

Characteristic of Ghees Obtained from Different Post-Clarification Temperatures

N. Suwarat and W. Tungjaroenchai

Abstract—Physical and chemical characteristics of ghee produced from a direct cream method with different post-clarification heating temperatures and times were studied. Fresh cream from buffalo's milk was inoculated with freeze dried mixed cultures of *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis* and *Leuconostoc mesenteroides* subsp. *cremoris*, and incubated at $32\pm0.5^\circ\text{C}$ for 4 hr. Fermented cream samples were heated to render butter oil (ghee), and post-clarification heatings were conducted at temperatures of 100 ± 5 , 110 ± 5 , or $120\pm5^\circ\text{C}$, for 5, 10, or 15 min, respectively. Free fatty acid values (FFA) and oxidative stability in hours significantly increased ($P < 0.05$). With increasing temperature and prolonged periods of time. The oxidative stability values increased from 15.45 ± 0.17 to 28.60 ± 0.34 h., while FFA values increased from 0.22 ± 0.12 to $0.36\pm0.03\%$ (expressed as oleic acid). Changes in moisture content of ghee samples were not affected ($P > 0.05$) by post-clarification heatings. Both temperature and time of post-clarification effected changes in L, a, and b values. Values of lightness (L) ranged 100.95 ± 0.07 to 94.91 ± 0.05 , yellow (b) ranged 23.17 ± 0.05 to 48.32 ± 0.07 , and green (a) ranged -6.46 ± 0.02 to -7.44 ± 0.03 , respectively.

Index Terms—Ghee, color, free fatty acid value, oxidative stability.

I. INTRODUCTION

Ghee, the most famous traditional dairy product in India and many countries in Middle East. It is made from milk, cream, or butter of several animal species [1]. Ghee processing was made either with or without fermenting and rendering oil from milk, cream or butter by direct heating. Consequences were milk protein precipitation, dehydration and clarification of oil. Generally, about 95% of milk fat melted almost completely at body temperature, leading to a clean mouth feels without waxy sensation [2]. Milk from different animal species affected on ghee quality. [3]. Four methods of making ghee were known: pre-stratification method (PS), creamery butter method (CB), direct cream method (DC), desi method or milk butter method (MB) [4], [5]. Direct heating has been selected to render or separate oil or ghee from milk cream. Keeping quality of butter oil was governed by several factors as the ripening of cream, method of manufacture, clarification temperature, and permeability of packaging materials to air and moisture, and type of animal feed [4], [6].

The aim of present study was to determine the physical

and chemical characteristics, and oxidative stability of ghee obtained from different post-clarification heatings.

II. MATERIALS AND METHODS

A. Materials

Buffalo's milk and cream were obtained from Murrah Farm in Cha-Choeng-Sao province of Thailand. Samples were transported and kept at $4\pm3^\circ\text{C}$ prior uses. Freeze-dried cultures of Lactic acid bacteria (LAB): *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis* and *Leuconostoc mesenteroides* subsp. *cremoris* (Danisco, Denmark), were used for preparation of fermented cream.

B. Preparation of Fermented Cream

Buffalo's milk and milk cream were analyzed for fat contents using Mojonnier method [7]. The cream was standardized to 25% fat, and then pasteurized at $69\pm2^\circ\text{C}$ for 30 min [8]. The cream was cooled and inoculation with 0.02 (%w/w) of the freeze-dry cultures. Incubation was controlled at $32\pm0.5^\circ\text{C}$ for 4 h, and then kept at $4\pm3^\circ\text{C}$, before further heating and rendering of butter oil (ghee) within 24 hr.

C. Heating the Fermented Cream and Rendering for Oil (Ghee)

The fermented cream was slowly heated and stirred in a Teflon pan. Post-clarification temperatures were 100 ± 5 , 110 ± 5 , or $120\pm5^\circ\text{C}$, for 5, 10, or 15 min, respectively. Total of nine experimental units for ghee were prepared. Ghee was separated through a filter unit with vacuum pump (Sonata WJ-20, Canada), and kept in brown glass bottles. Headspace on ghee surface was purged with nitrogen gas.

D. Physical and Chemical Analyses of Ghee

Color values of ghee samples were measured at $40\pm3^\circ\text{C}$ accordingly to Hunter color measurement (Hunter Lab, Color Quest XE). Free fatty acid (FFA) was determined by titration method, as defined in AOCS (1999) [9] Official method (Ca 5a-40) as oleic acid. Moisture content of ghee by an oven method AOAC (2002a) [10] was measured.

E. Oxidation Stability Test of Ghee

Determination of an oxidative stability of ghee by Rancimat equipment (Germany, model 743), was based on volatile acids from oxidation reaction passed through DI water, in which conductivity values were detected. Heating block was held constant at 110°C . A rate of air flow through liquid butter oil (ghee) was 10 l/h. Prior to the testing, frozen samples of ghee were thawed at $40\text{--}50^\circ\text{C}$, and a 3 ± 0.0020 g of ghee sample was taken for the analysis.

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F. Experimental Design and Statistical Analysis

A Factorial arrangement in Complete Randomize Design (CRD) with 3 different heating (post-clarification) temperatures (100 ± 5 , 110 ± 5 , or 120 ± 5 °C) and time (5, 10, or 15 min), and triplicate samplings were employed. Experimental data were expressed as mean \pm SD. An ANOVA test ($p < 0.05$) was applied to moisture content, milk fat, FFA and oxidative stability. Trial version SPSS 20 (IBM, USA) statistical package was used.

III. RESULT AND DISCUSSION

A. Physical and Chemical Characteristics of Ghee

Ghee samples produced in this experiment were colorless. Ghee from buffalo's cream lacked of carotenoids, however it contained biliverdin and bilirubin which gave a greenish tint [5], which corresponded to the colors measured by the Hunter Lab (Color Quest XE) (Table I). The results of the color values showed trends of high lightness (L), yellow and green, as consequences of different post-clarification heating conditions. Increasing of heating temperatures and periods of time resulted in significant differences in color values ($P < 0.05$) of ghee samples.

Increasing the post-clarification temperatures and times decreased in the L values, but increased in both values of a and b (Table I). Apparent color of ghee was intensified by both temperature and time of heating. However, several factors could cause changes in color of frying oil. Increased in the color intensity of oil was due to accumulation of nonvolatile decomposition products, such as oxidized triacylglycerols and FFA upon heating [11], [12]. Generally, frying oil and particles of fried foods lead to overheating, and were broken down to small particles as coloring substances [13]. Moreover, Nagao *et al.* (2006) reported that high mineral content tended to induce browning oil in a shorter time, and more intensively than low mineral content. Amino acids were implicated in oil color deterioration, such as methionine (Met) was found the most strongly coloring amino acid, while glutamine (Glu) was the least [13].

According to IDF (1977), defined ghee as a product exclusively obtained from milk, cream or butter from various animal species by means of processes which resulted in almost total removal of moisture and solids-not-fat, and which gave the product a particular physical structure. The standard specified ghee to have 96% minimum milk fat, 0.3% maximum moisture, 0.3% maximum FFA (expressed as oleic acid), and a peroxide value (PV) less than 1.0 [5].

TABLE I: COLOR MEASUREMENT OF GHEES FROM THE DIFFERENT HEATING CONDITIONS

Temperature (± 5 °C)	Time (min)	L	a	b
100	5	100.95	-6.46	23.17
	10	99.59	-6.69	24.19
	15	98.25	-6.70	25.87
110	5	100.62	-6.62	23.58
	10	99.36	-7.21	27.37
	15	97.81	-7.10	29.81
120	5	98.26	-7.44	31.51
	10	97.00	-7.20	31.92
	15	94.91	-6.78	48.33

Lightness (L), green to red (-a to +a), blue to yellow (-b to +b)

Due to heating of ghees for evaporation of moisture contained in buffalo milk cream, thus, moisture contents of ghee decreased with increasing heating temperature and time. However, both factors did not significantly influenced changes of final moisture contents of ghees (Table II). The presence of sufficient moisture and the activity of microbial lipases affect on the development of rancidity, meanwhile the storage stability of ghee is probably attributed to the low moisture content [14]. High moisture content of buffalo milk cream might led to high FFAs due to hydrolysis occurred during heating. The presence of air and water introduced as steam during heating oil, or frying process could accelerate oil deterioration [15]. However, ghee and butter fat kept much better than butter or ghee containing added moisture [16]. The heat treatment involved in the manufacture of ghee should destroy most bacteria, and the moisture content is too low to allow normal growth of most micro-organisms [14].

TABLE II: CHEMICAL PROPERTIES OF GHEES FROM THE DIFFERENT HEATING CONDITIONS

Temperature (°C)	Time (min)	Moisture content (%)	Free fatty acid (% expressed as oleic acid)	Oxidative stability (h)
100	5	0.14 ± 0.09	0.22 ± 0.12	15.45 ± 0.17
	10	0.14 ± 0.07	0.23 ± 0.06	16.14 ± 0.14
	15	0.12 ± 0.04	0.24 ± 0.04	19.29 ± 0.05
110	5	0.16 ± 0.02	0.24 ± 0.09	19.31 ± 0.35
	10	0.15 ± 0.03	0.32 ± 0.05	20.57 ± 1.26
	15	0.12 ± 0.02	0.35 ± 0.02	22.13 ± 0.01
120	5	0.12 ± 0.03	0.33 ± 0.14	24.38 ± 0.22
	10	0.09 ± 0.03	0.35 ± 0.07	26.37 ± 0.26
	15	0.12 ± 0.06	0.36 ± 0.03	28.60 ± 0.34

Means \pm standard deviation from triplicate analysis.

On the other hand, either the temperature or period of time increased, FFAs were significantly increased ($P < 0.05$) (Table II). The FFAs varied from 0.22 ± 0.12 to $0.35 \pm 0.02\%$ expressed as oleic acid. Bubbles of gas and steam generated during heating the fermented cream, and oil rendering were similar to the phenomenon of a deep fat frying reaction. High temperature for frying increased FFA [11], when oxidation, polymerization, hydrolysis which was causes of FFA changes. Oil was also oxidized to form hydroperoxide, conjugated dienoic acids, epoxides, hydroxides, aldehydes, and ketones. Increases in these volatile compounds development could attribute to increases in FFA content of oil. These compounds may undergo fission into smaller fragments or may remain in the triglyceride molecules, and cross-link with each other, leading to dimeric and higher polymeric triglycerides [15]. Furthermore, one of the increasing the FFAs causes could be a result of production of organic acids (e.g. lactic acid) by lactic acid bacteria [14]. FFAs were undesirable in milkfat products because the shorter-chain homologues are primarily responsible for the rancid flavour typified by butyric acid. The short-chain FFAs have been reported to catalyze the fat oxidation reaction. Conversely, the absence of short-chain FFAs in

ghee was possibly due to volatilization during the heat clarification of butter [5].

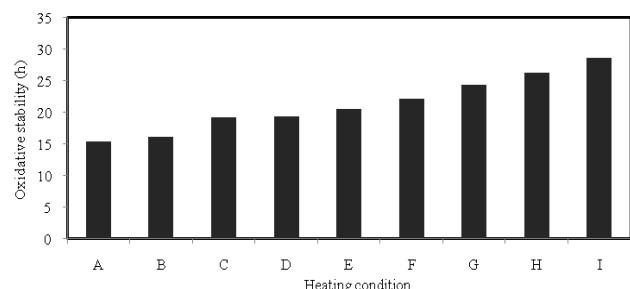


Fig. 1. Oxidative stability of ghees which were produced by the different heating conditions, 100 °C 5 min (A), 100 °C 10 min (B), 100 °C 15 min (C), 110 °C 5 min (D), 110 °C 10 min (E), 110 °C 15 min (F), 120 °C 5 min (G), 120 °C 10 min (H) and 120 °C 15 min (I).

Results of the oxidative stability (OSI) of ghees determined by Rancimat equipment were shown in Fig. 1. In this analysis, a stream of filtered, cleaned and dried air was bubbled into a reaction vessel containing the ghee sample. This vessel was placed in an electric heating block. Effluent air containing volatile organic acids from the heated ghee sample were collected in a measuring vessel with 60 mL of distilled water. The conductivity of the water was continuously detected and recorded, the OSI was automatically determined by the apparatus [17]. The OSI of ghee samples varied from 15.45 ± 0.17 to 28.60 ± 0.34 h, the higher temperature and longer period of time, the higher values of OSI in hours. (Table II). Although some papers reported that high temperature with prolong period of time affected high yield, color and oxidative stability tended to changed continuously Degradation of oil was normally induced by moisture, high temperature, crust formation and various structural, textural and chemical changes in the product, and degradation of frying medium [18]. Degradation of oil resulted in smaller molecules such as methyl heptanoate (C7:0), methyl octanoate (C8:0). Interestingly, the ratio between both methyl esters was very useful to deduce the extent of degradation of the main fatty acids –oleic and linoleic acids – present in edible fats and oils [19]. Animal fat contained higher content of saturated fatty acids compared to those of vegetable oil. Oxidation of unsaturated oil resulted in peroxides, small molecules of hydrocarbon. In contrast, oxidation of saturated fatty acids resulted in smaller/shorter molecules of fatty acids and other hydrocarbons

The higher temperature used in manufacturing of ghee, the more pronounced was the heated flavor and odor in the finished product. The higher temperatures also seemed to yield ghee with better keeping qualities; this may have been due to the less complete removal of the moisture with the lower temperatures [5], [16].

IV. CONCLUSION

Post-clarification heating at 100 ± 5 °C for 5, 10, or 15 min, or 110 ± 5 °C for 5 min, was considered an optimal condition for ghee. Although, the moisture content of ghees in each condition were rather be stable, FFAs were in the most accordance.....???? , IDF (1977). Therefore,

post-clarification temperature was one of important factors to be considered for the keeping quality of ghee product.

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REFERENCES

- [1] G. S. Rajorhia, "GHEE," *Encyclopedia of Food Sciences and Nutrition*, pp. 2883-2889, 2003.
- [2] C. R. Rachana, B. S. NathA, M. V. Reshma, and C. Armughan, "Variation in grainy texture of commercial ghee in relation to laboratory ghee and its blends," *J. International Dairy Technology*, vol. 6, pp. 1-8, 2012.
- [3] S. De, B. Brahma, S. Polley, A. Mukherjee, D. Banerjee, M. Gohaina, K. P. Singh, R. Singh, T. K. Datta, and S. L. Goswami, "Simplex and duplex PCR assays for species specific identification of cattle and buffalo milk and cheese," *J. Food Control*, vol. 22, pp. 690-696, 2011.
- [4] N. C. Ganguli and M. K. Jain, "Ghee: Its Chemistry, Processing and Technology," *J. Dairy Science*, vol. 56, pp. 19-25, 1972.
- [5] M. L. Sserunjogi, R. K. Abrahamsen, and J. Narvhus, "A Review Paper: Current Knowledge of Ghee and Related Products," *J. International Dairy*, vol. 8, pp. 677-688, 1998.
- [6] A. M. Fearon and D. E. Johnston, "A comparison of 3 instrumental techniques to evaluate butter spreadability," *J. Food Qual*, vol. 12, pp. 23-38, 1989.
- [7] *Milk Determination of fat content – Gravimetric method (Reference method)*, IDF standard 1E-2010.
- [8] *Milk Determination of fat content – Pasteurization: Definition and Method (Reference method)*, IDFA standard 2009.
- [9] *American Oil Chemistry Society*, AOCS standard 1999.
- [10] *Association of Official Analytical Chemists*, AOAC standard 2002a.
- [11] E. Choe and D. B. Min, "Chemistry of Deep-Fat Frying Oils," *J. Food Science*, vol. 72, pp. 86, 2007.
- [12] S. M. Abdulkarim, K. Long, O. M. Lai, S. K. S. Muhammad, and H. M. Ghazali, "Frying quality and stability of high-oleic Moringa oleifera seed oil in comparison with other vegetable oils," *J. Food Chemistry*, vol. 105, pp. 1382-1389, 2007.
- [13] T. Nagao, Y. Ayako, T. Mutsunobu, and M. Masafumi, "Color Deterioration of Oil during Frying," *J. oleo science*, vol. 55, pp. 51-57, 2006.
- [14] K. Aysegul and J. Issa, "Some chemical and microbiological properties of ghee produced in Eastern Anatolia," *J. Dairy Technology*, vol. 61, pp. 300-306, 2008.
- [15] B. Innawong, P. Mallikarjunan, and J. E. Marcy, "The determination of frying oil quality using a chemosensory system," *LWT - Food Science and Technology*, vol. 37, pp. 35-41, 2004.
- [16] P. H. Wishram and B.W. Hammer, "The Keeping Qualities of Ghee," *J. Dairy Science*, vol. 11, pp. 143-154, 1972.
- [17] P. J. Garc á-Moreno, R. Pérez-G ávez, A. Guadix, and E. M. Guadix, "Influence of the parameters of the Rancimat test on the determination of the oxidative stability index of cod liver oil," *LWT - Food Science and Technology*, vol. 51, pp. 303-308, 2013.
- [18] S. Paul, G. S. Mittal, and M. S. Chinnan, "Regulating the use of degraded oil/fat in deep-fat/oil food frying," *J. Food Science and Nutrition*, vol. 37, pp. 635-662.
- [19] J. Velasco, S. Marmesat, G. Murquez-Ruiz, and M. Carmen Dobarganes, "Formation of short-chain glycerol-bound oxidation products and oxidised monomeric triacylglycerols during deep-frying and occurrence in used frying fats," *J. Lipid science and Technology*, vol. 106, pp. 728-735, 2004.



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