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Docosahexaenoic acid content is significantly higher in *ghrita* prepared by traditional Ayurvedic method

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ABSTRACT

Background: Ghee (clarified butter) also known as *ghrita*, has been utilized for thousands of years in Ayurveda. Ghee is mostly prepared by traditional method in Indian households or by direct cream method at industry level. Ayurvedic classics mention that *ghrita* made from cow milk is superior. However, there is no scientific comparison available on preparation methods and essential fatty acids content of *ghrita.* **Objective:** To investigate fatty acid composition of *ghrita* prepared by traditional/Ayurvedic method and commercial method (direct cream method). **Materials and Methods:** Fatty Acid Methyl Esters (FAME) extracted from *ghrita* samples were analysed on Gas Chromatography (GC) Shimadzu B using capillary column BPX70 (0.32 mm*60 m, ID of 0.25 mm). The fatty acids in the samples were identified by comparing peaks with the external standard 68A (Nu-Chek-Prep, Inc.USA). Significant differences between the experimental groups were assessed by analysis of variance. **Results:** Distribution of fatty acids was compared in *ghrita* samples prepared by traditional method and direct cream method which is commercially used. Saturated fatty acids were predominant in both the groups. Mono unsaturated fatty acids and poly unsaturated fatty acids were in the range of 17-18% and 3-6% respectively. DHA content was significantly higher in ghee prepared by traditional method using curd starter fermentation. **Conclusion:** The findings suggested that *ghrita* prepared by traditional ayurvedic methods contains higher amount of DHA; Omega-3 long-chain polyunsaturated fatty acids, which is a major component of retinal and brain tissues and remains important in prevention of various diseases.

Key words: Ayurveda, Docosahexaenoic acid, ghrita, Omega-3 fatty acids, traditional method

INTRODUCTION

Ghee or *Ghrita* is obtained by clarification of milk fats by heating and has longer shelf life at room temperature as compared with butter. Fatty acid profile of ghee is complex and shown to contain high levels of saturated fats and cholesterol. Composition varies from source of ghee such as goat milk, sheep milk, cow milk, and buffalo milk.[1,2]

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Ghee is mostly prepared by traditional method in Indian households or by direct cream method at industry level.[3] The quality of ghee depends on the type and quality of milk and method of preparation.[4,5] Ghee is fairly shelf-stable largely because of its low moisture content and possible anti-oxidative properties. Shelf life of ghee is also dependent on the method of preparation. The storing quality of desi ghee is better than that of direct cream or creamery butter ghee because of the presence of phospholipids.[6]

The physical and chemical characteristics of the butter and ghee from cow, goat, and sheep milk are reported.[1,7] Ghee contains 99-99.5% fats and less than 1% moisture, unsaponifiable matter and traces of charred casein, carotene, and fat-soluble vitamins.[8] Fatty acid profile determines many of the physical, organoleptic, and nutritional characteristics of milk and dairy products.[9] Milk fat contains over 400 individual fatty acids and their isomers. Cow milk contains large amounts of saturated fatty acids (SFA), particularly C14:0 and C16:0, and small amounts of mono unsaturated fatty acids (MUFA), poly unsaturated fatty acids (PUFA) and omega-3 fatty acids with beneficial effects on human health.[10]

Many attempts have been made to alter the fatty acid composition of milk fat from dairy cows to improve the long chain poly unsaturated fatty acids (LCPUFA) content.[11,12] The biosynthesis of LCPUFAs such as docosahexenoic acid (DHA) and arachidonic acid (AA) takes place by desaturation and elongation of precursors such as α-linolenic acid (ALA, 18:3n-3) and linoleic acid (LA, 18:2n-6), respectively. Precursors ALA and LA supplied with forage undergo bioconversion in rumen and mammary glands forming DHA and AA.[9] Major sources of DHA include fish such as mackerel, salmon, tuna, fish oil, and deep-sea algae.[13]

Ghee also known as *"Ghrita"* is extensively used in Ayurveda practice. Ghee is strongly recommended in daily diet and Ayurvedic therapeutics such as *"Panchakarma"* or *"Anupana"* (Vehicle) for various medicines. Traditionally ghee is made from cow milk or any other milk after churning curdled whole milk, separating the butter after fermentation, and clarifying it by heating it in pan on low flame. Alternatively, it is prepared by clarifying cream collected from raw whole milk at industrial scale. Ayurvedic classics describe eight kinds of ghee from eight different animal milk; among them ghee made from cow milk is said to be the superior.[14] The present study was carried out to investigate fatty acid composition of *"ghrita"* prepared by traditional household/Ayurvedic method[15] and commercial method (direct cream method).[8]

MATERIALS AND METHODS

Preparation of "*Ghrita*" by traditional ayurvedic method

Cow milk was boiled and cooled at room temperature. About 10% curd was added as starter culture, mixed, and incubated for 8-10 hrs at room temperature. Curd formed was manually churned until butter floated on top of the buttermilk. Butter was washed 3-4 times using water to remove residual butter milk and then heated in stainless steel container till it is clarified. Clear liquid *"ghrita"* was then decanted in a glass container.

Preparation of "*Ghee*" by direct cream method

Cream is separated from whole cow milk and heated in stainless steel pan. Clear molten *"ghrita"* is decanted and stored in glass container.

Analysis of fatty acids

Modified Bligh and Dyer method was used for fatty acid analysis.[16] Briefly, 0.1 ml of molten "ghrita" was taken into glass test tube, followed by addition of 10 ml of 0.6 N methanolic HCl. Samples were incubated at 80°C for 2 hrs. Samples were extracted with hexane three times. Hexane extracts were mixed together and concentrated under vacuum evaporation. Analysis of fatty acid methyl esters (FAME) was done on GC Shimadzu B using capillary column BPX70 (0.32 mm*60 m, ID of 0.25 mm). The fatty acids in the samples were identified by comparing peaks with the external standard 68A (Nu-Chek-Prep, Inc. USA).[17]

Statistical analysis

Data was analyzed using the Statistical Package for the Social Sciences (SPSS software version: 11, Chicago, IL). Significant differences between the experimental groups were assessed by analysis of variance. All data are expressed as mean ± standard error of mean (SEM); P value less than 0.05 was considered to be significant.

RESULTS

Distribution of fatty acids was compared with *ghrita* samples prepared by traditional method and direct cream method, which is commercially used. Saturated fatty acids were predominant fatty acids in both the groups. Mono unsaturated fatty acids (MUFA) and poly unsaturated fatty acids (PUFA) were in the range of 17-18% and 3-6%, respectively. No significant difference was observed between the two acids [Table 1].

Distribution of selected n-6 and n-3 fatty acids was analyzed in *ghrita* samples prepared by both the methods. It was observed that there was no significant difference in precursor fatty acids such as LA and ALA contents in *ghrita* samples. DHA content was significantly higher in ghee prepared by traditional method using curd starter fermentation [Table 2].

DISCUSSION

DHA contributes to reduced risk of diseases like heart attack, cancer, insulin resistance, arthritis,[18] and ADHD.[19] Ghee is generally used in Ayurveda. According to Ayurveda classics *ghrita* promotes longevity and protects body from various diseases.[20] It increases digestive fire *agni* and improves absorption and assimilation. It nourishes *dhatus*, improves memory, and has lubricating activity in joints. Most of these activities are known to be imparted

Table 1: Distribution of fatty acids in *Ghrita* prepared by traditional method and direct cream method

Fatty acid % distribution	*Ghrita* prepared by traditional method	*Ghrita* prepared by direct cream method
SFA	72.4	73.2
MUFA	18.6	17.5
n-6	5.38	6.3
n-3	3.7	3.1
n-3: n-6 ratio	0.69	0.5

SFA=Saturated fatty acids, MUFA=Mono unsaturated fatty acids

Table 2: Distribution of selected Omega-6 and Omega-3 fatty acids in *Ghrita* prepared by traditional method and direct cream method (*n*=3)

Fatty acid % distribution	*Ghrita* prepared by traditional method	*Ghrita* prepared by direct cream method
LA	5.1±0.544	6.2±1.29
AA	0.157±0.061	0.132±0.037
ALA	3.66±0.88	3.0±0.7
AA	0.218±0.096	0.169±0.95
DHA	0.083*±0.003	0.062±0.002

Values are Mean±SEM, * Value significant with $P \leq 0.05$, LA=Linoleic acid, AA=Arachidonic acid, ALA=α-linolenic acid, DHA=Docosahexaenoic acid

by anti-oxidants and essential fatty acids such as DHA. We observed consistent and reproducible increase in DHA content in ghee prepared by traditional method in controlled laboratory conditions, however, this needs to be confirmed at field level. Whether traditional method of ghee preparation protects unsaturated fatty acids from trans fat formation is unknown. Limitation of this study is that the trans fats and anti-oxidant levels of ghee samples prepared by both the methods were not measured.

We observed increase in DHA content of *ghrita* prepared by traditional fermentation method, which may be attributed to rich microbial flora used in starter curd culture. Lactic acid bacillus cultures along with added lactose and fructose are shown to promote formation of conjugated linoleic acid.[21] Further analyses of microbial community and fatty acid profile are required to establish presence of desaturase enzyme producing bacteria responsible for conversion of precursor fatty acid ALA to DHA. DHA along with fat-soluble vitamins, anti-oxidants, and conjugated linoleic acid (CLA) could be responsible for health benefits of *ghrita*.

There are other considerations for the traditional processing of ghee. For example, mango seed kernels have been used to increase the shelf life of ghee. It has now been found that phenolics and phospholipids isolated from mango seed kernel, when added jointly to buffalo ghee, prevented peroxidation of fats and helped in extending the shelf life of ghee.[22] Also, Ayurveda mentions about enhanced therapeutic qualities of very old cow ghee. It will be interesting to study the composition of very old cow ghee as well as physicochemical and organoleptic characteristics at various time intervals. Use of traditional ayurvedic methods to prepare *ghrita* needs to be studied for its possible role in promoting health.

CONCLUSION

The findings suggested that ghrita prepared by traditional ayurvedic method contains higher amount of DHA with beneficial effects on human health.

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