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PHYSICOCHEMICAL STUDY AND STORAGE STABILITY OF HERBAL GHEE PREPARED WITH ETHANOLIC EXTRACT OF *Terminalia arjuna*

[1]Rosy Bansal and [2]Saijal Khosla

[1]Assistant Professor, GSSDGS Khalsa College, Patiala, [*]E-mail: rosydeepak@gmail.com and [2]Research Scholar MCM DAV College Chandigarh, [*]E-mail:saijalkhosla1306@gmail.com, Corresponding Author: ***Rosy Bansal***

Abstract: *The following analysis entitled "Physicochemical study and Storage Stability of Herbal Ghee prepared with Ethanolic extract of Terminalia arjuna" was conducted with the objective to enhance the shelf life and anti-oxidant ability of clarified butterfat (ghee) using accelerated oxidation tests. Various tests were conducted to check the storage stability of both the control and arjuna ghee i.e peroxide value, FFA content, phytosterol content. By these studies it was concluded that herbal ghee was effective in retarding the autooxidation of buffalo ghee during its storage. It was also observed that herbal ghee had an ability to enhance the antioxidant poiential of ghee in terms of radical scavenging activity and had a significant improve in phytosterol content in ghee along compared to the control. Also storage stabity of arjuna ghe was about 9 days ±80^{0}C as compared to 3 days of the control.After all these studies was suggested that ethanolic extact of T.arjuna could be used as an antioxidant and enhancing the phytosterol content in ghee.Freshly prepared butter fat arjuna ghee possess good potiential to act as free radical scavenger and thus could help in prevention of ,any free radical related disorders.*

Key Words*: Clarified butterfat, peroxide value, phtosterol content, storage stability, autooxidation*

Introduction: Ghee Beginning from the vedic times (3000 to 2000B.C) there is ample recorded evidence to show that ghee were extensively used by the early inhabitants of India both in their dietry and religious practices. It is worth noting that the utilization of milk fat in form of ghee, so admirably suited to this country, should have been hit upon in such early times. This unique position occupy by ghee may be described to its being not only the best form of the preservation of milk fat under tropical climate, but to its constituting in addition the only source of animal fat in otherwise predominantly vegetarian diet. The large production of ghee is due to:

- Concentration of milk production in rural are which are far away from the nearest urban consuming area.
- Lack of all weather and refrigerated transport facilities.
- Unfavourable climate condition i.e. high temperature and humidity for most parts of year causing rapid spoilage of milk
- Its long keeping quality under tropical storage conditions and ordinary packaging.
- Market demand.

Definition: According to P.F.A rules ghee is the pure clarified fat derived solely from milk or from desi butter or from cream to which no colouring matter is added. Ghee is heavily utilized in *Ayurveda* for numerous medical applications, including the treatment of allergy, skin, and respiratory diseases. Many Ayurvedic preparations are made by cooking herbs into ghee. Ghee carries the therapeutic properties of herbs to all the body's tissues. It is an excellent *anupana* (vehicle) for transporting herbs to the deeper tissue layers of the body. Proper digestion, absorption, and delivery to a target organ system are crucial in obtaining the maximum benefit from any therapeutic formulation; the lipophilic action of ghee facilitates transportation to a target organ and final delivery inside the cell since the cell membrane also contains lipid. A study that compared different forms of herbs and herb extracts found that the efficacy increased when

they were used with ghee, compared to usage in powder or tablet form.

Arjuna terminalia: Arjuna is an important medicinal tree known throughout the Indian subcontinent since the Vedic period (1700-550 BC).The bark, leaves and fruits of *T. arjuna* have been used in Indian traditional system of medicine the Ayurveda, for treatment of many systemic ailments, notably in heart diseases [1 & 2]. The arjuna is about 20–25 metres tall; usually has a buttressed trunk, and forms a wide canopy at the crown, from which branches drop downwards. It has oblong, conical leaves which are green on the top and brown below; smooth, grey bark; it has pale yellow flowers which appear between March and June; its glabrous, 2.5 to 5 cm fibrous woody fruit, divided into five wings, appears between September and November. The arjuna is usually found growing on river banks or near dry river beds in West Bengal and south and central India.

Every parts useful medicinal properties Arjun holds a reputed position in both Ayurvedic and Yunani Systems of medicine. According to Ayurveda it is alexiteric, styptic, tonic, anthelmintic, and useful in fractures, uclers, heart diseases, biliousness, urinary discharges, asthma, tumours, leucoderma, anaemia, excessive prespiration etc. According to Yunani system of medicine, it is used both externally and internally in gleet and urinary discharges. It is used as expectorant, aphrodisiac, tonic and diuretic.

Composition: The tree bark, the main medicinal component, contains: Active Constituents. Terminalia's active constituents include tannins, cardenolide, triterpenoid saponins (arjunic acid, arjunolic acid, arjungenin, arjunglycosides), flavonoids (arjunone, arjunolone, luteolin), gallic acid, ellagic acid, oligomeric proanthocyanidins (OPCs), phytosterols, calcium Arjuna is a good source of phytosterol, namely, -sitosterol which lowers down the cholesterol in blood serum mediated through inhibition of cholesterol absorption resulting from the higher solubility of phytosterols than of cholesterol in bile salt micelles [3,4].

Arjuna bark extract has also been reported to contain numerous functional constituents e.g. tannins, triterpenoids, saponins (termed as arjunic acid, arjunolic acid, arjungenin, arjunglycosides), flavonoids (arjunolone, arjunon, luteolin), gallic acid, ellagic acid, oligomeric proanthocyanidins (OPCs), calcium, magnesium, zinc and copper [5]. Also Arjuna bark decreases the level of serum triglycerides and cholesterol, recovers the level of high density lipoprotein (HDL), acts as an anti-ischemic agent, relieves myocardial necrosis, modulates platelet aggre-gation and also acts as an effective antioxidant [6]. The crude bark of T. arjuna augments endogenous antioxidant compounds of rat heart and prevents it from oxidative stress [7]. Flavonoids present in T. arjuna bark have been reported to exert antioxidant, anti-inflammatory and lipid lowering effects while glycosides are cardiotonic, thus making T. arjuna unique amongst most commonly used medicinal plants in Indian subcontinent [8].

Fig: Dried bark of *Arjuna terminalia*

Health benefits

Anti-inflammatory properties: Arjuna bark has anti-inflammatory properties which act as COX inhibitors and non-steroidal anti-inflammatory agents, and displayed both analgesic and anti-inflammatory properties.

Controls cholesterol: Studies have shown that Arjuna tree is effective in bringing down LDL cholesterol levels. The hypocholesterolaemic effect was comparable to vitamin E.

Cardiac protection: Arjuna bark has been traditionally prescribed for heart problems. Recent studies have shown that Arjuna was very effective in controlling refractory chronic congestive heart failure.

Keeps diabetes in check: Studies have shown that the extracts from Arjuna bark were very

effective in controlling diabetes. The research concluded Arjuna to be a potent diabetes reducing agent.

Liver protection: The antioxidants present in Arjuna bark acted as nullifying agents against fluoride damage caused to the liver. Arjuna bark extracts were so effective that fluoride levels had come down to almost normal after just 10 days.

Treats asthma: According to Ayurveda, Arjuna bark can be very effective for asthama.

Diarrhoea and dysentery: Arjuna bark powder can also be effective in reducing both diarrhoea and dysentery. Not more than 20 to 30 g of powder should be taken.

Fractures and contusions: According to Ayurveda, Arjuna bark is effective in restoring strength to the bones which have been fractured. Powdered dry bark of Arjuna can be taken along with honey for this.

Novelty of invention: So far no process has been developed whereby the active principles of the herb Arjuna can be incorporated into ghee without essentially altering the sensory status of the later. Ayurvedic herbal ghee is medicinal preparation carrying the herb extract which renders the product sensorily much different from normal ghee. The functional components of the herb Arjuna are incorporated during ghee making requiring no post-clarification treatment. Colour and flavour of the product are similar to those of conventional ghee unlike the presently marketed herbal ghee which has an unacceptable flavour and appearance.Arjuna herbal ghee was developed as a health food combining the medicinal value of *Arjuna terminalia* and the nutritional virtues of ghee. And also to improve its storage stabily by delaying the rancidity and radical scavenging assay so as to obtain a product which along with health benefits is oxidatively stable.

Presently, the herbal ghee being marketed in the country is mostly sold as medicine for cure of certain ailments and is therefore classified as ‘medicinal ghee’. They have typical flavour, bitter or pungent taste with a dark colour. Such therapeutic preparations are therefore not acceptable for routine use. Preparations which can serve as the health promoting items of the diet would therefore have to be essentially prophylactic foods based on the same principles. There was thus a need for developing processes for large-scale manufacture of ghee incorporating herbal properties, but without affecting sensory and physical properties. However, overcoming the adverse impact of herb incorporation on the sensory profile of the product was the major challenge. Development of Arjuna herbal ghee with appropriate technological alterations was a successful attempt in this direction.

Objective of the project: Oxidative deterioration of ghee is one of the major factors that limit the storage life of ghee [9,10]. The onset of rancidity in ghee is mainly due to the oxidation of unsaturated glycerides leading to development of peroxides and/or due to hydrolysis of glycerides resulting in increased levels of free fatty acids (FFA) [11,12]. Synthetic antioxidants such as butylated hydroxyanisole (BHA), propyl gallate and tertiary butyl hydroquinone (TBHQ) are often used in ghee to prevent oxidative deterioration [10, 13]. However, scientific studies have shown that application of synthetic antioxidants in foods may cause damage to liver and have been responsible for carcinogenesis [14]. These reasons have directed the attention towards the use of edible plant resources as safer and natural antioxidants; also consumer demand for natural food ingredients has resulted in extensive research on naturally occurring antioxidants. Recently, the use of natural antioxidants in the food industries has increased rapidly and consequently many related studies have been reported [15,16]. Numerous herbs have the potential to retard lipid oxidation during storage of foods which is usually mediated through their intrinsic antioxidant activity and the addition of herb and spice extracts in milk and milk products is evolving rapidly [17, 13]. This study was therefore undertaken to assess the effects of ethanolic extract of *T. arjuna* to understand its potential use as an antioxidant and phytosterol enhancer in clarified butterfat prepared from cow and buffalo milk during accelerated oxidation conditions. And thus combacing he oxidative and rancidity problems in ghe thus increasing its storahe and supplying it with nhanced nutrients.

Materials and Methods

Ethanolic Arjuna Extract: Alcoholic Arjuna extract was prepared by cold macerating one part of Arjuna bark with four parts of absolute ethyl alcohol for 72 h at room temperature (30±2°C) followed by filtering using muslin cloth ensuring that no part of the bark powder had retained in the filtrate. The filtrate was then dried at 65°C in a tray drier for 12 h. The dried alcoholic Arjuna extract was then packed in air tight glass container and stored in refrigerator.

Preparation of Arjuna Ghee: Pure butter bricks were procured from the local market of Chandigarh. It was then melted at low flame to avoid burning, with constant stiring. It was then heated till the time it starts boiling. Then ethanolic extract of arjuna was added at the time of ghee formation. Then the ghee containing the extract was filtered using muslin cloth. Ghee without the arjuna extract served as control. The prepared ghee samples were stored in hot air oven at 80 ± 1^0C for accelerated storage statbilty and were then analysed at intervals of 0 2, 4, 6, 8,10 days for peroxide value, FFA and phytosterol content. Other test also like iodine value and melting point and acid value were performed for physicochemical properties of ghee.

Chemicals Used: Potassium iodide, starch, ethyl alcohol, phenolphthalein, chloroform, sodium hydroxide ethyl acetate, sodium thiosulphate, and glacial acetic acid). Sulphuric acid, acetic anhydride and cholesterol. All the chemicals and reagents were of analytical grade.

Peroxide Value: Peroxide value represents the primary reaction products of lipid oxidation, which can be measured by their ability to liberate iodine from potassium iodide. . The peroxide value of ghee was determined by Lea's method

Procedure: Weigh small amount of ghee sample in a conical flask.add 25ml of solvent(2 volumes of glacial acetic acid and one volume of chloroform). And displace the air above the liquid with CO_2.Add 1ml of potassium iodide solution in stopper flask and allow it to stand for 1min.(with shaking). Now add 35ml of water and titrate the liberated iodine with 0.1N sodium thiosulphate solution, using starch as indicator.shake vigorously at the end to remove the traces of iodine from chloroform layer. Carry out a blank determination simultaneously.

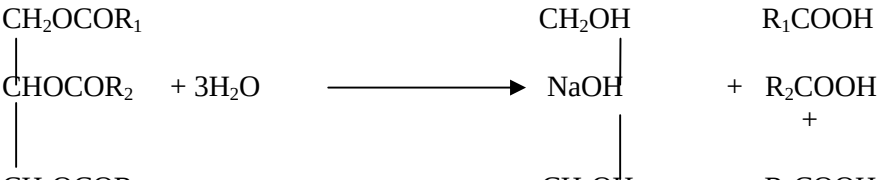
Calculation

$$\textbf{Peroxide value} = \frac{\textbf{(sample titre-blank titre)*normality of sodium thiosulphate*1000}}{\textbf{weight of sample taken}}$$

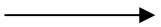
FFA content

FFA content of ghee samples was stimated by acid base titration with alkali NaOH usinf phenolpthalene as indicator .

Reaction 1



Reaction2



Procedure: Weigh 10g of the ghee sample in 250 mil conical flask in another flask bring 50 ml of ethanol to the boiling point and while still above 70^0C,neutralize it to phenolpthalene (using 0.5ml) with 0.1N NaOH.add the neutralized alcohol to flask containing ghee sample and mix the contents of the flask. Bring the mixture to boil and while it is still hot,titrate with 0.1N NaOH, shaking vigorously during titration. The end point of the titration is reached when the addition of single drop of produces a slight, but definite colour change persisting for 15sec. The acidity of ghee is frequently expressed as the percentage of free fatty acids in sample, calculated as oleic acid, using the formula

Free fatty acids=T/M *2.82

Where

T=volume of 0.1N alkali required for titration in ml

M= mass in g of ghee sample taken

Percentage FFA= 2.82V/W

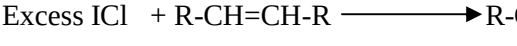
Phytosterol Content: Phytosterol in ghee was determined by direct colorimetric method.

Iodine Value: Measure of the double bonds in oils and fats. Generally expressed as number of grams of iodine that will react with double bonds in 100g of fat and oil. Two methods involved in the determination of iodine are;

Wij"s Method: It used iodine chloride and is a recommended method by AOCS METHODS.

Procedure: Add a solution of iodine monochloridein acetic acid to a test portion of oil or fat dissolved in CCl_4 or cyclohexane

Allow the mixture to stand for a specific time period.



Added potassiumiodide solution to reduce remaining ICl to free Iodine. ICl will react with KI to give

$ICl + 2KI \longrightarrow KCl + KI + I_2$

Titrate the liberated iodine with standardized solution of sodium thiosulphte using starch indicator.

I_2 + starch $+Na_2S_2O_3 \longrightarrow 2NaI + Starch + Na_2S_4O_6$

(blue) (colourless)

Iodine value is calculated by the formula

$$I.V = \frac{B-S * N * 12.69}{\text{Wt of sample (in gms)}}$$

Where

B= blank titre value

S=sample titre value

N= normality of $Na_2S_2O_3$

Moisture content: The moisture content is the loss in mass, expressed as a percentage by mass when the product is heated in a hot air oven at 105+-1oc to constant mass.

Procedure: Weigh accurately about 10g of the sample into a moisture dish which has been dried previously and weighed. Place in an air oven for 1hour at 105+-1oc. Remove the dish from the oven,cool in a dessicator and weigh. Repeat the process by keeping the dish in an oven for half hour each time, cool and weigh till two successive weighings do not exceed 1mg.

Calculations

$$\text{Moisture \& volatile matter \%/w} = \frac{(M_1 - M_2) * 100}{(M_1 - M)}$$

Where

M_1 = mass in g of dish with ghee before drying

M_2 = mass in g of dish with ghee after drying

M =mass in g of empty dish

Acid value: The number of mg of KOH required neutralizing the free fatty acids present in 1g of ghee sample.

Procedure: Weigh accurately appropriate amount of cooled sample in 250 ml conical flask and add 50-100ml of freshly neutralized hot ethyl alcohol and add about 1ml of phenolpthlene indicator solution. Boil the contents for 5minutes and titrate while hot against 0.1N standard alkali solution of KOH or NaOH and shake vigorously while titrating.

Acid value= 56.1VN/W

Where

V= volume of alkali required for titration in ml:

W= weight in g, of sample taken

Meting Point: Melting point of ghee was calculated using calorimeter. The melting point of fat depends on the chain length of constituent fatty acids and degree of unsaturation.It may be stated in general that greater the degree of unsaturation of the constituent fatty acids the lower is the melting point of fat.Melting point was determined by calorimetric method

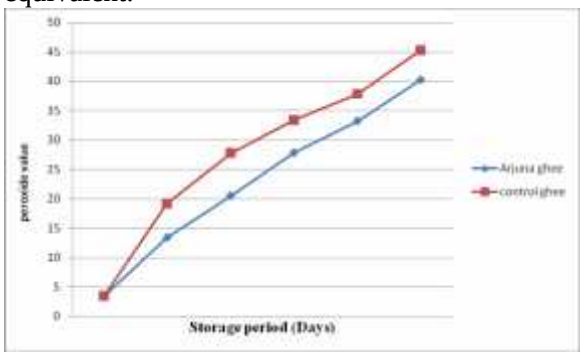
Procedure: Melt the sample and filter, to remove impurities and traces of moisture. Make sure the sample is absolutely dry. Mix the sample thoroughly. Introduce the capillary tube into the molten sample, so that the column of sample is about 10mm long, sucked into the tube. Chill the tube containing the sample immediately by touching the tube against a peice of use till fat solidifies. Place the tube in a small beaker and hold it for 1hour either in refrigerator or water maintained at a temperature of 4 to 10^0C. Remove the tube and attach with a rubber band to thermometer bulb, so that the lower end of capillary tube and thermometer bulb are at same levels. Take water in a “thistle” tube and immerse the thermometer with capillary tube containing the sample of fat.

Results and Discussion

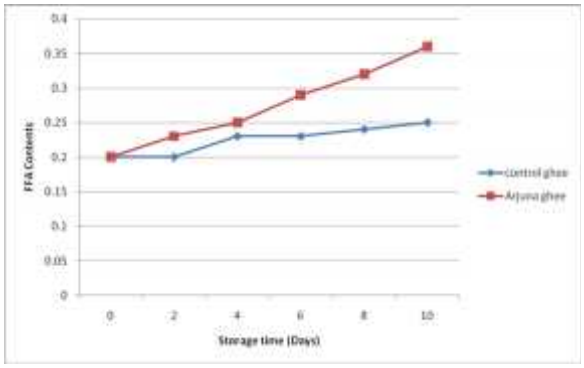
Peroxide Value: The changes in peroxide value expressed as milliequivalent of oxygen per kg (meq.O_2/kg) of the fat during storage are shown in figure 1. It was observed that extracts of arjuna significantly lowered the peroxide value of experimental ghee samples throughout the storage period at 80 ± 1^0C as compared to the control. The peroxide content of the control increased from 3.45 to45.33.32meq.O2/kg ater two days of storage so this high increase in the peroxide value indicated an high amount of oxidation in the product, while on the other hand the peroxide value of the experimental sample increased from 3.45 to 40.20 meq.o2/kg respectively after 10 days of storage. This indicated that the incorporation of ethanolic extract of Arjuna in ghee is very effective in retarding peroxide development as compared to control samples.

The presence of high concentration of alcohol soluble flavonoids, e.g., arjunolone, arjunon, luteolin, gallic acid, ellagic acid, oligomeric proanthocyanidins (OPCs) which are from the group of polyphenol is the probable reason of antioxidant attribute of Arjuna extract. Reported

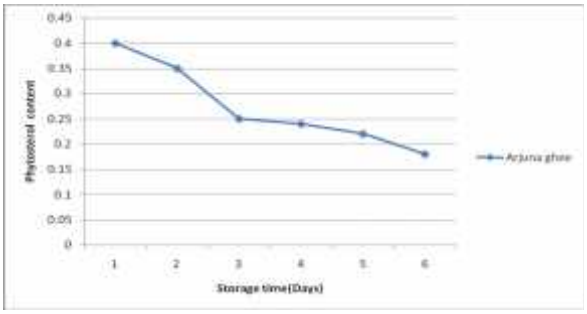
that the methanolic extract of *T. arjuna* contained 817.49±8.11 mg/g gallic acid equivalent total phenolic compounds and 199.122±8.282 mg/g quercetin equivalent of flavonoids. They also observed that the methanol extract of *T. arjuna* possesses very high antioxidant capacity to the tune of 377.66±1.89 mg/g ascorbic acid equivalent.[8]



FFA Content: The FFA content of ghee is a measurement of extent of hydrolytic and lipolytic rancidity in ghee. The initial mean FFA content for control and experimental Arjuna ghee was 0.20% The FFA content then increased during the storage period. The FFA content of control ghee was increased from 0.20 to 0.23% oleic acid at the end of two days of storage. Whereas, the FFA content of buffalo however remained the same.



Phytosterol Content: Our study reported that the phytosterol content of initial value of 0.35 mg/g in arjuna ghee was decreased to 0.16 after 10 days of storage. It was observed that there was a significant difference amongst phytosterol content before and after storage. The phytosterol present in Arjuna mainly consists of phytosterol having one double bond in the sterol ring structure [3]. Several studies have revealed that ring-unsaturated sterols such as sitosterol and stigmasterol are much more reactive than sitostanol [18, 19] due to the higher reactivity of the allylic secondary carbon centers. This would explain the gradual loss of phyto-sterols during storage in our study.



Melting Point: Normally the melting point of buffalo ghee is 104^0F and by the addition of herbal extract into the ghee the melting point was raised from 104^0F to 107^0F.

Smoke Point: The smoke of control ghee was 443^0F whereas the smoke point of the experimental sample was increased to 486^0F due to the presence of volatile compounds.

Iodine Value: The iodine value of control ghee was 28.7 and there was no larger difference in the iodine value of the experimental sample.

Acid Value: The acid value of ghee was calculated to be 4.8g

Highlights

- Addition of ethanolic extract of *T. arjuna* bark was highly effective in retarding the auto-oxidation of both cow and buffalo ghee during storage. Ethanolic extract of Arjuna has significant ability to enhance the antioxidant potential of ghee.
- It also improved the phytosterol content in ghee which decreased during storage. The shelf life of the Arjuna herbal ghee at 80±1°C was 9 days as compared to 3 days at 80±1°C in control ghee samples.
- The findings suggested that ethanolic extract of *T. arjuna* could be used as a natural antioxidant in ghee and enhancing the phytosterol content in ghee. Freshly prepared cow milk Arjuna ghee possesses good potential to act as free radical scavenger and thus could help in prevention of many free radical related disorders.

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