

Full Length Research Paper

## The effects of alcoholic extract of Arjuna (*Terminalia arjuna* Wight & Arn.) bark on stability of clarified butterfat

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**Potential of ethanolic extract of *Terminalia arjuna* Wight & Arn bark to enhanced the shelf life and anti-oxidant ability of clarified butterfat (ghee) was evaluated using accelerated oxidation tests. Seven percent by weight of ethanolic extract of *T. arjuna* Wight & Arn bark was effective in retarding the auto-oxidation of both cow and buffalo ghee during storage. It had significant ( $P<0.05$ ) ability to enhance the antioxidant potential of ghee in terms of its radical scavenging activity. It also improved phytosterol, content in ghee. Shelf life of the Arjuna ghee samples was 8 days at  $80\pm 1^\circ\text{C}$  as compared 2 days in the control. Findings suggested that ethanolic extract of *T. arjuna* Wight & Arn could be used as a natural antioxidant 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.**

**Key words:** Arjuna, ghee, antioxidant and radical scavenging activity, phytosterol content, oxidative stability.

### INTRODUCTION

Arjuna (*Terminalia arjuna* Wight & Arn) 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 (Warrier et al., 1996; Nema and Garg, 2011). Arjuna is a good source of phytosterol, namely,  $\beta$ -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 (Anjaneyulu and Prasad, 1982; Ghani, 2003). 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 (Miller, 1998). It has been observed that 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 aggregation and also acts as an effective antioxidant (Sumitra et al., 2001). The crude bark of *T. arjuna* augments endogenous antioxidant compounds of rat heart and prevents it from oxidative stress (Gauthaman et al., 2001). Flavonoids present in *T. arjuna* bark have been reported to exert antioxidant, anti-inflammatory and lipid lowering effects while glycosides are cardiogenic, thus making *T. arjuna* unique amongst most commonly used medicinal plants in Indian subcontinent (Shahriar et al., 2012). The total phenolic compound content of *T. arjuna* leaves, bark and fruits is reported to be very high, that is, to the tune of 72.0 to 167.2 mg/kg (Bajpai et al., 2005). The phenolic compound in herbs act as antioxidants,

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because of their redox properties which allow them to act as reducing agents, hydrogen donors, free radical quenchers and metal chelators (Banerjee and Bonde, 2011).

Clarified butter fat commonly known as ghee in India means the pure clarified fat derived solely from milk or curd or from desi (cooking) butter or from cream (FSSR, 2011). Ghee is one of the most widely used dairy products in India. Oxidative deterioration of ghee is one of the major factors that limit the storage life of ghee (Mehta, 2006; Puravankara et al., 2000). 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) (Amr, 1991; Muir, 1996). Synthetic antioxidants such as butylated hydroxyanisole (BHA), propyl gallate and tertiary butyl hydroquinone (TBHQ) are often used in ghee to prevent oxidative deterioration (Puravankara et al., 2000; Pawar et al., 2012).

However, scientific studies have shown that application of synthetic antioxidants in foods may cause damage to liver and have been responsible for carcinogenesis (Sherwin, 1990). 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 (Jeong et al., 2004; Iqbal et al., 2007). 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 (Pokorny and Korczak, 2001; Pawar et al., 2012). 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.

## MATERIALS AND METHODS

### Plant

Arjuna bark was procured from the local market of Karnal, Haryana, India. 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 cheese 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 until used.

### Chemicals and reagents

Potassium iodide, starch, ethyl alcohol, phenolphthalein powder,

sodium hydroxide, 2, 2-Diphenyl-1-picrylhydrazyl (DPPH), ethyl acetate were obtained from Sigma Chemical Co. (St Louis, MO, USA). Chloroform, sodium thiosulphate, and glacial acetic acid were procured from Loba Chemie Pvt Ltd., (Mumbai, India). Sulphuric acid, acetic anhydride and cholesterol was obtained from Qualigens Fine Chemicals (Mumbai, India). All the chemicals and reagents were of analytical grade.

### Preparation of Arjuna herbal ghee

Milk was procured from the Cattle Yard of National Dairy Research Institute, Karnal, Haryana, India. It was preheated to 45°C; cream was separated using a mechanical cream separator and standardized to 40% fat using skim milk of the same species and then cooled to 4°C and aged overnight at this temperature. Then, the aged cream was churned into butter that contained 80% fat. The produced butter was melted properly and ethanolic extract of Arjuna was added at 7% of the fat taken and clarified at 113 to 115°C, followed by filtering through cheese cloth. Ghee without any added Arjuna extract served as control. The prepared ghee samples stored in hot air oven at 80±1°C for accelerated storage stability test and were analyzed at regular intervals of 0, 2, 4, 6, 8 and 10 days for peroxide value, radical scavenging activity by DPPH assay, FFA and phytosterol content.

### 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 as described in IS: 3508 (1966).

### Radical scavenging activity (DPPH Assay)

Antioxidant potential was assessed by the Arjuna herb's capacity to quench the free DPPH radicals. The process of inhibiting the autoxidation of lipids by antioxidants is related to the antioxidants ability to break radical formation reaction. Therefore, antioxidant potential could also be estimated in systems in which radicals are generated chemically. The capacity of antioxidants to quench DPPH radical in ghee was determined during accelerated temperature storage according to the procedure described by Espin et al. (2000).

### FFA content

FFA content of ghee samples were determined by the method described in Indian standard (IS: 3508-1966) in terms of percent oleic acid.

### Phytosterol content

Phytosterol in ghee was determined by direct colorimetric method as described by Sabir et al. (2003).

### Statistical analysis

The data obtained during the experiments were analyzed statistically for both one and two way analysis of variance (ANOVA) using SPSS software (Version 20, Chicago, USA) and the data were expressed as mean values with standard error.

## RESULTS AND DISCUSSION

### Peroxide value (PV)

The changes in peroxide value expressed in milliequivalents of oxygen per kg (meq.O<sub>2</sub>/kg) of fat during storage are as shown in Figure 1. It was observed that the ethanolic extracts of Arjuna significantly ( $P<0.05$ ) lowered the peroxide value of the experimental ghee samples throughout the storage period at  $80\pm 1^\circ\text{C}$  as compared to the control. The peroxide value of control ghee increased from 3.73 to 45.33 meq.O<sub>2</sub>/kg of fat after two days of storage, and the product becomes totally oxidized ( $PV>40$ ) and sensorially unacceptable, hence any further analysis was discontinued. Whereas, the PV of buffalo Arjuna ghee and cow Arjuna ghee was increased from 3.73 to 43.20 and 5.33 to 41.60 meq.O<sub>2</sub>/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. Shahriar et al. (2012) reported that the methanolic extract of *T. arjuna* contained  $817.49\pm 8.11$  mg/g gallic acid equivalent total phenolic compounds and  $199.122\pm 8.282$  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  $377.66\pm 1.89$  mg/g ascorbic acid equivalent.

In a similar type of finding Siddiq et al. (2005) reported that the addition of methanolic (80 and 100%) and acetone (80 and 100%) extracts of seeds of *Moringa oleifera* (drumstick) to sunflower oil significantly inhibited the development of peroxides under accelerated conditions. In Southern Indian villages, people use the fresh leaves of *M. oleifera* during preparation of cow and buffalo ghee to increase its shelf life (Perumal and Becker, 2003). Merai et al. (2003) reported that addition of 0.6% of silica gel charcoal treated fraction of *Ocimum tenuiflorum* (holy basil) leaves powder to ghee was more effective than the BHA at 0.02% until the peroxide value of 5 meq.O<sub>2</sub>/kg of fat was reached.

### Radical scavenging activity (RSA) by DPPH assay

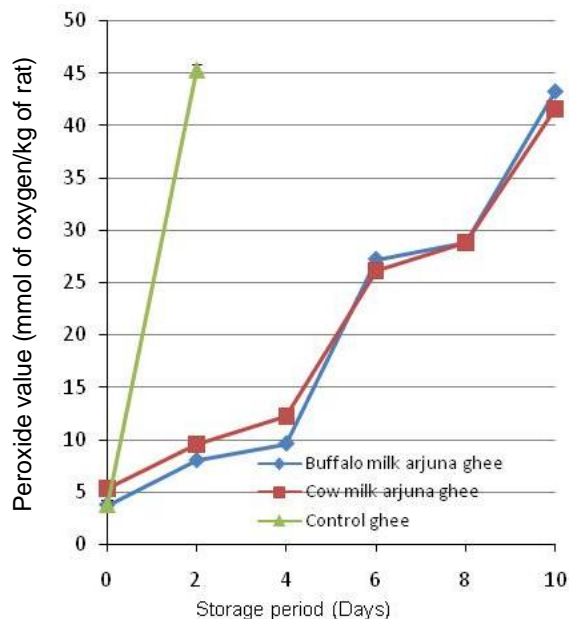
In the present study, antioxidant potential of the Arjuna extract in ghee was assessed by measuring its capacity to quench the free DPPH radicals. The process of inhibiting the autooxidation of lipids by antioxidants is related to the antioxidant's ability to break the radical formation reaction (Sulieman et al., 2006). The changes in percent radical scavenging activity during storage are

as shown in Figure 2. At the beginning of the study, cow Arjuna ghee had the highest potential to quench the free DPPH radicals followed by buffalo Arjuna ghee and control ghee. Even during the storage, the ability of cow and buffalo Arjuna ghee to quench the DPPH free radicals followed the same order. The percent radical scavenging activity of control ghee, buffalo Arjuna ghee and cow Arjuna ghee was reduced from 23.62 to 6.29, 27.75 to 10.51 and 58.24 to 27.29, respectively at the end of the storage period. Statistically, the addition of Arjuna herb extract, intervals of storage and their interaction contributed significantly ( $P<0.05$ ) towards the changes in RSA in ghee.

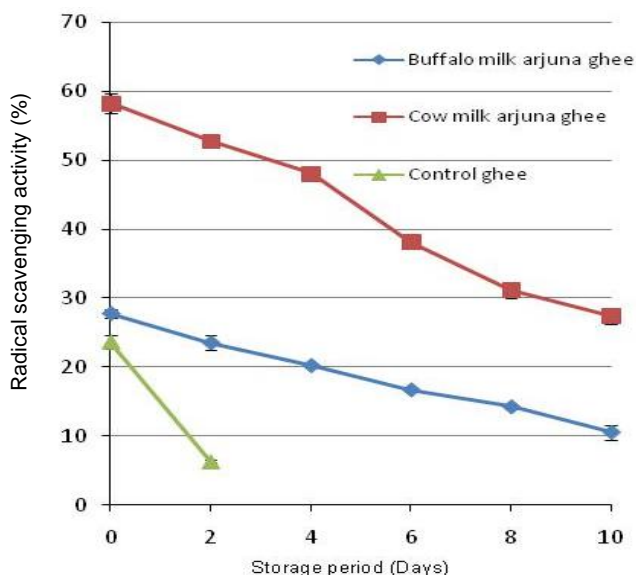
The results obtained in this test suggested that ethanolic extract of Arjuna has significant ( $P<0.05$ ) ability to enhance the antioxidant property in terms of radical scavenging ability of ghee and this was more pronounced in case of cow ghee than in buffalo ghee. The ethanolic extract of Arjuna was highly effective in the process of inhibiting the auto-oxidation of ghee by breaking the radical formation reaction. The finding could not be compared *per se*, as to the best of our knowledge no such work has been reported in ghee till date for Arjuna herb. However, a study conducted by Pawar et al. (2012) for evaluating the effect of *Asparagus racemosus* (Shatavari) on storage stability of ghee, the samples incorporated with ethanolic extract of Shatavari showed a strong activity in quenching DPPH radicals than the aqueous extract of the same herb. In a similar study conducted by Purohit (2011) for evaluating the effect of herb extract *Withania somnifera* (Ashwagandha) incorporation on storage stability of ghee, the samples incorporated with ethanolic extract of Ashwagandha was found to be more effective in quenching DPPH radicals than that of aqueous extract of the same before and after exposing to accelerated oxidation conditions. Our results showed that 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 e.g. cataract, atherosclerosis, diabetes, diseases of the gastrointestinal tract, aging of skin, Alzheimer's and other neurologic disorders.

### FFA content

The FFA content of ghee is a measurement of extent of hydrolytic and lipolytic rancidity in ghee. The changes in FFA content in ghee samples during storage are presented in Figure 3. The initial mean FFA content for control and buffalo milk Arjuna ghee was 0.21% and that of cow Arjuna ghee was 0.49% oleic acid. The FFA content increased during the entire storage period in all the ghee samples. The FFA content of control ghee was increased from 0.21 to 0.23% oleic acid at the end of two days of storage. Whereas, the FFA content of buffalo Arjuna ghee and cow Arjuna ghee using Arjuna alcoholic

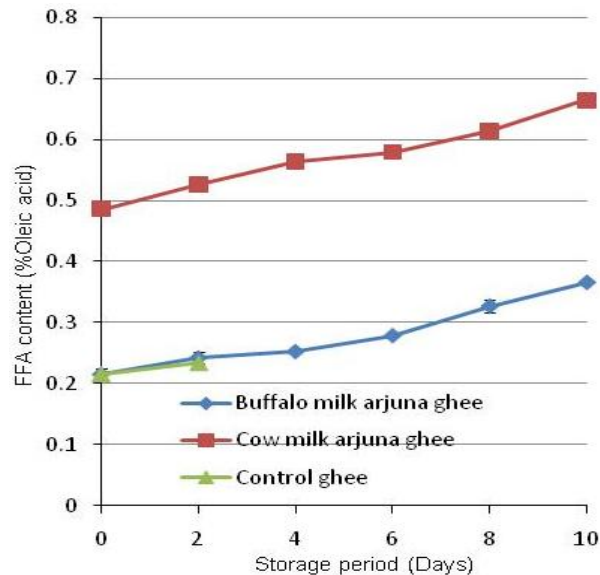


**Figure 1.** The changes in peroxide value expressed ghee sample stored at  $80\pm 1^\circ\text{C}$ .



**Figure 2.** Changes in percentage radical scavenging activity of ghee samples stored at  $80\pm 1^\circ\text{C}$ .

extract was increased from 0.21 to 0.37 and 0.49 to 0.67%, respectively at the end of the 10 days storage period. Statistically, the addition of Arjuna bark extract, intervals of storage and their interaction contributed significantly ( $P < 0.05$ ) towards the changes in FFA content in ghee. Yadav and Srinivasan (1992) reported that the FFA content of ghee normally ranges between 0.23 and 1.0% oleic acid. Hence, the FFA content of the

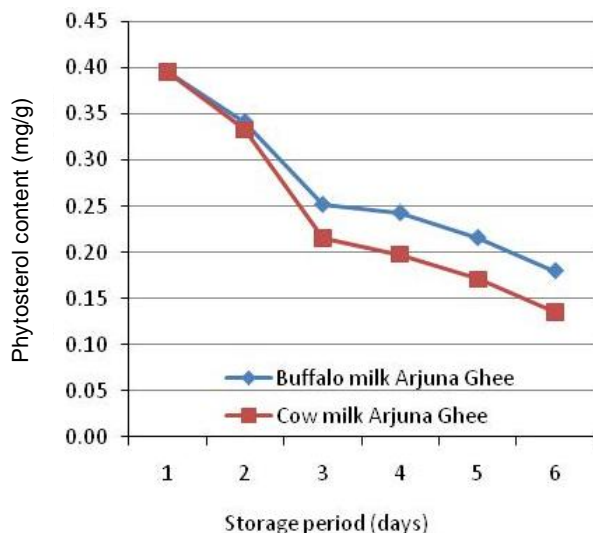


**Figure 3.** Changes in FFA content of ghee samples stored at  $80\pm 1^\circ\text{C}$ .

samples was found to be within the normal range during storage. Also, Wadhwa and Jain (1990) observed that the average total FFA content of cow ghee was higher than that of buffalo ghee. Similar findings have also been reported for other buffalo milk products e.g. the lipolytic changes during ripening in cheddar cheese made from buffalo milk were reported to much slower than those in similar cheese made from cow milk (Sahai, 1996).

### Phytosterol content

Phytosterols or plant sterols have the same basic function in plants as cholesterol in animals; that is, they play a key role in cell membrane function. Phytosterols are well known for their hypocholesterolemic properties. It has also been reported that phytosterols have anticancer properties and act as immune system modulators. The changes in phytosterol content of buffalo Arjuna ghee and cow Arjuna ghee prepared incorporating ethanolic extract of Arjuna bark is presented in Figure 4. The phytosterol content of an initial value of 0.39 mg/g in freshly prepared Arjuna ghee decreased gradually to 0.18 and 0.13 mg/g in buffalo and cow Arjuna ghee, respectively after 10 days of storage at  $80\pm 1^\circ\text{C}$ . It was observed that there was a significant difference ( $P < 0.05$ ) amongst phytosterol content of both types of ghee, that is, buffalo Arjuna ghee and cow Arjuna ghee. The phytosterol present in Arjuna mainly consists of  $\beta$ -sitosterol having one double bond in the sterol ring structure (Anjaneyulu and Prasad, 1982). Several studies have revealed that ring-unsaturated sterols such as sitosterol and stigmasterol are much more reactive than sitostanol (Dutta et al., 2006; Soupas et al., 2007) due to



**Figure 4.** Changes in phytosterol content of ghee samples stored at  $80\pm 1^{\circ}\text{C}$ .

the higher reactivity of the allylic secondary carbon centers. This would explain the gradual loss of phytosterols during storage in this study.

## Conclusion

It could be concluded that addition of ethanolic extract of *T. arjuna* bark at 7% by the weight 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 and this was more pronounced in case of cow ghee than in buffalo ghee. It also improved the phytosterol content in ghee which decreased during storage. The shelf life of the Arjuna herbal ghee at  $80\pm 1^{\circ}\text{C}$  was 8 days as compared to 2 days at  $80\pm 1^{\circ}\text{C}$  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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