RESEARCH ARTICLE Asian Journal of Dairy and Food Research, Volume 43 Issue 3: 453-461 (September 2024)

Study of Phytochemical Attributes of Ashwagandha Ghrita from Desi Cow Milk

Vishal Kumar[1], Tarun Verma[1], Suresh Chandra[2], Anita Wanjari[3], Vikas Patel[1], Saurabh Singh[1]

10.18805/ajdfr.DR-2229

ABSTRACT

Background: The dairy industry constantly works to manufacture healthier products to fulfill consumer demand. Ashwagandha therapeutic and medical qualities are highly recognized. The current study examines the phytochemical attributes of Ashwagandha (*Withania Somnifera*) ghrita from desi cow milk.

Methods: The Ashwagandha ghrita was prepared by following the guidelines of the Central Council for Research in Ayurvedic Sciences (CCRAS). Cow ghee contains 0.20% moisture, 99.85% milk fat and 0.20% free fatty acids as oleic acid. The butyro refractometer value is 41, the Reichert-Meissel value is 31, the Polenske value is 1.18, the Peroxide value is 0.75 and DPPH is 75.6. The Ashwagandha ghee contains 0.18% moisture, 99.82% milk fat and 0.40% free fatty acids as oleic acid. It has a Butyro Refractometer value of 42, Reichert-Meissel value of 32, Polenske value of 1.19, Peroxide value of 0.80, DPPH of 83.98, total phenolic content (GAE/ml) of 62.85 µg and phytosterol content of 0.33 mg/g.

Result: During investigation, it was found that the nutritional profile of Ashwagandha ghrita is better as compared to cow ghee. The sensory evaluation (flavor, texture, color, freedom from suspended solid and overall acceptability) was found significant ($p<0.05$) which was evaluated by a trained panel. Ashwagandha may be used as a natural ingredient for the development of Ashwagandha ghee with high antioxidant activity.

Key words: Antioxidant activity, Ashwagandha, Free fatty acid, Ghee, Phytochemical.

INTRODUCTION

In rural areas worldwide, more than 80% of people use plant-based traditional medicines such as Ayurveda, Unani and traditional Chinese medicine (Majeed, 2017). Ashwagandha [*Withania somnifera* (L.) Dunal] is an ancient medicinal herb used in Ayurvedic and Unani medicine. With over a hundred years of experience in cultivating, extracting and distributing ashwagandha, the world's largest producer of this herb is India. Other countries are consuming Ashwagandha in the form of pills, capsules and other formulations from India (Khabiya *et al.,* 2023). There are various pharmacological uses for this herb, including analgesics, antioxidants, anti-inflammatory, antidepressants, antimicrobials, anticonvulsants, anxiolytics and hypnotics, cardioprotective, nootropics, *etc*. (Yadav *et al.,* 2024; Langade *et al.,* 2023).

Ashwagandha roots are used in Ayurvedic medicine for their therapeutic properties. Ashwagandha roots contain phenolics, steroids, saponins, alkaloids, glycosides and volatile oils in addition to several hundred traditional phytochemicals (Yadav and Rai, 2018a; Naveed *et al.,* 2022). Besides having antioxidant and anti-inflammatory properties, these compounds also play a crucial role in producing therapeutic effects (Mandlik *et al.,* 2021). As a result, the usage of medicinal plants is becoming more widespread than it was previously, ushering in a new era in the treatment of several chronic illnesses, such as obesity, diabetes and hypertension (Choudhary *et al.,* 2023).

[1]Department of Dairy Science and Food Technology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi-221 005, Uttar Pradesh, India.

[2]Department of Agriculture Engineering, College of Post Harvest Technology and Food Processing, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut-250 110, Uttar Pradesh, India.

[3]Department of Rasashastra and Bhaishajya Kalpana, Mahatma Gandhi Ayurved College, Hospital and Research Centre, Salod (H), Datta Meghe Institute of Higher Education and Research, Wardha-442 001, Maharashtra, India.

Corresponding Author: Vishal Kumar, Department of Dairy Science and Food Technology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi-221 005, Uttar Pradesh, India. Email: vishalkumar@bhu.ac.in

How to cite this article: Kumar, V., Verma, T., Chandra, S., Wanjari, A., Patel, V. and Singh, S. (2024). Study of Phytochemical Attributes of Ashwagandha Ghrita from Desi Cow Milk. Asian Journal of Dairy and Food Research. 43(3): 453-461. doi: 10.18805/ajdfr.DR-2229.

Submitted: 23-04-2024 **Accepted:** 02-07-2024 **Online:** 14-08-2024

To maintain and improve health, nutrition and immunity, health-conscious consumers have shown a growing interest in making use of the therapeutic and functional properties of herbs. Spices and herbs were traditionally considered to be medicinal foods (Rani *et al.,* 2023). The trend of fortifying dairy products with these herbs to take advantage

of their therapeutic and functional properties has also gained attraction (Paswan *et al.,* 2021; Yadav and Rai, 2018b). Milk and dairy products are increasingly popular due to their reputation as a nearly complete source of nutrition, often described as a "nutrient reservoir"(Gupta *et al.*, 2022). Ghee is one of the crucial components of the Ayurvedic medical system. Ghee is the Indian term for clarified butter fat, which is made by boiling it off. Buffalo and cow milk, either individually or in combination, are typically used to make it. In India, approximately 50-55% of the country's total milk production is utilized in the creation of traditional dairy products (Singh *et al.*, 2023). Ghee is made from about 30 to 35 per cent of the milk produced in India (Laghari *et al.,* 2023). Ghee and butter, in particular, can capture all of the therapeutic and functional properties of the integrated bio-actives without altering their qualities (Pandhi *et al.,* 2021).

In the current millennium, the dairy industry is shifting from a focus on bulk commodities to an emphasis on innovative and flavorful value-added products. This transition is marked by the development of niche segments that prioritize unique taste, color and visual appeal (Sahana and Vijayalaxmi, 2024). Ghee serves as a basis for the Ayurvedic medication ghrita, which dissolves, extracts, or retains the active medicinal ingredients. Ghee preparations with added medicinal properties, known as ghritas, are made with the fat-soluble parts of the components (Prajapati *et al.,* 2017). Dairy products could provide consumers with not just the usual nutritional benefits but also various additional health benefits. The purpose of this study was to develop herbal ghee with cow's milk and ashwagandha ghrita. The product was also assessed for characterization, antioxidant, phenolic activity and sensory characteristics.

MATERIALS AND METHODS

The experimental research was conducted in the laboratory of the Department of Dairy Science and Food Technology at the Institute of Agricultural Sciences, (BHU). Cow milk was purchased from dairy farm at BHU Varanasi. Ashwagandha root was procured from the local market of Varanasi. All the chemicals used during the study were of analytical grade and were procured from Hi Media Laboratories, Pvt. Ltd., Mumbai, India.

Preparation of ashwagandha kwath

The process outlined in the Ayurvedic Sarsangraha was followed to make ashwagandha kwath (Gurav *et al.,* 2020) as shown in Fig 1. Ashwagandha roots were dried in the sun for a day to remove moisture. They were then crushed into coarse powder using a mortar and pestle. The coarse powder was separated and the remaining ashwagandha roots were further ground into fine powder using a mixer and grinder. Approximately 200 g of the coarse ashwagandha root powder was measured and soaked overnight in four liters of water. The next day, the container with the soaked ashwagandha was heated on a gas burner until the liquid was reduced to half its original volume, which took about 6 hour. This liquid was then filtered and set aside.

Preparation of ashwagandha ghee

The methods for the formation of ghee and preparation of the sample were according to De (2005) with slight modifications are shown in Fig 1 and 2. In a separate pot, several liters of milk were boiled. To this boiling milk, 200 g of ghee and 50 g of finely powdered ashwagandha were added. In addition to the liquid, the one-liter decoction (liquid) prepared earlier was added too. The mixture was boiled on low heat for an additional 2 hour. After boiling, it was left to cool overnight. The next morning, heating was resumed and occasional stirring was done. During the heating, a frothy layer formed on the surface of the mixture and the milk started to curdle, forming a solid consistency after about 6 hours of continuous boiling. By the 10th hour of reduction, a cohesive mud-like paste had formed at the bottom of the container. Continuous stirring was done to prevent the paste from charring. As time passed, around the 12th hour of stirring, the frothy layer on the surface began to disappear. We kept on heating the mixture until every bit of water had evaporated from the ghee and the ghee started to separate from the paste. As it reached the final stages, the ghee became clear and transparent and there were no bubbles or froth left. To make sure that all the water had completely evaporated, we took a small amount of the paste and burned it. If there were no crackling sounds, it showed that all the water had been successfully removed. This step was crucial to confirm that the ghee was pure and didn't contain any remaining water.

Characterization of ashwagandha root powder

Using standard AOAC procedures (2007), chemical analysis of dehydrated ashwagandha (*Withania somnifera*) was conducted to approximate the concentration of moisture, ash, fat, protein and fiber. The calorimetric method was used to determine iron, while the titration method was used to determine calcium. Using the 2, 6-Dichlorophenol dye method, Vitamin C was determined.

Characterization of ghee

Moisture and fat

The moisture and fat percent of Ashwagandha ghee were determined by the AOAC (2007) method.

Butyro refractometer, reichert-meissl and polenske value

The butyro refractometer (BR) was used to determine the butyro refractometer (BR) reading of the ghee samples. Using the conventional procedure by BIS (1981), the Reichert-Meissl (RM) and Polenske values of ghee samples were calculated.

Oxidative stability (Peroxide Value)

Ghee peroxide value was ascertained using Lea's method, which is described in the BIS method (IS:3508, 1966), to assess oxidative stability.

Vitamins and cholesterol

The vitamins and cholesterol of ghee were determined by the AOAC method as described in (AOAC, 2000).

Sodium, potassium, calcium and iron

With slight modifications, the procedure outlined by (Vasanth *et al.*, 1971) was used to ascertain the mineral content of ghee.

Quantification of antioxidant and phenolic properties of ghee

DPPH was calculated using the scavenging activity method developed by (Patel *et al.,* 2022). 200 µl of prepared samples were analyzed at a wavelength of 517 nm for this study. A UV-VIS spectrophotometer (Shimadzu, Japan) was used to measure the absorbance.

$$\text{Percentage DPPH Inhibition} = \frac{A0\text{-}A1}{A0} \times 100$$

Where:

A0= Absorbance of the control.

A1= Observed final absorbance of the extracted sample at the wavelength of 517 nm.

In the blank experiment, 95% Ethyl acetate was used.

Total phenolic content

The total phenolic content (TPC) was determined according to the method described by Krawitzky *et al.* (2014) with slight modifications. For this, 100 µl prepared sample was taken after that, a UV-VIS spectrophotometer (Shimadzu, Japan) was set to detect absorbance at 760 nm wavelength. Finally, the total phenolic content in the sample was expressed in micrograms of gallic acid equivalent (GAE) per milliliter (ml) of the extract. This measurement helps assess the concentration of phenolic compounds, which are important antioxidants, in the sample.

Statistical analysis

To measure the phenolic, antioxidant and characterization qualities of ashwagandha ghee, the experiment was carried out in triplicate. To ascertain the significance of sample differences, OP Stat software was used. Significance levels were decided at ($p<0.05$).

RESULTS AND DISCUSSION

Chemical composition

The comparative chemical composition of control ghee and ashwagandha ghee is presented in Table 1. The control ghee and ashwagandha ghee had almost the same moisture content, 0.20% in control ghee and 0.18% in ashwagandha ghee. The fat content was very high and was comparable in both ghees, with control ghee containing 99.85% and ashwagandha ghee containing 99.82% fat. The free fatty acid content of control ghee was 0.20% compared to 0.40% of ashwagandha ghee. The Butyro refractometer reading of the control ghee was 41 while the ashwagandha ghee was 42. The Reichert-Miesel value of the control ghee was 31, while the ashwagandha ghee was slightly higher. Both ghees had similar Polanske-value, control ghee was 1.18 and ashwagandha ghee had 1.19. The percentage of DPPH inhibition in control ghee (75.6%) was lower compared to Ashwagandha ghee (83.98%). The total phenolic content of ashwagandha ghee was 62.85 µg GAE/ml, while control ghee showed no detectable amount. The phytosterol content of ashwagandha ghee was 0.33 mg/g. The peroxide value of control ghee was 0.75, whereas ashwagandha ghee had a slightly higher value of 0.80.

A comparison between the fat content of regular cow ghee and ghee enriched with ashwagandha can reveal any changes in the lipid profile brought about by the herbal enrichment. General research on the nutritional profiles and composition of ghee was discussed by (Kumar *et al.,* 2018) which proves that supplementation with herbs in ghee reduces the moisture content. Readings from a Butyro refractometer at 40°C are used to determine the composition and purity of ghee. It evaluates how much solid fat is in the ghee (Gandhi and Lal, 2017). Variations in this value between regular cow ghee and ghee enhanced with ashwagandha could be a sign of changes in the composition and crystalline structure (Ramya *et al.,* 2019). One metric used to evaluate the volatile fatty acid concentration of ghee is the Reichert-Meissl value. It is a sign of the ghee's quality and freshness (Nekera *et al.,* 2023). Variations in the amounts of volatile fatty acids between the enriched and regular ghee can provide information on the stability and possible oxidation of the goods (Kumbhare *et al.,* 2021). Studies on volatile fatty acids in dairy products can be cited to support information on the Reichert-Meissl value and its implications for the quality of ghee (Gandhi *et al.,* 2014). The Polenske value evaluates the amount of volatile fatty acids while also taking the existence of unsaturated fatty acids into account, much like the Reichert-Meissl value. Variations in the fatty acid composition and oxidative stability between the two forms of ghee may be indicated by differences in Polenske values (Ahmed *et al.,* 2020). As with the Reichert-Meissl value, pertinent research on fatty acid content and oxidative stability might be consulted when discussing the Polenske value (Ahmed *et al.,* 2020). The peroxide value is a measure used to determine the extent of primary oxidation in fats and oils. Increased oxidative stress is indicated by elevated peroxide levels (Gotoh and Wada, 2006). Peroxide readings from regular cow ghee and ghee enhanced with ashwagandha can be compared to provide insight into the products' oxidative stability. Research on lipid oxidation in dairy products can be cited to support a thorough discussion of peroxide value (Pawar *et al.,* 2014).

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay quantifies a material's antioxidant capacity (Shahidi and Zhong, 2015). When comparing ashwagandha-enriched ghee to regular cow ghee, a higher percentage of DPPH inhibition would suggest that the addition of ashwagandha has boosted the antioxidant ability due to the presence of

Withaferin and other compounds. Research studies on the antioxidant qualities of herbs and functional foods were cited to support discussions on the DPPH assay and antioxidant activity (Gurav *et al.,* 2020).

Phenolic chemicals enhance a product's capacity to function as an antioxidant. A comparison of total phenolic content can reveal information about the overall richness of antioxidants (Piluzza and Bullitta, 2011); greater values in ghee supplemented with ashwagandha suggest possible health benefits. Studies on the phenolic composition of herbs and their contributions to antioxidant capacity were used to support information on total phenolic content and its significance (Pawar *et al.,* 2014).

Nutritional profile

The nutritional profile of control ghee and ashwagandha ghee is presented in Table 2. The micronutrients such as sodium, potassium, calcium and iron were found to be high in ashwagandha ghee as compared to control ghee, while the Vitamin A, cholesterol and energy value were found to be high in control ghee as compared to ashwagandha ghee.

When compared to the control ghee, ashwagandha ghee has noticeably higher levels of salt, potassium, calcium and iron. This could be explained by ashwagandha's natural mineral composition, which is high in important minerals (Nekera *et al.,* 2023). Ashwagandha ghee's improved mineral composition may help it fulfill its potential as a nutrient-dense dietary item. When compared to ashwagandha ghee, the control ghee has a higher vitamin A content. The variance might result from the unique ways that ashwagandha is extracted and processed, which could affect how much of certain vitamins are retained (Singh *et al.,* 2011). To clarify the impact of ashwagandha on the vitamin makeup of ghee, more investigation is required.

Ashwagandha ghee has less cholesterol than ghee which is used as a control. This result is consistent with earlier research that suggested some herbal extracts, such as ashwagandha, may have the ability to decrease cholesterol. Ashwagandha ghee's possible cholesterol-lowering qualities may be a factor in its advantages for cardiometabolic health (Sharma *et al.,* 2010). Ashwagandha ghee has a somewhat lower total energy content than control ghee. This could be explained by the unique makeup of ashwagandha and how it might affect the ghee product's total energy density (Singh *et al.,* 2011). To fully comprehend the impact of ashwagandha on energy metabolism, more research on the metabolic effects of ghee is necessary (Sharma *et al.,* 2010).

According to comparative nutritional research, ashwagandha ghee has a unique nutritional profile that is enhanced with important minerals and may have certain health advantages. Variations in vitamin content, however, call for additional research. The benefits of Ashwagandha ghee as a functional food are evident in its impact on cardiovascular health and energy balance. These benefits are underscored by the notable differences in cholesterol levels and energy content.

Shelf-life evaluation by sensory and antioxidative parameters

The sensory parameters and several antioxidative parameters of control ghee and ashwagandha ghee during the storage period have been presented in Table 3. The flavor score of control ghee (9.01±0.02) was found higher than ashwagandha ghee (8.80±0.02). On the opposite, the texture score was slightly higher in ashwagandha ghee (24.55±0.10) as compared to control ghee (24.25±0.02). The score of color and appearance was higher in control ghee (9.05±0.02) as compared to ashwagandha ghee (8.87±0.02). There were no significant differences in the score of overall acceptability. The score of overall acceptability was slightly higher in ashwagandha ghee (94.20±0.02) as compared to control ghee (94.15±0.02). From the study, it was revealed that as the days pass during storage the antioxidant values in the form of FFA, DPPH and peroxide values decrease significantly. The same pattern was also observed for the organoleptic parameters as well.

Sensory evaluation is a way to scientifically study and understand how our senses (like sight, smell, taste, touch and hearing) experience things, such as food or materials (Tzia *et al.,* 2023). It helps us to measure, analyze and

Table 1: Comparative chemical composition of the optimized product.

Chemical compositions	Control ghee	Ashwagandha ghee
Moisture (%)	0.20±0.01	0.18±0.01
Milk fat (%)	99.85±0.02	99.82±0.02
Free fatty acids (% Oleic acid)	0.20±0.01	0.40±0.02
Butyro refractometer reading at 40°C	41±20	42±20
Reichert-meissl value	31±20	32±20
Polenske-value	1.18±0.02	1.19±0.02
% DPPH inhibition	75.67±0.02	83.98±0.02
Total phenolic content (µg GAE/ml)	-	62.85±0.02
Phytosterol content (mg/g)	-	0.33±0.02
Peroxide Value	0.75±0.02	0.80±0.02

All the Values are in mean±SD (n= triplicate).

make sense of what we feel, see, smell, taste, or hear when we interact with something. The temperature at which fat (ghee) is stored significantly impacts both sensory acceptance and oxidative degradation. The special taste of ghee comes from a mix of different compounds created when it is made (Pena-Serna and Restrepo-Betancur, 2020). These compounds, like free fatty acids, carbonyls and lactones, give ghee its unique aroma (Bumbadiya *et al.,* 2023). Ghee takes on a different flavor and aroma when produced with Ashwagandha containing certain bioactive chemical compounds. Our findings agreed with Rajnikant, (2005) findings that the flavor score of ghee significantly decreased with an increase in the amount of arjuna herb in cow ghee. The addition of ashwagandha may be the reason for the minor reduction in flavor intensity in Ashwagandha Ghee, as shown by the lower score when compared to control ghee (Gurav *et al.,* 2020). The unique flavor profile of the herb may affect the ghee's overall flavor. Research indicates that the perception and acceptance of flavor may be affected by herbal additions. It's interesting to note that Ashwagandha Ghee has a little better texture score than Control Ghee. This outcome might be the result of Ashwagandha's special makeup, which could alter the ghee's texture and consistency (Gurav *et al.,* 2020).

One important factor influencing the stability and shelf life of ghee products is their moisture level. Being less in moisture content is generally preferable since it lowers the chance of lipid oxidation and microbial development (Nekera *et al.,* 2023). An analysis of the effects of the herbal enrichment on moisture levels can be obtained by contrasting regular cow ghee with ghee supplemented with ashwagandha powder. Research on lipid stability and microbial development in dairy products can be used to examine how moisture content affects the quality of ghee (Pena-Serna and Restrepo-Betancur, 2020). The main component of ghee is fat and the amount of fat present is a significant indicator of its nutritional worth (Nekera *et al.,* 2023).

Cow ghee has a characteristic golden-yellow color because of the presence of carotenoids (Misra *et al.,* 2023). For the color attribute, it has been found that ghee prepared from Ashwagandha powder had a stronger tendency toward reddish brown. According to (Saha *et al.,* 2012), ghee's dark brown color may be caused by tannins contained in the aqueous extract of Ashwagandha root powder. Ashwagandha ghee's lower color and appearance scores indicate a noticeable difference from control ghee. The incorporation of ashwagandha extracts may cause the ghee's color to change, which consumers may notice. This result is corroborated by research on how herbal extracts affect food products' visual characteristics (Gurav *et al.,* 2020).

Both control ghee and Ashwagandha ghee have similar overall acceptability values, although the little differences in each metric. This implies that minor variations in flavor, texture and appearance do not considerably impact consumers' overall acceptance of the products. The strong approval scores suggest that customers are still receptive to Ashwagandha ghee.

The number of carboxylic acid groups in fatty acid compounds is determined by the term "acid value." Over some time, as oil or fat oxidizes, triglycerides are transformed into fatty acids and glycerol, increasing the total acid value. It can be explained by the presence of acidic phyto-constituents, the catalytic influence of iron (from the manufacturing vessel), or the production of free fatty acids from triglycerides present in ghee (Gurav *et al.,* 2020). These may be responsible for the greater acidity of prepared ashwagandha ghee during storage when compared to control ghee (Gurav *et al.,* 2020).

The storage condition affects the lipid oxidation of fat, oils, or fatty acids (Catalá, 2010). The level of lipid peroxidation, or auto-oxidation, in unsaturated fats and oils, is the primary indicator of rancidity and is measured in terms of peroxide value (Pena-Serna and Restrepo-Betancur, 2020). High acidity indicates a high peroxide value because free unsaturated acids oxidize more quickly and easily than identical acids in intact glycerides (Oluremi *et al.,* 2013). It can be presumed that the maximum oxidative degradation of ghee is caused by the catalytic activity of iron and/or high temperature (Kochhar, 2016). It showed that the ashwagandha root provided a degree of protection against oxidative and catalytic damage as well as the production of peroxide (Gurav *et al.,* 2020). In our study, we found similar results to a research paper by (Siddiq *et al.,* 2005). They discovered that when they added extracts from the *Moringa oleifera* plant to sunflower oil, it significantly prevented the formation of harmful substances called peroxides when the oil was exposed to unfavorable conditions that speed up spoilage. Among the different extracts they tested, the one made with 80% methanol was the most effective for keeping the peroxide levels low (Siddiq *et al.,* 2005). The reason for these variations in results when using different solvents to make extracts could be that *Moringa oleifera* contains both water-soluble and fat-soluble antioxidants and these compounds work differently. Interestingly, in some parts of southern India, people use fresh *Moringa oleifera* leaves when making ghee from cow and buffalo milk. They do this to help the ghee last longer on the shelf. So, our study aligns with Siddiq's findings

Table 2: Comparative nutritional composition of the optimized product.

Nutritional profile	Control ghee	Ashwagandha ghee
Cholestrol (mg)	65±20	42±20
Vitamin A (IU)	3280±20	2970±20
Sodium (mg)	1.90±0.02	4.75±0.02
Potassium (mg)	1.10±0.02	2.89±0.01
Calcium (mg)	1.48±0.02	3.72±0.02
Iron (mg)	0.45±0.02	2.36±0.02
Total energy (Kcal)	895.50±0.02	853.20±0.02

All the values are in mean±SD (n= triplicate).

Table 3: Comparative shelf-life study of control ghee and ashwagandha ghee.

Parameters	Storage period (days)	Control ghee	Ashwagandha ghee
Peroxide value	0	1.58±0.02	1.82±0.02
	5	1.78±0.02	1.86±0.02
	15	3.65±0.02	1.95±0.02
	20	5.91±0.02	2.87±0.02
	25	8.16±0.02	4.02±0.02
	30	11.18±0.02	5.28±0.02
Flavour	0	9.00±0.02	8.80±0.02
	5	8.82±0.02	8.65±0.02
	15	8.45±0.02	8.25±0.02
	20	7.94±0.02	7.62±0.02
	25	7.52±0.02	7.26±0.02
	30	7.10±0.02	6.82±0.02
Texture	0	24.25±0.02	24.55±0.02
	5	25.45±0.02	25.75±0.02
	15	26.15±0.02	26.45±0.02
	20	26.35±0.02	26.65±0.02
	25	26.45±0.02	26.75±0.02
	30	26.55±0.02	26.85±0.02
Colour and appearance	0	9.00±0.02	8.8±0.02
	5	8.75±0.02	8.65±0.02
	15	8.55±0.02	8.45±0.02
	20	8.36±0.02	8.19±0.02
	25	8.24±0.02	8.04±0.02
	30	8.03±0.02	7.92±0.02
Ghee residue	0	9.80±0.02	9.75±0.02
	5	9.80±0.02	9.75±0.02
	15	9.65±0.02	9.6±0.02
	20	9.55±0.02	9.48±0.02
	25	9.47±0.02	9.42±0.02
	30	9.36±0.02	9.31±0.02
Overall acceptability	0	94.25±0.02	94±0.02
	5	93.55±0.02	93.15±0.02
	15	92.75±0.02	92.15±0.02
	20	91.86±0.02	91.26±0.02
	25	90.65±0.02	90.05±0.02
	30	90.15±0.02	89.75±0.02
Moisture content	0	0.18±0.02	0.16±0.02
	5	0.17±0.02	0.15±0.02
	15	0.16±0.02	0.14±0.02
	20	0.15±0.02	0.13±0.02
	25	0.14±0.02	0.12±0.02
	30	0.13±0.02	0.11±0.02
Free fatty acid content	0	0.33±0.02	1.45±0.02
	5	0.33±0.02	1.45±0.02
	15	0.46±0.02	1.45±0.02
	20	0.53±0.02	1.68±0.02
	25	0.64±0.02	2.25±0.02
	30	0.75±0.02	2.82±0.02
% DPPH inhibition	0	75.67±0.02	83.98±0.02
	5	73.87±0.02	82.65±0.02
	15	71.77±0.02	80.54±0.02
	20	70.57±0.02	79.25±0.02
	25	69.32±0.02	77.16±0.02
	30	68.04±0.02	76.13±0.02

All the values are in mean±SD (n= triplicate).

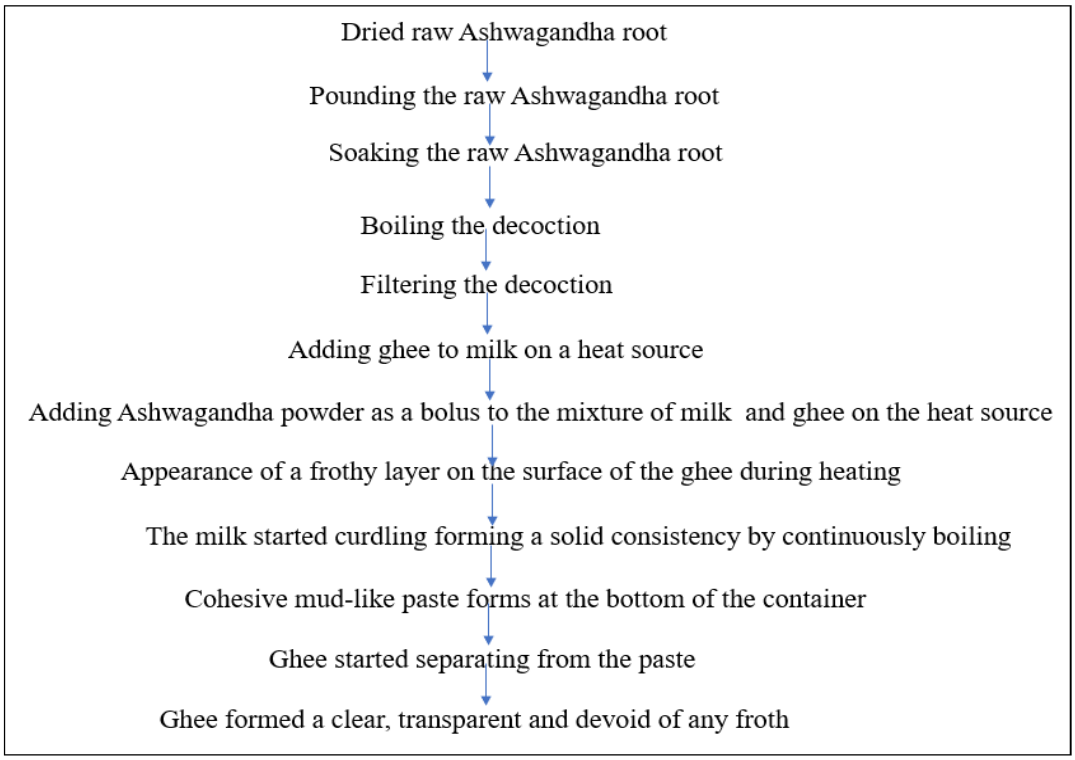

Fig 1: Flow chart for manufacturing of Ashwagandha root powder ghee.

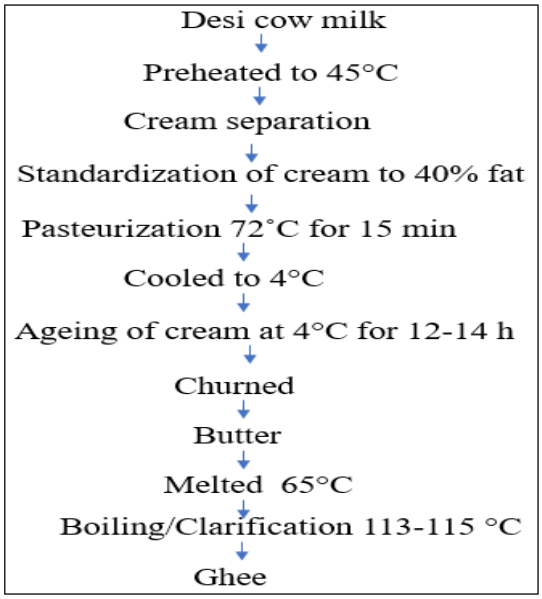

Fig 2: Flow chart for manufacturing of cow milk ghee.

about the *Moringa oleifera* extracts being good at preserving oil quality and it also highlights a traditional practice in southern India where these leaves are used to extend the shelf life of ghee (Siddhuraju and Becker, 2003). The study by Merai *et al*. (2003), found that adding 0.6 percent of a special fraction of Tulsi leaves powder treated with silica gel charcoal to ghee was better at preserving the ghee's quality than using a chemical antioxidant like BHA at a concentration of 0.02 per cent. It was more effective in preventing the ghee from getting rancid until it reached a peroxide level of 5 milliequivalents of peroxide oxygen. These studies favor the research that by adding the ashwagandha extract we could able to extend the shelf life of the herb-enriched ghee.

In a study examining how adding an extract from the herb *Asparagus racemosus*, also known as Shatavari, affects the storage stability of ghee, it was discovered that ghee samples containing the ethanolic extract of Shatavari demonstrated stronger antioxidant activity (Pawar *et al.,* 2012). The ethanolic extract of Shatavari was more effective in preserving the quality of the ghee and preventing it from becoming rancid, both at the beginning and the end of the storage period, compared to the aqueous extract of the same herb. This suggests that using the ethanolic extract of Shatavari could be a better choice for enhancing the shelf life and stability of ghee. This means that these herbal extracts were better at neutralizing harmful DPPH radicals both when the ghee was fresh (on day zero) and even after it had undergone oxidation (at the end of 21 days) at a temperature of 80±10°C.

CONCLUSION

In this study, herbal ghee containing Ashwagandha may result in a functional food item with improved antioxidant qualities and a changed nutritional makeup. These results are significant for the dairy industry since ashwagandha's great stability of sensory qualities and bioactive components

allow it to be further incorporated into a variety of dairy-based products. Furthermore, the sensory characteristics of ghee showed that the incorporation of ashwagandha did not significantly alter the texture and body, color and appearance, flavor and mouthfeel, or overall acceptability. Ashwagandha ghee is a promising newcomer to the continually expanding field of functional foods, combining ancient knowledge with modern nutritional science.

Conflict of interest

There is no conflict of interest among authors.

REFERENCES

Ahmed, S., Hamid, M.A. and Rahman, M.M. (2020). Assessment of ghee adulterated with oils and fats in Bangladesh. Journal of Advanced Veterinary and Animal Research. 7(4): 678-684.

AOAC, (2000). Peroxide Value of Oils and Fats 985.33.12. Official Methods of Analysis of AOAC International (17th edn.) USA: Maryland.

AOAC, (2007). Determination of Moisture, Ash, Protein and Fat. Official Methods of Analysis Association of Official Analytical Chemist's, 18th edition Washington DC, USA.

BIS (1981). ISI Handbook of Food Analysis: Part XI Dairy Products. Indian Standards Institution.

Bumbadiya, M.R., Maji, S., Sao, K. and Ranvir, S.G. (2023). Butter Oil (Ghee): Composition, Processing and Physicochemical Changes during Storage. In The Chemistry of Milk and Milk Products, Apple Academic Press. pp: 159-184.

Catalá, A. (2010). A synopsis of the process of lipid peroxidation since the discovery of the essential fatty acids. Biochemical and Biophysical Research Communications. 399(3): 318-323.

Choudhary, D., Mashkey, V.K., Goutam, E., Shrivastava, M., Rawat, M., Kumari, A. and Tripathi, V. (2023). Medicinal orchids: Traditional uses and recent advances. Annals of Phytomedicine. 12(1): 1-9.

De, S. (2005). Outlines of Dairy Technology. Oxford University Press, New Delhi.

Gandhi, K. and Lal, D. (2017). Butyro-refractometer (BR) reading linked with solvent fractionation technique as an aid to detect adulteration of palm olein and sheep body fat in ghee. Indian Journal of Natural Products and Resources. 8(3): 276-281.

Gandhi, K., Upadhyay, N., Aghav, D., Sharma, V. and Lal, D. (2014). Detection of adulteration of ghee (clarified milk fat) with palmolein and sheep body fat using Reichert-Meissl (RM) value coupled with solvent fractionation technique. Indian Journal of Dairy Science. 67(5): 387-393.

Gotoh, N. and Wada, S. (2006). The importance of peroxide value in assessing food quality and food safety. JAOCS, Journal of the American Oil Chemists' Society. 83(5): 473.

Gupta, P.K., Pandey, R.K., Panta, R., Rathaur, A. and Tinde, L.K. (2022). Effect of storage temperature on microbiological quality of optimized almond supplemented paneer kheer. Asian Journal of Dairy and Food Research. doi: 10.18805/ajdfr.DR-1852.

Gurav, N.S., Gurav, S.S. and Sakharwade, S.N. (2020). Studies on ashwagandha ghrita with reference to murcchana process and storage conditions. Journal of Ayurveda and Integrative Medicine. 11(3): 243-249.

Gurav, N.S., Gurav, S.S. and Sakharwade, S.N. (2020). Studies on ashwagandha ghrita with reference to murcchana process and storage conditions. Journal of Ayurveda and Integrative Medicine. 11(3): 243-249.

IS:3508 (1966). Indian Standards, Methods for Sampling and Test for Ghee (Butterfat). New Delhi: Bureau of Indian Standards.

Khabiya, R., Choudhary, G.P., Jnanesha, A.C., Kumar, A., Lal, R.K. (2023). An insight into the potential varieties of Ashwagandha (Indian ginseng) for better therapeutic efficacy. Acta Ecologica Sinica. 44(3): 444-450.

Kochhar, P. (2016). Thermal stability of fats for high temperature applications. In Functional Dietary Lipids, Woodhead Publishing. pp: 103-148.

Krawitzky, M., Arias, E., Peiro, J.M., Negueruela, A.I., Val, J. and Oria, R. (2014). Determination of color, antioxidant activity and phenolic profile of different fruit tissue of Spanish 'Verde Doncella'apple cultivar. International Journal of Food Properties. 17(10): 2298-2311.

Kumar, A., Tripathi, S., Hans, N., Pattnaik, F. and Naik, S.N. (2018). Ghee: Its Properties, Importance and Health Benefits. Lipid Universe. 6: 1-14.

Kumbhare, S., Prasad, W., Khamrui, K., Wani, A.D. and Sahu, J. (2021). Recent innovations in functionality and shelf life enhancement of ghee, clarified butter fat. Journal of Food Science and Technology. 60: 1-13.

Laghari, Z.H., Mahesar, S.A., Kori, A.H., Sherazi, S.T.H., Panhwar, T. (2023). Chemistry and Physicochemical Properties. Ghee: Chemistry, Technology and Health Aspects. 227.

Langade, D., Dawane, J. and Dhande, P. (2023). Sub-acute toxicity of Ashwagandha (Withania somnifera) root extract in wistar rats. Toxicology Reports. 11: 389-395.

Majeed, M. (2017). Evidence-based medicinal plant products for the health care of world population. Annals of Phytomedicine. 6(1): 1-4.

Mandlik, D.S. and Namdeo, A.G. (2021). Pharmacological evaluation of Ashwagandha highlighting its healthcare claims, safety and toxicity aspects. Journal of Dietary Supplements. 18(2): 183-226.

Merai, M., Vitthal, B. and Sharma, R.S. (2003). Extraction of antioxygenic principles from tulsi leaves and their effects on oxidative stability of ghee. Journal of Food Science and Technology. 40(1): 52-57.

Misra, M., Harsoliya, M. and Khunt, D. (2023). Chemistry and Properties. Ghee: Chemistry, Technology and Health Aspects. 237.

Naveed, M.A., Kim, J., Ansari, M.A., Kim, I., Massoud, Y., Kim, J. and Rho, J. (2022). Single-step fabricable flexible metadisplays for sensitive chemical/biomedical packaging security and beyond. ACS Applied Materials and Interfaces. 14(27): 31194-31202.

Nekera, K.D., Tola, Y.B., Gemechu, A.T. and Kuyu, C.G. (2023). Chemical, microbial and sensory characteristics of traditional cow ghee as affected by modified atmospheric packaging. LWT- Food Science and Technology.190: 115-558.

Oluremi, O.I., Solomon, A.O. and Saheed, A.A. (2013). Fatty acids, metal composition and physico-chemical parameters of Igbemo Ekiti rice bran oil. Journal of Environmental Chemistry and Ecotoxicology. 5(3): 39-46.

Pandhi, S. and Arvind (2021). Functional dairy foods: The way forward after COVID-19. Annals of Phytomedicine.10(S1): S251-S256.

Paswan, V.K., Rose, H., Singh, C.S., Yamini, S. and Rathaur, A. (2021). Herbs and Spices Fortified Functional Dairy Products. Herbs and Spices-new Processing Technologies. doi: http://dx.doi.org/10.5772/intechopen.98775.

Patel, V., Rai, D.C., Singh, U.P. and Rathaur, A. (2022). Development of functional ice cream using niger [*Guizotia abyssinica* (Lf) Cass.] seed oil microcapsules. Annals of Phytomedicine. 11(1): 588-594.

Pawar, N., Arora, S., Bijoy, R.R. and Wadhwa, B.K. (2012). The effects of *asparagus racemosus* (shatavari) extract on oxidative stability of ghee, in relation to added natural and synthetic antioxidants. International Journal of Dairy Technology. 65(2): 293-299.

Pawar, N., Gandhi, K., Purohit, A., Arora, S. and Singh, R.R.B. (2014). Effect of added herb extracts on oxidative stability of ghee (butter oil) during accelerated oxidation condition. Journal of Food Science and Technology. 51: 2727-2733.

Pena-serna, C. and Restrepo-Betancur, L.F. (2020). Chemical, physicochemical, microbiological and sensory characterization of cow and buffalo ghee. Food Science and Technology. Campinas. 40(Suppl. 2): 444-450.

Piluzza, G. and Bullitta, S. (2011). Correlations between phenolic content and antioxidant properties in twenty-four plant species of traditional ethnoveterinary use in the Mediterranean area. Pharmaceutical Biology. 49(3): 240-247.

Prajapati, P.K., Sharma, R., Amrutia, A. and Patgiri, B.J. (2017). Physicochemical screening and shelf life evaluation of Kunkumâdi Ghrta prepared using Kesaraand Nâgakesara. Ancient Science of Life. 36(3): 129-135.

Rajnikant, (2005). Development of process for herbal ghee. M.Tech Thesis Submitted to National Dairy Research Institute (Deemed University), Karnal.

Ramya, S.B., Baskaran, D., Vijayarani, K., Palanidorai, R. and Ramasamy, D. (2019). A study on physico-chemical properties of uthukuli ghee. International Journal of Current Microbiology and Applied Sciences. 8(4): 2090-9.

Rani, J., Kaur, P. and Chuwa, C. (2023). Nutritional benefits of herbs and spices to the human beings. Annals of Phytomedicine. 12(1): 187-197.

Saha, A., Pawar, V.M. and Jayaraman, S. (2012). Characterisation of polyphenols in Terminalia arjuna bark extract. Indian Journal of Pharmaceutical Sciences. 74(4): 339.

Sahana, H.S. and Vijayalaxmi, K.G. (2024). Development of Gulabjamun with Incorporation of Foxtail Millet. Asian Journal of Dairy and Food Research. 43: 2. doi: 10.18805/ajdfr.DR-1882.

Shahidi, F. and Zhong, Y. (2015). Measurement of antioxidant activity. Journal of functional Foods. 18: 757-781.

Sharma, H., Zhang, X. and Dwivedi, C. (2010). The effect of ghee (clarified butter) on serum lipid levels and microsomal lipid peroxidation. AYU (An international quarterly journal of research in Ayurveda). 31(2): 134-140.

Siddhuraju, P. and Becker, K. (2003). Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. Journal of Agricultural and Food Chemistry. 51(8): 2144-2155.

Siddiq, A., Anwar, F., Manzoor, M. and Fatima, A. (2005). Antioxidant activity of different solvent extracts of moringa oleifera leaves under accelerated storage of sunflower oil. Asian Journal of Plant Sciences. 4(6): 630-635.

Singh, B., Yadav, M.P.S., Yadav, P., Prakash, V., Rai, D.C. and Rathaur, A. (2024). Development of Chhana Spread by Incorporating Moringa (*Moringa oleifera* L.) leaves extract as a source of antioxidants and phenolics. Asian Journal of Dairy and Food Research. 43(1): 142-147. doi: 10.18805/ajdfr.DR-2082.

Singh, N., Bhalla, M., de Jager, P. and Gilca, M. (2011). An overview on ashwagandha: A Rasayana (rejuvenator) of Ayurveda. African Journal of Traditional, Complementary and Alternative Medicines. 8(S): 208-213.

Tzia, C., Giannou, V., Kekes, T., Chranioti, C. and Katsouli, M. (2023). Sensory Science and its Perceptual Properties. Natural Flavours, Fragrances and Perfumes: Chemistry, Production and Sensory Approach. 165-189. https://doi.org/10.1002/9783527824816.ch10.

Vasanth, S., Bharathi, R.B., Purushothaman, K.K. and Narayanaswami, V. (1971). Chemical analysis of talaka bhasma. Journal of Research in Indian Medicine. 5(2): 237-241.

Yadav, A.K. and Rai, D.C. (2018a). *In vitro* screening of ashwagandha root extracts for the maximum functional components. The Pharma Innovation Journal. 7(2): 12-16.

Yadav, A.K. and Rai, D.C. (2018b). Comparative study of the physico-chemical and functional properties of ashwagandha root extract enriched Shrikhand and control Shrikhand during storage. Journal of Pharmacognosy and Phytochemistry. 7(2): 1172-1179.

Yadav, A.K., Rai, D.C. and Rathaur, A. (2024). Process optimization for the development of ashwagandha root extract enriched shrikhand using response surface methodology. Journal of Food Chemistry and Nanotechnology. 10(S1): S47-S56.