Original Article

Physico - Chemical Analysis of Purana Ghrita (Old Clarified Butter) with special reference to Fatty Acid Profile

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Abstract

Background: In Ayurveda, fats and oils have been utilized for numerous therapeutic purposes. Among fats, cow ghee is mentioned to be utilized in fresh and old forms. Old ghee is specifically indicated in epilepsy and mania. These indications represent high lipid solubility of old ghee and its action on CNS. Few analytical researches are available on fresh cow ghee but no research is done on 15 years old cow ghee.
Aim: Present work aimed on Physico-chemical analysis of *Purana Ghrita* (Old Clarified Butter) with special reference to fatty acid profile and its comparison with fresh ghee.
Material and methods: Fresh cow ghee was self prepared and compared for physico-chemical and fatty acid profile with old cow ghee which was also self prepared in 2003 and stored in airtight glass bottle.
Observation and results: Colour and odour of fresh ghee and old ghee are yellowish white with specific odour of cow ghee and creamish white with distinguishing odour than fresh cow ghee respectively. Specific gravity, Refractive Index, Iodine value, Saponification value, Acid value and Peroxide value of fresh and old ghee were 0.91, 1.459, 30.67, 203.76, 1.62, 0 and 1.07, 1.465, 42.26, 347.28, 75.88, 37.54 respectively. The proportion of Capric acid, Lauric acid, Stearic acid and Oleic acid is higher in fresh Ghee than *Puran* Ghee. On the other hand, *Puran* Ghee showed much higher proportion of Myristic acid, Palmitic acid and linoleic acid than fresh Ghee.
Conclusions: Both fresh and old ghee contain therapeutically significant constitutes. The differences in the proportion of these constitutes indicates use of fresh ghee as anti-oxidant and in gastro-intestinal disorders; and old ghee can be claimed as beneficial in CNS disorders.
Key words: Fresh ghee, Old ghee, Fatty acid profile therapeutic use use of old ghee.

Introduction:
Ghee is the elixir of life. The history of consumption of ghee is very old. Regarding value of ghee in day to day life, the references are found in old classical books "Vedas" (approximately 3rd century BC). It occupies the unique place in the life of human and so closely woven in life that practically it is inseparable.

Four types of unctuous substances are mentioned in Ayurveda. ie, *Ghee*, *Taila*(Oil), *Vasa* (Animal fat) and *Majja* (Bone marrow). Out of these, Ghee is considered as superior owing to its special attributes ie, *Samskarsya Anuvaertanm* of mean that Ghee carries the properties of the drugs without leaving its inherent properties.[1] This is unique nature of ghee which makes its wider application in different disease conditions by processing it with specific drugs as per condition. Ayurveda recommends cow's ghee as best for both food and medicinal purpose.[2] According to Ayurveda ghee promotes longevity and protects the body from various diseases. It increases the digestive fire (*Agni*) and improves absorption and assimilation. It nourishes the *Ojas*, the subtle essence of all the body tissues. It improves memory and strengthens the brain and nervous system. It lubricates the connective tissues there by rendering the body more flexible. With regard the three *Doshas*, ghee pacifies *Vata* & *Pitta* and is acceptable for *Kapha* in moderation.[3]

Ayurveda texts classified ghee in to two types viz., *Nava* (new) and *Purana* (old) ghee. Freshly prepared ghee is *Madhura* in *Rasa* & *Vipaka* and alleviates *Vata* and *Pitta* where as old ghee is *Katu* and *Tikta* in *Rasa* and *Katu* in *Vipaka* and alleviates *Vata* , *Pitta* and *Kapha*. Thus older the ghee, better the qualities. As per Bhava Prakasha, ghee stored for one year in air tight container is called *Purana* Ghee[4]. As per Charaka, it should be 10 years old and useful in the treatment of *Unmad* (psychosis) [5]

The old ghee is considered more therapeutic

value in eye diseases. Yogratnakar[6] , Bhavprakash[7] mention as the ghee gets old, its increases in potency. It is mentioned that ghee is preserved for 100 yrs it attains the power to cure all the diseases. When administered according to the prescribed procedure, it increases, thousand times in potency and develops manifold utilities.[8]

References are available for the contents and analytical values of ghee. But till date no efforts are actually made to evaluate the analytical properties of Purana Ghee in spite of its rich therapeutic value. Considering this fact, an attempt has been made to study and explore the value of Ayurvedic concept of Purana Ghee by using modern analytical parameters.

Objectives of the study:

I) To assess the physical and chemical properties of *Puran Ghee*.

ii) To compare the properties of *Purana Ghee* with fresh ghee and to establish Pharmacopeial standards of *Purana Ghee* .

Materials & Methods :

Materials-: Collection of *Puran Ghee*:

Purana Ghee was taken from investigator himself who has prepared the *Ghee* 15 yrs back (February 2003) and stored in a glass container. Organoleptic characters, physicochemical analysis, microbial contamination were studied in analytical lab as per API standards. Fatty acids profiling was done at Anocon laboratories Pvt Ltd , Nagpur.

Method Preparation of *"Ghee"*

Cow milk was boiled and cooled at room temperature. About 5 % curd was added as starter culture, mixed, and incubated for 8-10 hrs at room temperature. Curd formed was manually churned with wooden churner until butter floated on top of the buttermilk. Butter was separated from buttermilk and washed 3-4 times using water to remove residual butter milk and then heated in stainless steel container till it is clarified. Clear liquid "*Ghee*" was then decanted, filtered through a piece of cloth and stored in a glass container. (Table 1 & 2)

Table 1 : Ingredients and quantity of each batch for Ghee preparation (Total 3 batches)

S.N.	Milk	Curd added for fermentation	Proportion	Butter obtained after churning	Ghee obtained
1	1 lit	50 gm	20:1	190 gm	85 gm
2	1 lit	50 gm	20:1	179 gm	78 gm
3	1 lit	50 gm	20:1	182 gm	80 gm
Average		50 gm	20:1	183.6 gm	81 gm

Table no.2: Observations of *Ghee preparation*

Sr.	Observational parameters	Batch A	Batch B	Batch C	Average
1	Total quantity of Cow milk	1 lit	1 lit	1 lit	1 lit
2	Quantity of Curd added	50 gm	50 gm	50 gm	50 gm
3	Temperature of milk during addition of curd	34 °C	34 °C	34 °C	34 °C
4	Average temperature while boiling milk	97 °C	98 °C	97 °C	97.5 °C
5	Duration of heating of milk	15 min	16 min	15 min	15.5 min
6	Obtained quantity of butter	190 gm	179 gm	182 gm	183.66 gm
7	Average temperature while heating butter	87 °C	88 °C	86 °C	87 °C
8	Maximum temperature while heating	120 °C	118 °C	121 °C	119.66 °C
9	Duration of heating butter	28 min	29 min	31 min	29.33 min
10	Total duration of heating	43 min	45 min	46 min	44.66 min
11	Obtained quantity of Ghee	85 gm	83 gm	82 gm	83.33 gm
12	Weight loss (in comparison to butter)	105 gm	96 gm	100 gm	100.33 gm
13	% weight loss	55.26	53.63	54.94	54.61
14	Temperature during filtration	45 °C	46 °C	45 °C	45.33 °C

a) Analytical Study:

Analytical study was done to establish the basic standards for *Puran Ghee* as there is no pharmacopeia standard guideline. The Ghee sample was first tasted for organoleptic parameters such as odour and colour. (Table no. 3) Physicochemical analysis includes Weight per ml (Specific gravity), Refractive Index,, Iodine value, saponification Value, Acid value,& peroxide value. (Table no. 4) Microbial specification were tasted to validate its safety for internal as well as external use. Enteriobacteriaceae, Total fungus count, E-coli, Salmonella, *Staphyliococus Aureus* and *Psudomonas Aueruginosa* were performed as par CCRAS parameters. (Table no. 5) Analysis of samples was conducted as par API standards in analytical lab of Mahatma Gandhi Ayurved Collage hospital and Research center, Salod (H) Wardha, Maharsashastra. Under Fatty acids profiling Capric acid, Lauric acid, Myristic acid, Palmitic acid, Stearic acid, Oleic acid & linoleic acid were tested. (Table no.6)

Observation & Results:

Table no.3: Average observations of organoleptic characters of milk, butter, fresh Ghee and *Puran Ghee*

Sr.	Parameter	Milk	Butter	Fresh Ghee	*Puran Ghee*
1	Colour	White	White	Yellowish white	Creamish white
2	Touch	Smooth	Smooth	Smooth	Smooth
3	Taste	Slightly Sweet	Sour	Slightly Sweet	Slightly Sweet
4	Appearance	Non transparent	Non transparent	Translucent	Translucent
5	Odour	Cow milk odour	Rancid	Specific odour of cow ghee	Distinguishing odour than fresh cow ghee

Table no. 4: Average results of Physico-chemical parameters of *Puran Ghee*

Parameters	Pharmacopeia standard	Obtained values (gm/ml)		Inference
		Fresh Ghee	*Puran* Ghee	
Specific gravity	Not available	0.91	1.07	Acceptable
Refractive Index	Not available	1.459	1.465	Acceptable
Iodine value	Not available	30.67	42.26	Acceptable
Saponification value	Not available	203.76	347.28	Acceptable
Acid value	Not available	1.62	75.88	Acceptable
Peroxide value	Not available	0	37.54	Acceptable

Table 5: Average results of Microbiological specification of *Puran* Ghee

Parameters	Pharmacopeia standard	Fresh Ghee	*Puran* Ghee
Total viable count	Maximum 10^5 / gm	No growth	No growth
Enterobacteriaceae	10^3 / gm	Absent	Absent
Total fungus count	Maximum 10^3 / gm	Absent	Absent
E-coli	Maximum 10 / gm	Absent	Absent
Salmonella	None	Absent	Absent
Staphyliococus aureus	Absent	Absent	Absent
Psudomonas aueruginosa	Absent	Absent	Absent

Table 6 : Average results of Fatty Acid Profiling of *Puran Ghee*

S.N.	Test parameter	Measurement unit	Test method	Fresh Ghee	*Puran* Ghee
1.	Capric acid	g/100g	GCMS/MS	3.0	0.003
2.	Lauric acid	g/100g	GCMS/MS	8.8	0.053
3.	Myristic acid	g/100g	GCMS/MS	9.9	18.158
4.	Palmitic acid	g/100g	GCMS/MS	26.1	63.287
5.	Stearic acid	g/100g	GCMS/MS	9.1	5.629
6.	Oleic acid	g/100g	GCMS/MS	24.7	9.811
7.	linoleic acid	g/100g	GCMS/MS	1.8	2.059

Discussion:

Every substance possesses specific combination of single or several constitute and this is basis of significance of analytical methods. It is also understood that, time, effect of environmental factors and artificial processing's changes either the arrangement, proportion or even the constitutes. Due to this the physico0chemical analysis of various food products, cosmetics and medicines is made mandatory by regulatory authorities. Such analysis helps in identification, authentication, preparation of new compounds, establishing the correlation of material properties and the values obtained in analytical parameters as well as to make assumptions related to utility and characteristics of a material. In medical field, the analytical studies help to forms the basis of standardization. In the context of Ayurveda medicines, the classical texts have mentioned the pharmacological properties of numerous drugs as well as formulations and analytical studies helps to understand the rationality behind the processing, specific combination, their proportion etc. However, there are few descriptions in Ayurveda where time itself is allowed to do changes in a substance and utilization of such time processed substance is indicated by ancient seers. *Guggul* (*Commifera mukul*), *Bhallatak* (*Semicarpus anacardium*), *Guda* (Jaggary) and Ghee are the well known examples of time processed drugs. Therefore to know the effect of time on physic-chemical parameters of such drugs, present work was done in which sample of *Puran* (15 years old Ghee) was analyzed.

The procedures adopted during market samples of Ghee are not known and the time passed after freshly preparing Ghee and purchasing may lead minor deviation in analytical profile. Therefore for comparative study, fresh Ghee was prepared in three batches to establish standard manufacturing procedure of fresh Ghee as well as to avoid any change in chemical profile of Ghee. Average 83.33 gm ghee was obtained from 1 lit milk and one batch was selected for analytical study in comparison to *Puran* Ghee. There is significant difference detected in colour and odour of fresh (yellowish white with specific odour of cow ghee) and *Puran* Ghee (Creamish white with distinguishing odour than fresh cow ghee). Colour is indicative of specific chemical profile and significant change in chemical profile leads to change in colour. The chemical constitutes are related to pharmaco-therapeutic attributes, hence the difference in colour of both samples is indicative of the difference in chemical nature and thus difference in therapeutic properties too.

The specific gravity of a liquid is the weight of given volume of the liquid at 25°C (unless otherwise specified) compared with the weight of an equal volume of water at the same temperature[9]. The specific gravity of a sample can be correlated to density of arranged molecules, total solid content in a sample or increased ratio of mass/volume. In Ayurvedic point of view the specific gravity can be correlated to *Guru* (heavy for digestion) and *Snigdh* (slimy, soft or fatty) attributes. It can be interpreted that the time has increased the density of molecules or reduced the proportion of low density molecules. Presence of high density molecules (possibly high density lipoproteins) in *Puran* Ghrita represents its more beneficial over fresh Ghrita.

Refractive Index (RI) is mostly applied for identify a particular substance, confirm its purity, or measure its concentration[10]. RI at 40 ^{0}C can also be correlated with presence turbid materials. There is negligible difference between RI of both samples which indicates the similar concentration of turbid materials. The iodine value in chemistry is the mass of iodine in grams that is consumed by 100 grams of a chemical substance. Iodine numbers are often used to determine the amount of unsaturation in fatty acids. This unsaturation is in the form of double bonds, which react with iodine compounds. The higher the iodine number, the more C=C bonds are present in the fat[11]. Saponification value represents the number of milligrams of potassium hydroxide required to saponify 1g of fat under the conditions specified[12]. Detection of peroxide gives the initial evidence of rancidity in unsaturated fats and oils[13]. There is considerable difference in Iodine and Saponification value of the studied samples. Both values are much higher in *Puran* Ghee than fresh Ghee. This fact is representative of more stable nature of *Puran* Ghee.

Acid value of fresh and *Puran* Ghee also showed large difference (1.62 and 75.88 gm/ml respectively). Acid value is a common parameter in the specification of fats and oils. It is defined as the weight of KOH in mg needed to neutralize the organic acids present in 1g of fat and it is a measure of the free fatty acids (FFA) present in the fat or oil. An increment in the amount of FFA in a sample of oil or fat indicates hydrolysis of triglycerides. Such reaction occurs by the action of lipase enzyme and it is and indicator of inadequate processing and storage conditions (i.e., high temperature and relative humidity, tissue damage). The source of the enzyme can be the tissue from which the oil or fat was extracted or it can be a contaminant from other cells including microorganisms. Besides FFA, hydrolysis of triglycerides produces glycerol[14]. The higher acid value in *Puran* Ghee time based and enzyme induced hydrolysis of triglycerides and thus high proportion of glycerol. Glycerol is taken by mouth for weight loss, improving exercise performance, helping the body replace water lost during diarrhea and vomiting, and reducing pressure inside the eye in people with glaucoma[15]. According recent research work, Glycerol is found to be an alternative to dexamethasone for patients receiving brain irradiation for metastatic disease[16]. From these evidences it is clear that claims mentioned regarding more beneficial properties and therapeutic potentials of *Purana* Ghee are accurate, however as Ghee is not 100% Glycerol hence its level of efficacy need to be scientifically established by experimental and clinical studies.

Both studied samples have no any microbiological contamination which indicates that the preservation of *Puran* Ghee was well done and satisfactory care taken during preparation of fresh Ghee. In analytical profile for fatty acids, it is detected that the proportion of Capric acid, Lauric acid, Stearic acid and

Oleic acid is higher in fresh Ghee than *Puran* Ghee. Capric acid is a medium-chain fatty acid found in saturated fats. Small amounts are present in cow's milk and goat's milk. Capric acid, together with other medium-chain triglycerides, is responsible for its health benefits[17]. Medium-chain triglycerides such as capric acid are broken down quickly and processed in the liver, and can be used as a source of energy. Stearic acid has neutral effects on lipids and lipoproteins unlike the other long-chain saturated fatty acids[18]. A meta-analysis of 60 controlled trials1 has reported that when isocalorically substituted for dietary carbohydrate, stearic acid has a neutral, or even slight total cholesterol and LDL-C lowering effect relative to other long chain saturated fatty acids (i.e. lauric, myristic, and palmitic acid), all of which increase total cholesterol and LDL-C. A study conducted by Kelly et al reported that a high stearic acid diet (6.6% of Kcal, 19 g per day) compared with a diet high in palmitic acid (7.8% of Kcal, 22.5 g per day) beneficially affected thrombotic risk factors for CVD[19]. Lauric acid is a saturated fat used for treating viral infections including influenza (the flu); swine flu; avian flu; the common cold; fever blisters, cold sores, and genital herpes caused by herpes simplex virus (HSV); genital warts caused by human papillomavirus (HPV); and HIV/AIDS[20]. Oleic acid is a common monounsaturated fat in human diet. Monounsaturated fat consumption has been associated with decreased low-density lipoprotein (LDL) cholesterol, and possibly increased high-density lipoprotein (HDL) cholesterol[21]. Oleic acid may be responsible for the hypotensive (blood pressure reducing) effects of olive oil[22]. Higher proportion of Capric acid, Lauric acid, Stearic acid and Oleic acid in fresh Ghee represents its superiority with respect to properties observed in those fatty acids.

On the other hand, *Puran* Ghee showed much higher proportion of Myristic acid, Palmitic acid and linoleic acid than fresh Ghee. Various "human epidemiological studies have shown that myristic acid and lauric acid were the saturated fatty acids most strongly related to the average serum cholesterol concentrations in humans"[23], meaning they were positively correlated with higher cholesterol levels as well as raising triglycerides in plasma by some 20% increasing the risk for heart disease although some research points to myristic acid's positive effects on HDL cholesterol and hence improving HDL (good cholesterol) to total cholesterol ratio[24]. Palmitic acid is a saturated fatty acid commonly found in both animals and plants. There are controversial views regarding harmful and beneficial effect of Palmitic acid as it is virtually never consumed apart from other healthier fats, so its negative impact on health might have been over-estimated[25]. Linoleic acid belongs to one of the two families of essential fatty acids, which means that the human body cannot synthesize it from other food components[26]. Palmitic acid does not raise cholesterol if it is combined with linoleic acid[27]. From these evidences it is predictable that Myristic acid, Palmitic acid and linoleic acid present in *Purana* ghee must have health beneficial effects which are assigned to attributes of *Purana* ghee.

It is evident from classical literature that *Puran* ghee is rarely indicated for oral administration and mostly utilized for either local of by nasal route. However, too high proportion of tri-glycerides in *Puran* ghee represents its possible utility in CNS disorders. On the basis of findings of analytical profile of fresh and *Puran* ghee, it can be said that this study has formed the conceptual and analytical basis for further research on therapeutic potentials of *Puran* ghee which need to be studied on experimental and clinical ground.

Conclusion:

Time factor affects physico-chemical profile of ghee and thus changes its therapeutic potentials. Analytical profile of fresh ghee indicates its utility in gastro-intestinal disorders, as nutrient and as anti-oxidant. On the other hand it is observed values of analytical parameters of *Purana* ghee represent its possible beneficial use in CNS disorders such as epilepsy and mania. Present work has established analytical standards for 15 years old ghee and these parameters need to be utilized for further research on pre-clinical and clinical ground especially in CNS related researches.

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