Preparation and physicochemical characterization of ghee and murchita ghṛta

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Background: Ghṛta murchchana is a process of pre-treatment recommended in Ayurveda to purify ghee before it can be used for siddha ghṛta which is claimed to improve the properties of the ghee in general and that of the prepared siddha ghṛta.

Objective: This work is aimed at studying the physicochemical properties of ghee and murchchita ghṛta in order to understand the impact of ghṛta murchchana process.

Materials and methods: Ghee and murchchita ghṛta were prepared from the milk of local Pahadi, Jersey and Holstein cows. The samples were characterized by FTIR spectroscopy, differential scanning calorimetry and free fatty acid measurements.

Results: Among the samples studied, the Holstein cow ghee was found to contain the least amount of free acid (1.34%) whereas ghṛta murchchana process led to further decrease in the free acid content polymorphism was observed in the samples as evidenced by multiple melting points. In most cases, murchchita ghṛta was found to contain less solid fat than the corresponding ghee implying that the high melting compound was converted to low melting one during the process.

Conclusion: The observed lowering of free fatty acid and solid fat contents in the ghee samples may provide a possible validation to the performance enhancement of the ghṛta murchchana process.

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1. Introduction

Ghee is a specialized form of clarified butter indigenous to South Asia, usually prepared from milk of cows or buffalos [1]. Several commercial products (such as clarified butter, butter-oil and other indigenous milk products) commonly used in different regions are closely related to ghee. However, as demonstrated by Sawaya et al. [2] and Baker et al. [3], they must be understood as distinct products. Ghee is mainly used as food additive for pleasant smell and taste. It can substitute oil for frying, especially that involves high temperature as it has higher smoke point than the traditional oil.

Nevertheless, due to higher cost of ghee than oil, its use in cooking is limited only to high economic status population [4]. On the other hand, consumption of saturated-fat-rich food is usually associated with chronic heart disease and insulin resistance in certain population, although the impact of trace components on these effects are yet to be ascertained [5,6]. It could be of interest to develop ghee to decrease its cost and make it available to more important population.

Ayurveda, the traditional medicinal practice in South Asia, considers ghee as a superior food. Ghee (alone or with other herbs), preferably obtained from cow milk, has been recommended in Ayurveda as medicine to improve memory, digestion and intellectual performance [7,8]. Moreover, ghee is used in combination with different kinds of herbs for formulating wide variety of drugs while maintaining its inherent qualities [9]. Ghee is a component of two common Aṣṭavaṇḍu mixtures: paṅca-gavya which is a blend of five
cow products namely milk, yoghurt, urine, dung and ghee; and \textit{paicamata} which comprises milk, yoghurt, honey, sugar and ghee. Old ghee, 100 years or older, is believed to have enhanced remedial properties and can be applied topically to cure the ailments of skin, ear, head and reproductive organs [10]. Ghee based formulations have also been found effective as antibiotic to prevent surgical site infection [11].

Traditionally, ghee is obtained from milk using extensive procedure including fermentation of boiled milk (with cream included) with curd containing \textit{Lactobacillus} followed by churning of the yoghurt hence obtained. The butter is then separated from buttermilk and simmered to obtain ghee. We have to notice that there are also other methods used to prepare ghee which may have different compositions than the traditional one [12,13]. Moreover, the origin of milk (goat, cow and buffalo [14–16]), the choice of the processing technique, and the storage method, leads to different fatty acids distribution and as a consequence to different final product in term of taste. For instance, β-carotene present in cow’s ghee gives it characteristic yellow tinge, which is lacking in ghee from buffalo milk. It has been observed that, not only among cattle, quality of ghee also differs among the breeds of cows. Sindhi cow ghee was found to be more stable than that from Jersey cow although the latter was found to be superior while in fresh state [17]. It is of interest to know that Sindhi cow ghee have more unsaturated fatty acid than Thari cow ghee [18]. Due to differences in methods used in above works, Sindhi and Thari cow ghee, however, could not be precisely compared.

Our interest in the present work lies in the process of \textit{sneha kalpana}, which is a drug formulation procedure, by boiling herbs with ghee, oil, animal fat or bone marrow as substrate [19], ghee being the most preferred choice [20]. \textit{Siddha ghrta} so prepared is used in a wide variety of conditions [21]. However, before its use, Ayurveda demands the ghee be purified. \textit{Ghrta murchana} is the process of purification of ghee by treatment with certain herbs to obtain \textit{murchita ghrta}, the ghee suitable to be used for preparing drug formulations [9]. \textit{Ghrta murchana} is believed to improve the extraction power and shelf-life of ghee as well as to improve the efficacy of medicinal formulations and to remove any unpleasant odor [9]. Although clinical impacts of \textit{ghrta murchana} have been studied [22,23], only limited attempts have been made to determine the changes that occur during the \textit{murchana} process. Further, no attempts have been made to understand the dynamics of this process and to establish structure–property relationship.

This study aims at understanding the physiochemical properties of the ghees and \textit{murchita ghrta} in order to provide reliable evidence for or against the claimed effects [9] of \textit{ghrta murchana} process.

2. Methodology

Three different cow breeds viz. Holstein, Jersey and Local Pahadi reared on same farm was selected for milk collection. The collected milk was boiled and allowed to cool to room temperature. 100 mg of thermophilic culture (Chr Hansen STI-13 containing \textit{Streptococcus thermophilus}) was added and incubated at 30 °C for 12 h to form yoghurt. Commercial culture was chosen to ensure reproducibility. \textit{Lactobacillus} is generally found to be used in such experiment.

\textit{S. thermophilus} was preferred over \textit{Lactobacillus} in the present case as the former was more widely used culture in local market and hence easily available. Diluted yoghurt was then churned at 1400 rpm and butter separated manually. The butter was simmered at 115 °C until the boiling stopped and residue turned brown. The mixture was filtrated and the filtrate obtained was the required ghee sample. The sample preparation overview is shown in Fig. 1.

Commercial product from Kathmandu, the Sita Ram’s Pure Ghee Batch No 3/2017, was used as reference. \textit{Murchita ghrta} was prepared from the ghee sample by boiling ghee with herbs in the proportion mentioned in Table 1 using standard method described in \textit{bhaisajya kalpana vijnana} [9]. Briefly, Ghee was heated with constant stirring until the typical boiling sound stopped. \textit{Curcuma longa} suspension was added and the ghee was boiled until its volume was reduced to one-fourth. Same process was repeated with \textit{Emblica officinalis} juice. Remaining herbs and water were then added. The heat was cut off when water vapour disappeared and herbs could be rolled into wick. The mixture was filtered to obtain \textit{murchita ghrta} as filtrate. Hence, four \textit{murchita ghrta} samples were obtained from four ghee samples, which are indexed in Table 2.

The samples were characterized by free fatty acid (FFA) content analysis, Fourier Transform infrared (FTIR) spectroscopy and differential scanning calorimetry (DSC). FFA was determined by IUPAC standard methods for the analysis of oils, fats and derivatives with some modification [24]. Briefly, 0.5 g of sample was dissolved in 50 mL of 1:1 (v/v) mixture of ethanol and diethyl ether. The solution was titrated against 0.01 N NaOH solution with 1% phenolphthalein as indicator to determine the equivalent free fatty acid value as mass percentage equivalent of oleic acid.

FTIR spectra of solid samples were obtained from 400 cm$^{-1}$ to 4000 cm$^{-1}$ with 2 cm$^{-1}$ resolution. IRTRacer-100 from Shimadzu was used to obtain the FTIR spectra using pellets of sample with KBr. Mettler Toledo DSC12E was used to obtain the calorimetric data. The specimen was heated on hermetically sealed aluminium crucible with air as reference. DSC heating curve was obtained from with a heating rate of 5 °C min$^{-1}$. The sample was previously heated to 80 °C, held for 15 min, cooled and held at 10 °C to remove thermal memory of solid. Heating, cooling and holding time was same in each step. Solid fat content (SFC), the mass percentage of fat present as solid, was determined by partial integration of DSC curve [25,26].

![Fig. 1. Schematic diagram showing the different stages for preparation of ghee and murchita ghrta samples.](image)

Table 1: Ingredients used in the present work for murchita ghrta preparation and their concentration.

| S.N. | Nepali Name | English/Botanical Name | Parts by weight |
|------|-------------|------------------------|-----------------|
| 1    | ghyu        | Ghee                   | 100.00          |
| 2    | pani        | Water                  | 400.00          |
| 3    | amalū        | Emblica officinalis     | 6.25            |
| 4    | haledo      | Curcuma longa           | 6.25            |
| 5    | nigrumamthe  | Cyprus rotundus         | 6.25            |
| 6    | barro       | Terminalia chebula      | 6.25            |
| 7    | barro       | Terminalia bellirica    | 6.25            |
| 8    | kāgati      | Citrus limon            | 6.25            |
3. Result and discussion

The yield of each product during ghee preparation with respect to boiled milk is shown in Table 2. Ghee from Local cow milk (LG) was obtained with the yield of 2.86%, which is the highest yield obtained among the test samples. All the breeds of cow gave the yield of ghee between 2% and 3% with Holstein cow having the least yield. Local cow milk (LG) also gave the highest yield with respect to mass of ghee and Jersey cow the intermediate yield.

All the samples showed decrease in mass after ghṛta mūrchna despite addition of herb components during the ghee processing. The yield of mūrchna ghṛta also followed the same trend as that obtained for the ghee of corresponding cow with Local cow (LM) giving the highest yield of mūrchna ghṛta (1.81%). Local cow’s ghee (LG) also gave the highest yield with respect to mass of ghee (63.2%) while Holstein cow’s ghee gave the least yield (39.7%). The samples can easily be grouped in two categories with the raw ghee with high yield and mūrchna ghṛta with low yield. Lower yield of mūrchna ghṛta than that of corresponding ghee indicates some sort of decomposition process occurring during mūrchna process. However, no new functional groups are obtained as demonstrated by the lack of new peak in the FTIR spectrum. Hence, it can be assumed that ghṛta mūrchna process results in changes hat the decomposition products are lost through evaporation and/or decant as solid residue. The photographs of the prepared samples are in Supplementary Information.

FVA values, which represent the fraction of unesterified fatty acids, are displayed in Table 2. Holstein cow ghee (HG) was found be least acidic with 1.34% oleic acid equivalent while market ghee (CG) was found to contain highest free acid (2.49%). Decrease in FFA value was seen in all samples after mūrchna except on the ghee of Jersey cow. After mūrchna the mūrchna ghṛta of Local cow ghee (LM) had the lowest free fatty acid. Free fatty acid in fat promotes microbial growth and decreases the shelf life of fat.

The lowering of fat content might be the reason why mūrchna process is claimed to increase the shelf-life of the fat. Thus, it justifies the recommendation of the mūrchna process for increasing of shelf-life of the ghee in Ayurveda [14]. Although higher liquid fraction in mūrchna ghṛta can make mūrchna ghṛta more susceptible to contamination than the corresponding ghee, this is not a big issue as the liquid phase is non-aqueous where microbial growth is highly inhibited [27,28].

FTIR spectra of the investigated samples are shown on Fig. 2. As the sample is rich in fat, we can expect the peaks from C–H and C–C bonds in the carbon skeleton. These include peaks at 723 cm⁻¹, 1169 cm⁻¹, 1238 cm⁻¹, 1377 cm⁻¹, 1463 cm⁻¹, 2852 cm⁻¹ and 2922 cm⁻¹ for C–H vibrations of –CH2- and –CH3 and groups. The peaks at 968 cm⁻¹, 1417 cm⁻¹, 1651 cm⁻¹ and 3006 cm⁻¹ indicates the sample also consists of (Fig. 3) unsaturated fatty acids such as ones depicted in Fig. 3b and c [29]. Indeed, among many types of fats, triacylglycerols are the most common ones, which are esters of glycerol with three different fatty acids. The nature of fatty acid chains of triacylglycerols determine the physical and chemical properties of the fats [30].

The C=O stretching of ester at 1745 cm⁻¹ and its overtone at 3468 cm⁻¹ confirms the presence of triacylglycerols [31]. The C=O stretching peak at 1238 cm⁻¹ further supports presence of ester group. Presence of shoulder around 1703 cm⁻¹ for the samples indicates the presence of free acids [32]. Absence of broad peak in the range of 3200 cm⁻¹ to 3700 cm⁻¹ shows that the sample is moisture free. The peaks centred at 2337 cm⁻¹ are due to atmospheric CO2 [33,34]. Thus, no peak appears or disappears after ghṛta mūrchna, which indicates that mūrchna does not change the functional groups.

![Fig. 2. FTIR spectra of ghee (solid) and mūrchna ghṛta (dotted) from milk of Holstein (HG/HM), Jersey (JG/JM) and Local (LG/LM) cow and commercial ghee (CG/CM).](image)

![Fig. 3. Schematic structures of some important chemical compounds associated with ghee.](image)

Table 2
Designation of the investigated samples with their specimen codes, relevant descriptions and free fatty acid (FFA) contents.

| Code   | Description                              | FFA (% of oleic acid) | Yield (% from milk) | Yield (% from ghee) |
|--------|------------------------------------------|-----------------------|---------------------|---------------------|
| CC     | Commercial ghee                          | 2.49                  | –                   | –                   |
| LG     | Ghee from Local cow milk (LG)            | 1.45                  | 2.86                | –                   |
| JG     | Ghee from Jersey cow milk (JG)           | 1.43                  | 2.68                | –                   |
| HG     | Ghee from Holstein cow milk (HG)         | 1.34                  | 2.19                | –                   |
| CM (from CG) | mūrchna ghṛta from CG | 1.09                  | –                   | 59.4                |
| LM (from LG) | mūrchna ghṛta from LG | 0.80                  | 1.81                | 63.2                |
| JM (from JG) | mūrchna ghṛta from JG | 1.61                  | 1.48                | 55.2                |
| HM (from HG) | mūrchna ghṛta from HG | 0.87                  | 0.87                | 39.7                |
DSC curves are shown on Fig. 4. The signals obtained show a series of endothermic and exothermic events. This is a complex signal already obtained on such complicated substances [25]. It is possible to distinguish two melting temperature domains with a crystallization session in between. Both melting domains are in fact a succession of multiple endothermic peaks. This is because many different polymorphic crystalline forms of fat are reached during sample preparation. The DSC curve resembles the curve obtained by Lopez et al. [35] which also has two melting domains but does not have a crystallization session.

In fact, three crystal structures called: α, β and β′ can be achieved from triacylglycerols [36]. The α-form has hexagonal cell for which the adjacent carbon chains can oscillate between parallel and perpendicular arrangements. It’s melting temperature is expected to appear from 17 °C to 22 °C and transition to β from 0 °C to 22 °C. The β′ crystal has orthorhombic cell with each carbon chain is perpendicular to the adjacent one. Its melting range is 20 °C–27 °C and β′ to β transition occurs between 5 °C and 27 °C. Finally, β form is triclinic cell with all carbon chain parallel. It melts between 29 °C and 34 °C. α is the least stable while the stability of β and β′ depends on the type of triacylglycerol. Usually for milk fats β′ is more stable than β. When heating, the crystals can melt directly to liquid or transition from α to β′ to β in that order [37,38]. Thus, the DSC curve obtained show the melting of α and β′ between 15 °C and 25 °C, a transformation of β′ to β around 30 °C and β melting above 35 °C. Finally, we observe that the same event occurs for all the samples in the same temperature domains, only the magnitude of each event seems different.

The SFC procedure performed on the DSC data (Fig. 4) gives the curve presented in Fig. 5. It can be immediately concluded that the murchchana process decreases the SFC at all the temperatures of all ghee except Jersey cow ghee. As low SFC improves the extraction efficiency of the ghee and promotes emulsification, murchchita ghrta can improve the medicine’s efficacy. At body temperature (37 °C), Jersey cow ghee has been found to contain only 10.6% solid fat which is the least among the ghee samples making it easiest to digest [39]. However, among the murchchita ghrta samples, murchchita ghrta from commercial ghee had the least solid fat at body temperature (3.7%). The β recrystallization from β′ can be clearly seen as a maximum in SFC curve (Fig. 5).

4. Conclusion

Ghee and murchchita ghrta samples prepared showed general similarity among each other with significant specific differences. The laboratory prepared samples contained less free acids than the commercial samples and the amount of free acids was found to decrease after ghrta murchchana process. As murchchita ghrta contains less free fatty acid and solid fat than the corresponding ghee, the traditional through the murchchana process can be justified. However, further extensive work with large sample size, full fatty acid profile and the effect of bacterial strain in fat composition is needed to further understand the dynamics of ghrta murchchana.

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Conflicts of Interest

None.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jaim.2020.06.004.

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