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Development of process for the production of *arjuna* herbal ghee from buffalo milk

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ABSTRACT

The present study was envisaged to develop a process of production of herbal ghee from buffalo milk using *arjuna* extract. Three different types of extracts *viz.* commercial aqueous *arjuna* extract powder, aqueous extract and alcoholic extract prepared in laboratory from *arjuna* bark were tried. It was observed that, amongst the three types of extracts when added at the level of 4% of the fat taken, ghee prepared using alcoholic extract was found superior. While selecting the level of alcoholic extract for maximizing the retention of phytosterol in ghee, it was found that there was no significant difference in overall acceptability scores of ghee samples prepared by adding the extract @ 5%, 6% and 7% by the weight of fat taken. Phytosterol content was found to be the highest, i.e. 0.38 and 0.47 mg/g when cream and butter were used as fat source at 7% level of addition of alcoholic extract, respectively. Based on the above results, the optimized product was the one that was prepared by addition of 7% alcoholic *arjuna* extract using creamery butter method. The chemical composition of the optimized product was found to be fat 99.92%, moisture 0.08%, free fatty acid 0.22% oleic acid, Butyro-Refractometer reading 41.5 at 40°C, Reichert-Meissl value 31.5 and phytosterol content 0.39 mg/g.

Key words: Herbal ghee, Phytosterol, Sensory attributes, *Terminalia arjuna*

Ghee is one of the most widely used dairy products in India and an important source of fat in the Indian diet. Ghee is a carrier of fat-soluble vitamins; A, D, E and K, which our body needs in very small quantities but cannot make for itself. Similarly, the essential fatty acids, which cannot be synthesized in our body, are also supplied by ghee. The various milk fat components, such as CLA, sphingomyelin, butyric acid, ether lipids having anticarcinogenic potential are also supplied by ghee (Alkalin and Tokusoglu 2003, Khanal and Olson 2004). Buffalo milk fat has been shown to contain a significantly higher amount of rumenic acid (the main conjugated linoleic acid) than cow milk. This may be the most important advantage of buffalo milk, as CLA isomers are regarded as anticarcinogenic, antiatherogenic, antiobesity and antidiabetic components (Ménard *et al.* 2010). Butyric acid present in ghee is a lower chain fatty acid present in milk of ruminants. Butyric acid is a well-known modulator of gene function (German 1999). It acts as anticarcinogen by regulating cell growth and inducing cell differentiation in a wide variety of neoplastic cell lines (Prasad 1980, Merrill 1991).

Despite of its numerous health benefits, over the past few years, ghee has received adverse publicity due to its high cholesterol content and saturated fatty acid contents. Both have been negatively implicated as perpetrators of arteriosclerosis (Sharma *et al*. 2010) hence hypertension. From the nutritionist's point of view, the removal of a whole food group from the diet, such as ghee simply to avoid cholesterol and saturated fatty acids is illogical and creates more difficulty for Indian people where ghee plays an important role in their diet hence nutrition.

Medicinal plants contain active chemical constituents with high antioxidant property which may play an important role in the prevention of various degenerative diseases (Lukmanul *et al*. 2008). *Arjuna* (*Terminalia arjuna)* is such a medicinal tree native to Indian subcontinent. In ayurveda, *arjuna* has been used as a prophylactic agent against many systemic aliments, notably "hritshool" (angina) and related cardiac ailments (Balasubramanian 2003). The bark, leaves and fruits of *Terminalia arjuna* have been used in indigenous system of medicine for different ailments (Warrier *et al.* 1996). *Arjuna* is described as good source of phytosterol (plant sterol *i.e.* β-sitosterol) which lowers down the cholesterol in the blood serum (Hawk *et al.* 1954). *Arjuna* bark extract contains various functional constituents e.g. tannins, triterpenoids, saponins (arjunic acid, arjunolic acid, arjungenin, arjunglycosides), flavonoids (arjunolone, arjunon, luteolin), gallic acid, ellagic acid, oligomeric proanthocyanidins (OPCs), phytosterols, calcium, magnesium, zinc and copper (Miller 1998). *Arjuna* bark

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reported to decrease the level of serum triglycerides and cholesterol, recovers the level of high density lipoprotein (HDL), relieves myocardial necrosis, modulates platelet aggregation and also acts as an effective antioxidant (Balasubramanian 2003). As there is no single drug till date which alone or in combination offers definite and reliable protection cure from atherosclerotic cardiovascular disorders, the time is arrived to incorporate *Terminalia arjuna* in the overall management of coronary artery disease (CAD) and related cardiovascular disorders. Hence, the present work was carried out to standardize the process of a*rjuna* herbal buffalo milk ghee production using direct cream and creamery butter method and to compare the physico-chemical attributes of the product with conventional ghee and herbal ghee prepared with *arjuna* bark.

MATERIALS AND METHODS

Raw materials: Arjuna bark was procured from the local market of Karnal, Haryana and used as the raw ingredient for the preparation of aqueous and alcoholic extracts. Buffalo milk was procured from the Cattle Yard of National Dairy Research Institute, Karnal, Haryana.

Commercial aqueous arjuna *extract:* Commercial aqueous extract of *arjuna* herb in powder form was purchased from a plant based nutraceutical manufacturer located at New Delhi.

Preparation of arjuna *extract:* Aqueous extract of *arjuna* herb was prepared by the method prescribed by Oberoi *et al*. (2011) with minor modifications. Aqueous extract was prepared by adding 10 g of pulverized crude a*rjuna* bark to 500 ml of boiling ultrapure water (Milli-Q) and boiled for 15–20 min, followed by cooling to 30°C and then centrifuged for 15 min at 1000 rpm. The supernatant was collected as the decoction stock solution and then dried at 65°C in a tray drier for 12 h.

Alcoholic extract of *arjuna* herb was prepared by cold macerating one part of a*rjuna* 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 a*rjuna* extracts were packed in air tight glass containers and stored in refrigerator until further use.

Preparation of arjuna *herbal ghee:* For each batch, 40 l milk was preheated to 45°C; cream (containing 60% fat) was separated using a mechanical cream separator. The cream was then pasteurized at 90°C for 15 sec and cooled to 4°C. In case of direct cream method of ghee preparation, the aqueous a*rjuna* extract was added directly to the cream at 45°C and clarified at 113–115°C for no hold, followed by filtration through cheese cloth. For the preparation of ghee using creamery butter method, cream was standardized to 40% fat using buffalo skim milk (0.2% fat, 8.5% SNF) cooled to 4°C and aged overnight at this temperature. The aged cream was churned into butter that contained 80% fat. The produced butter was melted properly and ethanolic extract of a*rjuna* was added to it and clarified at 113–115°C, followed by filtering through cheese cloth. The flow diagram for the production of herbal ghee is given in Fig. 1 (In each batch, 2.4 l of ghee was obtained).

Determination of phytosterol content: Phytosterol in ghee was determined by the direct colorimetric method as described by Sabir *et al*. (2003). A blank was run simultaneously. The standard curve (a straight line passing through the origin) was prepared by taking known amount of pure cholesterol, developing the colour with the LB

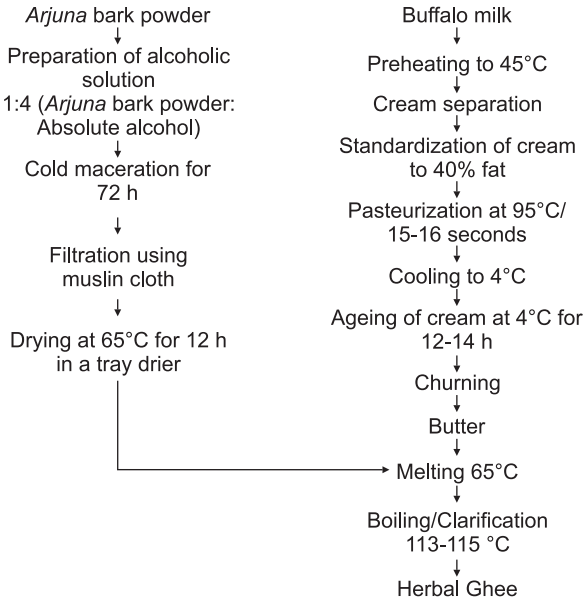

Fig. 1. Flow diagram for the production of herbal ghee.

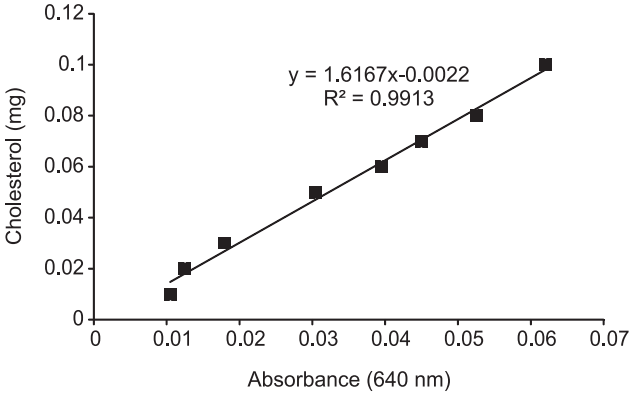

Fig. 2. Standard curve for estimation of total sterols.

reagent and recording the absorbance reading against the corresponding amount of cholesterol. The standard curve to determine total sterols in ghee is given in Fig. 2. The phytosterol content was then estimated by the method of difference in control and the herbal ghee samples.

Calculation of yield of ghee: The yield of ghee was calculated by calculating the percentage of fat retained in ghee from fat source butter as well as the fat recovered from ghee residue which again clarified and then blended with a*rjuna* ghee. In order to recover the fat from the ghee residue,

Table 1. Effect of type of extract and methods of ghee making on phytosterol content, yield and sensorial scores of *arjuna* ghee

	Direct cream method			Creamery butter method		
	Commercial aqueous extract	Laboratory made aqueous extract	Laboratory made alcoholic extract	Commercial aqueous extract	Laboratory made aqueous extract	Laboratory made alcoholic extract
Phytosterol content (mg/g)	0.22 ± 0.00^{c}	0.04 ± 0.01^{a}	0.09 ± 0.01^{b}	0.34 ± 0.01^{a}	0.03 ± 0.00^{c}	0.11 ± 0.00^{b}
Yield (%)	81.42 ± 1.40^{a,b}	74.58 ± 2.34^{b}	84.50 ± 2.60^{a}	81.97 ± 1.14^{c}	86.58 ± 0.40^{b}	90.03 ± 1.47^{a}
			Sensory attributes			
Flavour	38.33 ± 1.23^{b}	42.67 ± 0.78^{a}	43.56 ± 0.99^{a}	36.78 ± 1.54^{b}	41.67 ± 1.28^{a}	42.11 ± 1.5^{a}
Texture	27.44 ± 0.56^{a}	25.00± 0.62^{b}	26.56 ± 0.67^{a,b}	26.78 ± 0.40^{a}	26.44 ± 0.69^{a}	26.44 ± 0.44^{a}
Colour	8.00 ± 0.17	8.44 ± 0.18	8.33 ± 0.17	8.22 ± 0.28	8.22 ± 0.32	8.00 ± 0.29
Freedom from suspended solids	9.67 ± 0.17	9.44 ± 0.18	9.67 ± 0.17	9.56± 0.18	9.44 ± 0.24	9.67 ± 0.17
Overall acceptability	83.44 ± 1.46^{b}	85.56 ± 0.75^{a,b}	88.11 ± 1.20^{a}	81.33 ± 1.46^{a}	85.78 ± 1.98^{a}	86.22 ± 2.23^{a}

N = 3; $^{a, b, c}$means values with different superscripts within column differ significantly (P<0.05).

it was soaked in hot water and then stored in the refrigerator to solidify the fat. Then this fat layer was recovered.

Sensory evaluation: The herbal ghee samples were evaluated for their sensorial acceptability during the process of standardization. Sensory evaluation was carried out by presenting approximately 40 ml of herbal ghee samples (tempered to 40°C) to seven semi trained panelists selected from the faculty of Dairy Technology Division of National Dairy Research Institute, Karnal. Panel members were requested to judge each sample on the basis of flavour, body and texture, colour and appearance, suspended solids and overall acceptability and requested to indicate their score on a 100-point scale as prescribed by Bureau of Indian Standards (IS: 7770–1975).

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) and the data were expressed as mean values of three replicates with standard error.

RESULTS AND DISCUSSION

The optimization of the protocol for the production of herbal buffalo milk ghee using *arjuna* extract was carried out in two steps. In the first step, the extract was selected based on phytosterol content, yield and sensory attributes of ghee and in the second stage level of the extract was determined.

Selection of type of extract for the production of herbal ghee: To study the effect of type of *arjuna* extract on the sensory attributes of herbal ghee, three different types of extracts i.e. commercial aqueous extract, laboratory made aqueous extract and alcoholic extract were tried at 4% of fat of cream and butter. The effect of type of extract and methods of ghee making on phytosterol content, yield and sensorial scores of *arjuna* ghee is presented in Table 1.

In case of *arjuna* ghee manufactured employing direct cream as well as creamery butter method, it was observed that there was a significant difference (P<0.05) in the phytosterol content (mg/g) of the herbal ghee prepared using three different types of extracts. The retention of phytosterol was highest in case of ghee prepared using commercial aqueous extract powder followed by ghee prepared using laboratory made alcoholic extract and then ghee prepared using laboratory made aqueous extract.

In case of herbal ghee manufactured employing direct cream method, it was observed that there was a significant difference (P<0.05) in the percentage yield of the herbal ghee prepared from three different types of extracts. The percentage yield was highest in case of ghee prepared using commercial aqueous extract powder followed by ghee prepared using laboratory made alcoholic extract and then ghee prepared using aqueous extract. While in case of herbal ghee manufactured employing creamery butter method, it was observed that there was a significant difference (P<0.05) in the yield of the herbal ghee prepared using commercial aqueous extract powder to that of other two samples. The percentage yield was highest in case of ghee prepared from laboratory made alcoholic extract followed by ghee prepared from aqueous extract and then ghee prepared from commercial aqueous extract powder.

It is evident that in case of herbal ghee manufactured employing direct cream method as well as creamery butter method, the flavour score of the ghee prepared using commercial aqueous extract powder was less (P<0.05) compared to ghee prepared from laboratory made aqueous and alcoholic extract. It was observed that the bitterness/ astringency was more in case of ghee prepared using commercial aqueous extract powder than the other two *arjuna* ghee samples. Higher content of tannins in commercial aqueous extract powder compared to other two extracts may be the reason of astringency in ghee prepared using commercial aqueous extract powder as around 15 types of tannins and related compounds have been isolated from bark of *Terminalia arjuna* (Lin *et al*. 2001) which may be responsible for its astringency, wound healing and anti-microbial activity (Chaudhari and Mengi 2006). For the colour attribute, the intensity towards reddish brown was found to be more in case of ghee prepared from

commercial aqueous extract powder than the other two samples. The dark brown colour of ghee may be due to the presence of tannins as reported by Saha *et al*. (2012) that aqueous fraction of *Terminalia arjuna* was dark brown in colour indicating the presence of tannins or proanthocyanidins and intensity of reddish brown colour of ghee may be interpreted by higher concentration of tannins or proanthocyanidins in dehydrated form. In case of *arjuna* ghee made using direct cream method, overall acceptability of the herbal ghee was highest in case of ghee sample prepared using laboratory made alcoholic extract followed by ghee prepared using laboratory made aqueous extract and commercial aqueous extract powder, respectively. Texture wise it was found that the ghee prepared using commercial aqueous extract was superior than the other two samples. It was observed that overall acceptability of the herbal ghee significantly differed (P<0.05) from each other. Overall acceptability of the herbal ghee was more in case of ghee prepared using laboratory made aqueous extract followed by ghee prepared using laboratory made alcoholic extract and then for the ghee prepared using commercial aqueous extract powder irrespective of ghee production method used.

Based on the above results, it was found that the phytosterol content was highest in ghee prepared using commercial aqueous extract powder. The maximum yield was obtained when ghee was prepared using laboratory made alcoholic extract in both the production process that were adopted i.e. direct cream and creamery butter methods. Among the two processes, the yield was higher when ghee was prepared employing creamery butter method. Bitterness was more in case of ghee prepared using commercial aqueous extract powder than the other two samples and the ghee prepared from laboratory made aqueous extract was turbid and was much difficult to filter even after settling of the a*rjuna* extract residues and that lead to losses of fat at the time of filtration process. Hence, the ghee prepared employing creamery butter method from butter containing 80% fat produced from buffalo cream using laboratory made alcoholic extract had higher yield and better sensorial attributes than other samples. Hence, the ghee prepared from creamery butter method using laboratory made alcoholic extract was selected for the rest of the study.

Selection of level of extract for the production of herbal ghee: The effect of level of extract and methods of ghee making on phytosterol content, yield and sensorial scores of *arjuna* ghee is presented in Table 2.

It is also clear that there was a direct relationship between the rate of addition of alcoholic extract and the phytosterol content in herbal ghee. Rajnikant (2005) reported no significant difference in phytosterol content with increase in level of herb but there was a minute increase in phytosterol content observed with increase in herb level. Similar findings were reported by Shivaswamy (2009) also. It was observed that there was an inverse relationship between the rate of addition of laboratory made alcoholic extract and the yield when creamery butter method was used to prepare ghee. While, when the ghee was prepared using creamery butter method using laboratory made alcoholic extract at three different levels, there was a significant difference (P<0.05) in the yield of herbal ghee as the level of extract increased from 5 to 7%. It was also observed that there was an inverse relationship between rate of addition of alcoholic extract and the percentage yield of ghee.

It is evident that the sensorial attributes of *arjuna* ghee samples did not differ significantly except the flavour score of the samples prepared using direct cream method. Our results were in agreement with Rajnikant (2005) who reported that with increase in level of *arjuna* herb in cow ghee, flavour score of ghee decreased significantly (P<0.01). Similar findings were observed by Shivaswamy (2009) who reported a non-significant negative effect on flavour of *Triphala* ghee with level of ethanolic extract of *Triphala* herb. Sawale (2015) also reported that as the concentration of lyophilized *Pueraria tuberosa* extract in milk was increased from 0.2 to 0.5%, a significant drop (P<0.05) was observed in all the sensory attributes except sedimentation. Kumar *et al*. (2013) also reported that flavour preference of *Tulsi* extract based herbal ice-cream increased compared

Table 2. Effect of level of extract and methods of ghee making on phytosterol content, yield and sensorial scores of *arjuna* ghee

	Laboratory made alcoholic extract					
	Direct cream method			Creamery butter method		
	5%	6%	7%	5%	6%	7%
Phytosterol content (mg/g)	0.11 ± 0.000^{c}	0.26 ± 0.004^{b}	0.38 ± 0.004^{a}	0.12 ± 0.004^{c}	0.18 ± 0.008^{b}	0.47 ± 0.008^{a}
Yield (%)	81.41 ± 0.84^{a}	80.41 ± 0.47^{a}	78.97 ± 2.09^{a}	89.90 ± 0.06^{a}	88.58 ± 0.04^{b}	88.18 ± 0.02^{c}
	Sensory attributes					
Flavour	41.82 ± 0.81^{a}	41.55 ± 1.07^{a,b}	38.55 ± 1.28^{b}	43.22 ± 1.37^{a}	41.33 ± 1.35^{a}	41.67 ± 1.34^{a}
Texture	25.73 ± 0.57^{a}	25.91 ± 0.67^{a}	24.73 ± 0.51^{a}	25.67 ± 0.62^{a}	27.00 ± 0.71^{a}	25.78 ± 0.40^{a}
Colour	8.64 ± 0.28	8.73 ± 0.27	8.73 ± 0.27	8.44 ± 0.24	9.00 ± 0.29	8.56 ± 0.24
Freedom from suspended solids	9.36 ± 0.20	9.36 ± 0.20	9.64 ± 0.15	9.44 ± 0.24	9.56 ± 0.24	9.44 ± 0.24
Overall acceptability	85.64 ± 1.49	85.64 ± 1.85	82.64 ± 1.27	86.78 ± 1.79	86.89 ± 2.12	85.44 ± 1.63

N=3; $^{a, b, c}$means values with different superscripts within column differ significantly (P<0.05).

Table 3. Comparative chemical composition of the optimized product

Constituent	Amount	
	Control ghee	Buffalo milk a*rjuna* ghee
Moisture (%)	0.13	0.08
Milk fat (%)	99.87	99.92
Free fatty acids (% Oleic acid)	0.20	0.22
Butyro Refractometer reading at 40°C	41.5	41.5
Reichert-Meissl value	32	31.5
Phytosterol content (mg/g)	-	0.39

to plain ice-cream when *Tulsi* extract was used up to 3% level only while increase in level of extract (i.e. 4% level) led to decrease in flavour score due to presence of excessive amounts of *Tulsi* extract and intense flavour. However, the overall sensory acceptability score was highest at 5% level, followed by 6% and 7%. Similar findings were observed by Rajnikant (2005) who reported that there was a negative effect of level of *arjuna* herb on the overall acceptability of herbal ghee.

Comparison of the physico-chemical attributes of herbal ghee with conventional ghee: The comparative chemical composition of the product prepared using 7% laboratory made alcoholic extract with control buffalo ghee prepared by creamery butter method using laboratory made alcoholic *arjuna* extract is presented in Table 3. It can be concluded from the table that both the ghee samples complied with the standards prescribed by FSSR (2011). The fat content as well as free fatty acid content was higher in a*rjuna* ghee than the control ghee. RM value was less in optimized product compared to control ghee. The optimized product was having phytosterol content 0.39 mg/g.

Based on the above results it can be concluded that the phytosterol content was highest in ghee manufactured by creamery butter method using laboratory made alcoholic extract at the rate of 7% by weight of fat in butter. The yield of ghee was highest when alcoholic extract was added at 5% in both the methods of the ghee preparation i.e. direct cream and creamery butter method. However, the ghee prepared by creamery butter method from buffalo cream using laboratory made alcoholic extract at 7% had maximum phytosterol content with acceptable sensorial attributes.

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