 ± 0.79
		b* 51.87 ± 2.22	b* 48.00 ± 1.80

Data presented as mean ± SD (n = 3).

injected in gas chromatography (GC) for the determination of fatty acid profile of ghee. Before running the sample, Supelco 37 Component FAME mix was run as standard before running the sample to ascertain the retention time of various fatty acids. A comparison of the concentration of various fatty acids found in control and low-cholesterol ghee has been presented in Table 2, and the respective chromatograms have been depicted in Figure 1. The fatty acid composition of control and low-cholesterol ghee was almost similar with some minor differences in polyunsaturated fatty acid (PUFA) content. The unsaturated fatty acids of both the samples were majorly monounsaturated, but the total PUFAs estimated were slightly higher in control ghee (5.48%) than low-cholesterol ghee (4.61%). $C_{16:0}$ and *cis*-$C_{18:1}$ were the most predominant fatty acids in both the samples, representing around 57% of the total fatty acids. There was no major difference in the short-chain fatty acid ($C_{4:0,}$ $C_{6:0}$, and $C_{8:0}$) content in both the samples. The findings of the present investigation are in agreement with the findings of Seon *et al.* (2009), wherein they reported the similar concentration of short-chain fatty acids in control and low-cholesterol cheddar cheese made after treating cheese milk with cross-linked β-cyclodextrin. Fatty acid $C_{18:1}$-trans was slightly higher in low-cholesterol ghee as compared to control ghee. However, the content of linoleic acid ($C_{18:2}$ cis) was found to be higher in control ghee (2.81%) as compared to low-cholesterol ghee (2.31%). Among other unsaturated fatty acids, the content of $C_{18:3\ n3\alpha}$, $C_{20:1\ n9}$, and $C_{24:1n9}$ was also marginally higher in control ghee as compared to low-cholesterol ghee. Rest of the fatty acid profile of both the samples had no major differences. Lower content of unsaturated fatty acids in the low-cholesterol ghee may be due to the fact that high-speed mixing of β-cyclodextrin with butter using blender might have induced oxidation of some unsaturated fatty acids.

Triglycerides

The TG composition of control ghee and low-cholesterol ghee, as determined by GC, is shown in Table 3, and the respective chromatographs have been depicted in Figure 2. Fifteen major peaks of even carbon number TGs, ranging from C_{26} to C_{54}, were identified in both control and low-cholesterol ghee. Some unidentified peaks were also observed in the chromatographs of both the samples. However, these TGs were found in very low concentration. C_{50} was the most predominant TG in both control (11.87%) and low-cholesterol ghee (12.31%). Apart from C_{52}, other TGs which were found to be predominant in both control and low-cholesterol ghee were C_{36}, C_{38}, C_{40}, C_{48}, and C_{52}. These six TGs represented around 67% of the total TGs. C_{24} was present in a very small concentration in both control and low-cholesterol ghee samples. The TGs with smaller carbon number (C_{26}, C_{28}, C_{30}, and C_{32}) were found to be present in a very nominal amount. C_{28}, C_{32}, and C_{38} were present in higher concentration in control ghee as compared to low-cholesterol ghee, whereas low-cholesterol ghee had a higher concentration of C_{50} and C_{52}. However, these differences in TG profile of control and low-cholesterol ghee were not apparent. The present findings are in agreement with those reported by Gutierrez-

Table 2. Fatty acid profile of low-cholesterol and control ghee.

		Control ghee	Low-cholesterol ghee
Peak no.	Compound name	Concentration (%)	
1.	Butyric acid ($C_{4:0}$)	1.02 ± 0.05	1.00 ± 0.12
2.	Caproic acid ($C_{6:0}$)	1.15 ± 0.05	1.18 ± 0.13
3.	Caprylic acid ($C_{8:0}$)	0.91 ± 0.04	0.95 ± 0.09
4.	Capric acid $C_{10:0}$	2.39 ± 0.12	2.47 ± 0.25
5.	Undecylic acid $C_{11:0}$	0.22 ± 0.03	0.24 ± 0.03
6.	Lauric acid $C_{12:0}$	3.09 ± 0.16	3.33 ± 0.34
7.	Tridecylic acid $C_{13:0}$	0.06 ± 0.01	0.06 ± 0.01
8.	Myristic acid $C_{14:0}$	10.94 ± 0.41	11.26 ± 0.65
9.	Myristovaccenic acid $C_{14:1}$	0.83 ± 0.04	0.85 ± 0.06
10.	Pentadecylic acid $C_{15:0}$	0.92 ± 0.04	0.92 ± 0.02
11.	Pentadecenoic acid $C_{15:1}$	0.25 ± 0.01	0.25 ± 0.01
12.	Palmitic acid $C_{16:0}$	30.48 ± 0.06	30.55 ± 0.21
13.	Palmitoleic acid $C_{16:1}$	1.76 ± 0.04	1.78 ± 0.03
14.	Margaric acid $C_{17:0}$	0.51 ± 0.02	0.52 ± 0.04
15.	Heptadecenoic acid $C_{17:1}$	0.25 ± 0.02	0.26 ± 0.02
16.	Stearic acid $C_{18:0}$	11.59 ± 0.29	11.24 ± 0.52
17.	Elaidic acid $C_{18:1}$ trans	0.06 ± 0.02	0.28 ± 0.01
18.	Oleic acid $C_{18:1}$ cis	26.90 ± 0.52	27.16 ± 1.06
19.	Linolelaidic acid $C_{18:2}$ trans	0.04 ± 0.01	0.03 ± 0.01
20.	Linoleic acid $C_{18:2}$ cis	2.82 ± 0.04	2.31 ± 0.08
21.	Arachidic acid $C_{20:0}$	0.27 ± 0.02	0.25 ± 0.03
22.	α-linolenic acid $C_{18:3\ n3\alpha}$	0.73 ± 0.02	0.49 ± 0.03
23.	Gondoic acid $C_{20:1\ n9}$	0.19 ± 0.02	0.10 ± 0.01
24.	Heneicosylic acid $C_{21:0}$	0.61 ± 0.01	0.62 ± 0.02
25.	Dihomo-γ-linolenic acid $C_{20:3\ n6}$	0.26 ± 0.03	0.22 ± 0.04
26.	Behenic acid $C_{22:0}$ Arachidonic acid $C_{20:4\ n6}$	0.12 ± 0.01	0.11 ± 0.01
27.	Dihomo-α-linolenic acid $C_{20:3\ n3}$	0.27 ± 0.03	0.29 ± 0.03
28.	Docosadienoic acid $C_{22:2}$	1.10 ± 0.02	1.01 ± 0.10
29.	Lignoceric acid $C_{24:0}$	0.03 ± 0.01	0.03 ± 0.01
30.	Nervonic acid $C_{24:1\ n9}$	0.06 ± 0.01	0.05 ± 0.01
31.	Docosahexaenoic acid $C_{22:6\ n3}$	0.19 ± 0.03	0.21 ± 0.10
	Total saturated fatty acids (%)	64.24	64.66
	Total unsaturated fatty acids (%)	35.76	35.34
	Total monounsaturated fatty acids (%)	30.29	30.73
	Total polyunsaturated fatty acids (%)	5.47	4.61

Data presented as mean ± SD (n = 3).

Tolentino *et al.* (2007).[15] It is evident from Table 3 that the concentration and composition of TGs in both control and low-cholesterol ghee were almost similar.

Physicochemical characteristics

Table 1 depicts the various physicochemical properties of low-cholesterol and control ghee. As shown in Table 1, the cholesterol removal from butter using β-cyclodextrin did not considerably affect the RM value, Polenske value, BR reading at 40°C, moisture content, and FFA value of ghee. Moreover, values obtained for various physicochemical properties were well within the values specified in legal standards of ghee in India.[16,17] The findings of the present investigation were in agreement with the findings of Kumar *et al.* (2010),[2] wherein they had prepared low-cholesterol ghee using the cream as a base material.

The peroxide value of both control and low-cholesterol ghee was found to be nil, which indicated that cholesterol removal using β-cyclodextrin did not result in any oxidative deterioration. The same was reported earlier for low-cholesterol ghee made using the cream as a base material.[2,18] The iodine value of ghee was also not much affected by treatment with β-cyclodextrin. The iodine value of control ghee was 35.38 ± 0.16, while that of low-cholesterol ghee was 36.04 ± 0.14.

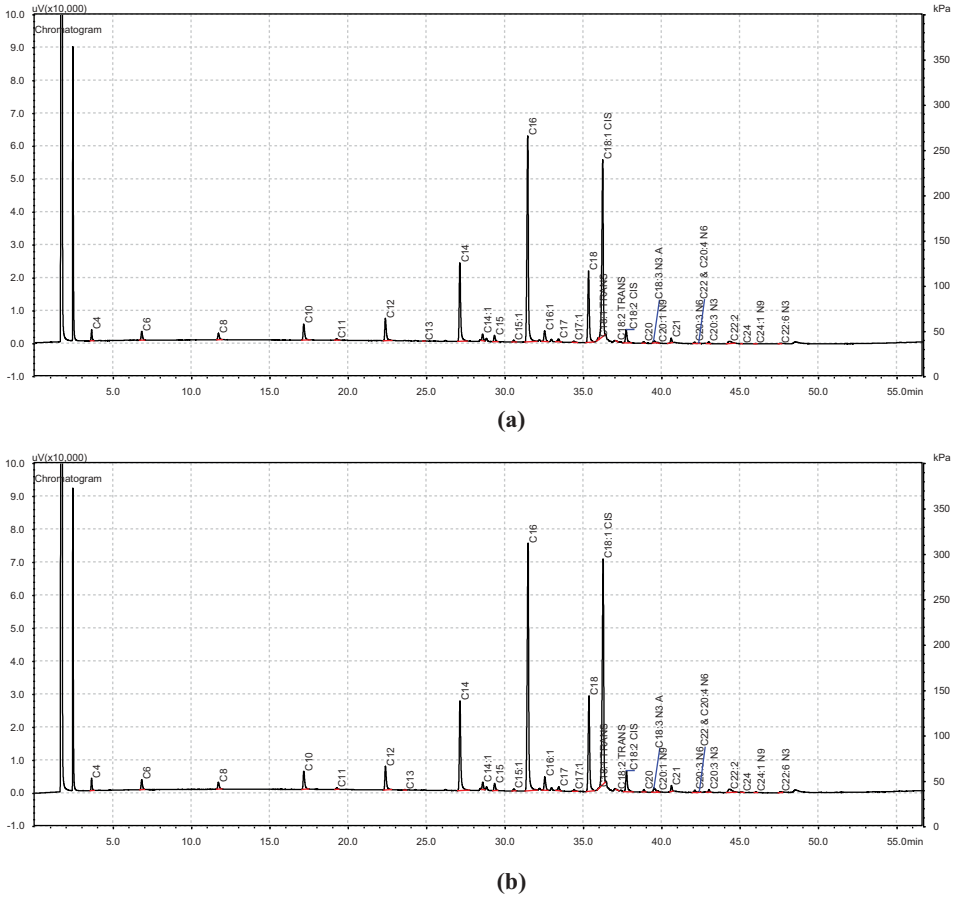


Figure 1. (a) Chromatogram of fatty acid methyl esters of low-cholesterol ghee. (b) Chromatogram of fatty acid methyl esters of control ghee.

Table 3. Triacylglycerol (TAG) profile of control and low-cholesterol ghee.

	Control ghee	Low-cholesterol ghee
TAGs	Concentration (%)	
C_{24}	0.05 ± 0.01	0.04 ± 0.00
C_{26}	0.20 ± 0.03	0.21 ± 0.02
C_{28}	0.62 ± 0.03	0.56 ± 0.02
C_{30}	1.01 ± 0.05	0.96 ± 0.05
C_{32}	2.21 ± 0.08	2.08 ± 0.06
C_{34}	4.79 ± 0.34	4.91 ± 0.13
C_{36}	9.41 ± 0.25	9.23 ± 0.16
C_{38}	11.75 ± 0.04	11.22 ± 0.26
C_{40}	9.74 ± 0.19	9.60 ± 0.20
C_{42}	6.88 ± 0.12	6.68 ± 0.30
C_{44}	6.14 ± 0.15	6.15 ± 0.09
C_{46}	6.94 ± 0.07	7.14 ± 0.05
C_{48}	8.68 ± 0.11	9.09 ± 0.09
C_{50}	11.87 ± 0.14	12.31 ± 0.13
C_{52}	11.85 ± 0.07	12.30 ± 0.29
C_{54}	7.50 ± 0.17	7.51 ± 0.53

Data presented as mean ± SD (n = 3).

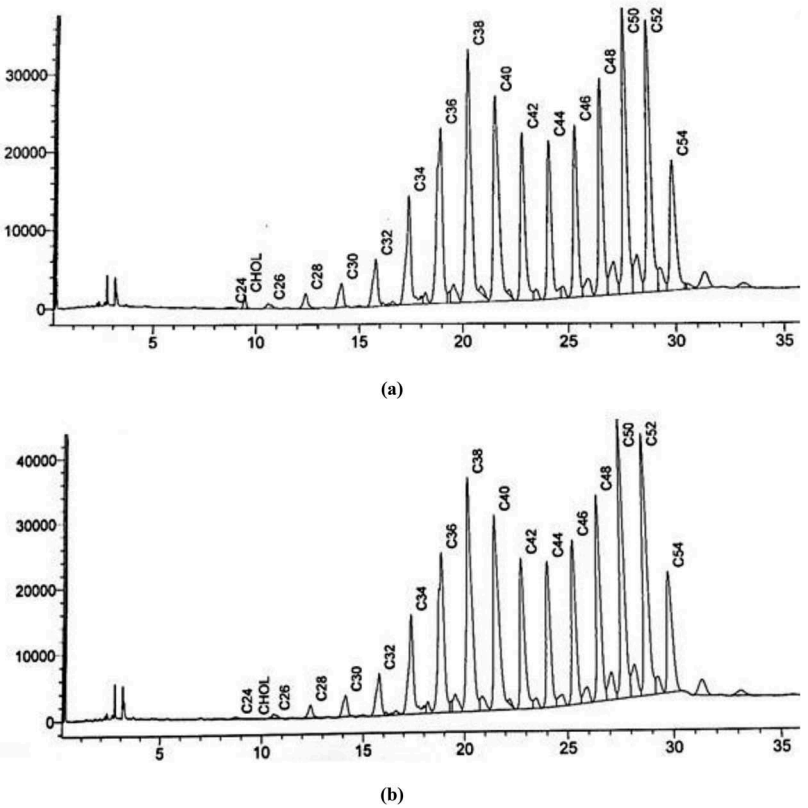


Figure 2. (a) Triglyceride profile chromatogram of control ghee. (b) Triglyceride profile chromatogram of low-cholesterol ghee.

Color value. The color value of low-cholesterol ghee has not been reported in the literature. Therefore, in the present investigation, an attempt was made to quantify the change in color of cow ghee as a result of cholesterol removal. The color value of ghee was expressed as L* (lightness value), a* (redness value), and b* (yellowness value). It is evident from the data presented in Table 1 that the values, i.e., L*, a*, and b*, were not very different from both the ghee samples, i.e., control and low-cholesterol ghee. This indicated that the process of cholesterol removal did not affect the typical color of cow ghee to the extent which will impact the consumer perception of cow ghee.

Conclusion

The present investigation on low-cholesterol ghee revealed that butter can also be used as a base material for the preparation of low-cholesterol ghee, achieving over 90% reduction in cholesterol content. The visual appearance of cow ghee was not affected by the process of cholesterol removal. Most of the physicochemical properties were also found to be almost unaltered. However, 75% loss of vitamin-D was observed on treatment of milk fat with β-cyclodextrin. The degree of unsaturation in low-cholesterol ghee was less, i.e., 4.61% vis′-a-vis 5.48% in control ghee. TG profile was also unaffected by the process of cholesterol removal.

Acknowledgments

The authors are thankful to Director, NDRI, Karnal, for providing infrastructure and other facilities to carry out research. The financial assistance provided by ICAR in the form of Junior Research Fellowship is also duly acknowledged.

References

[1] Kansal, V. K. Reaffirming: Health Benefits of Desi Ghee. *Indian Dairyman*. **2010**, *62*, 60–63.

[2] Kumar, M.; Sharma, V.; Lal, D.; Kumar, A.; Seth, R. A Comparison of the Physico-Chemical Properties of Low-Cholesterol Ghee with Standard Ghee from Cow and Buffalo Creams. *Int. J. Dairy Technol.* **2010**, *63*, 252–255. DOI: 10.1111/idt.2010.63.issue-2.

[3] Fox, P. F.; McSweeney, P. L. H. *Dairy Chemistry and Biochemistry*; Blackie Academic and Professional: London, **1998**.

[4] Griffin, H. D. Manipulation of Egg Yolk Cholesterol: A Physiologist's View. *World's Poultr. Sci. J.* **1992**, *48*, 101–112. DOI: 10.1079/WPS19920010.

[5] Houston, D. K.; Ding, J.; Lee, J. S.; Garcia, M.; Kanaya, A. M.; Tylavsky, F. A.; Newman, A. B.; Visser, M.; Kritchevsky, S. B. Dietary Fat and Cholesterol and Risk of Cardiovascular Disease in Older Adults: The Health ABC Study. *Nutr. Metab. Cardiovasc. Dis.* **2011**, *21*, 430–437. DOI: 10.1016/j.numecd.2009.11.007.

[6] Connor, S. L.; Connor, W. E. Dietary Cholesterol and Coronary Heart Disease. *Curr. Atherosclerosis Rep.* **2002**, *4*, 425–432.

[7] Orczewska-Dudek, S.; Bederska-Lojewska, D.; Pieszka, M.; Pietras, M. P. Cholesterol and Lipid Peroxides in Animal Products and Health implications-A Review. *Ann. Anim. Sci.* **2012**, *12*, 25–52. DOI: 10.2478/v10220-012-0003-9.

[8] ISO 17678 / IDF 202. Milk and Milk Products: Determination of Milk fat purity by gas Chromatographic analysis of triglycerides. International Dairy Federation. Brussels, **2010**

[9] Dias, H. M.; Berbicz, F.; Pedrochi, F.; Baesso, M. L.; Matioli, G. Butter Cholesterol Removal Using Different Complexation Methods with Betacyclodextrin, and the Contribution of Photoacoustic Spectroscopy to the Evaluation of the Complex. *Food Res. Int.* **2010**, *43*(4), 1104–1110. DOI: 10.1016/j.foodres.2010.02.002.

[10] Sharma, V.; Reddy, M. J. S.; Arora, S.; Kumar, A.; Lal, D.; Seth, R.; Wadhwa, B. K.; Sharma, G. S. Applicability of Enzymatic Diagnostic Kit for Estimation of Cholesterol in Ghee. *J. Food Sci. Technol.* **2009**, *46*, 244–246.

[11] IS: (SP: 18). *ISI Handbook of Food Analysis. Part XI: Dairy Products*; Bureau of Indian Standards, Manak Bhavan: New Delhi, **1981**.

[12] Parrish, D. B.; Richter, E. F. Determination of Vitamin D in Foods: A Review. *CRC Critical Reviews in Food Sci. Nutr.* **1979**, *12*, 29–57. DOI: 10.1080/10408397909527272.

[13] Assawarachan, R.; Noomhorm, A. Changes in Colour and Rheological Behavior of Pineapple Concentrate through Various Evaporation Methods. *Int. J. Agric. Biol. Eng.* **2010**, *3*, 1–11.

[14] O'fallon, J. V.; Busboom, J. R.; Nelson, M. L.; Gaskins, C. T. A Direct Method for Fatty Acid Methyl Ester Synthesis: Application to Wet Meat Tissues, Oils, and Feedstuffs. *J. Anim. Sci.* **2007**, *85*(6), 1511–1521. DOI: 10.2527/jas.2006-491.

[15] Gutierrez-Tolentino, R.; Vega Y Leon, S.; Diaz-Gonzalez., G.; Delgadillo-Gutierrez, H. J.; Urban-Carrillo, G.; Ramirez-Ayala, A.; Gonzalez-Cabrera, C.; Mendez-Ramirez, I. Detección de grasa extraña en grasa láctea por cromatografía de gases y estadística multivariable. *Agrociencia*. **2007**, *41*, 733–742.

[16] AGMARK. *Ghee Grading and Marking Rules, 1938 (As Amended in 1988)*; Government of India, Ministry of Food and Agriculture, Department of Agriculture: New Delhi, **1988**.

[17] FSSAI. **2017**. https://www.fssai.gov.in/dam/jcr:a9817c57-c8ee-4585-9dbd-fb10cc2af024/Direction_Operationalization_Milk_Standards_04_08_2017.pdf

[18] Meghwal, K.; Sharma, V.; Lal, D.; Arora, S.; Singh, M. Effect of Cholesterol Removal on Storage Stability of Cow Ghee. *Indian J. Dairy Sci.* **2011**, *64*, 396–399.