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RESEARCH PAPER

Evaluation of *Ashwagandha* herb to enhance shelf-life of *Ghee* against oxidative deterioration

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Abstract : *Ghee*, a crucial component in Indian cuisine, is prone to oxidative rancidity, affecting its shelf- life, flavor and nutritional quality. This research explores the potential of *Ashwagandha,* a medicinal herb, as a natural antioxidant to enhance *Ghee's* oxidative stability. The study involves the collection and preparation of *Ashwagandha* root, followed by the addition of its aqueous extract to cow cream during the ghee-making process. The herbal *Ghee* is then evaluated for acceptability based on various sensory parameters. Chemical analyses, including peroxide value, free fatty acid content, radical-scavenging activity using DPPH assay and total phenolic content, are conducted to assess the impact of *Ashwagandha* on *Ghee* quality and stability. The results show significant differences in peroxide value and free fatty acid content between control *Ghee* and *Ashwagandha*-infused ghee, highlighting the potential antioxidant effects of the herb.The study emphasizes the growing interest in utilizing natural, plant-based antioxidants to address concerns associated with synthetic antioxidants. While the addition of herbal extracts has challenges, such as flavor alteration and solvent residue, exploring alternative sources like *Ashwagandha* opens avenues for improving food preservation naturally. The findings contribute valuable insights into the potential use of herbs in enhancing the quality and shelf-life of food products.

Key Words : Ashwagandha, Antioxidant, Peroxide value, Oxidative stability

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INTRODUCTION

The anhydrous milk fat known as *Ghee* plays a significant role in Indian cuisine. *Ghee* is produced by heating cream or butter that has been churned out of fresh or matured cream, or dahi, which is made by fermenting milk with either specific starter cultures or native milk bacteria (Shakya *et al.*, 2022). In terms of composition,*Ghee* is a complex mixture of triacylglycerol, moisture, traces of metals like copper and iron, and minute amounts of free fatty acids, phospholipids, steroids, hydrocarbons, and carbonyl compounds (Gupta and Singh, 2024) *Ghee* is an excellent source of vital fatty acids, fat-soluble vitamins, and energy (Ilankumaran and Anand, 2012).

The main cause of *Ghee's* deterioration is oxidative rancidity. Auto-oxidation is another name for oxidative

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deterioration since, once started, it is essentially a self-catalyzed reaction. One of the primary elements limiting *Ghee* storage life is oxidative degradation (Kumbhare *et al.*, 2023). *Ghee* oxidative deterioration reduces the product's economic value while also destroying its delicious flavour and producing potentially harmful compounds (lipid peroxides, hydroxyl fatty acids, carbonyl compounds like malonaldehyde, cyclic monomers, dimers, polymers, polycyclic aromatic compounds (Mehta *et al.*, 2015). Consumption of such products leads to diarrhea, poor growth rate, promotion of tumor growth, and carcinogenic properties (Montané *et al.*, 2020). Thus, it's critical to ascertain *Ghee's* storage stability. Research efforts to increase the shelf-life of *Ghee* through several means are ongoing. Adding antioxidants is one of the most popular methods. Antioxidants are substances or systems that prevent the production of free radicals or stop them from spreading by one or more of a number of different methods. This delays the onset of autoxidation. (1) scavenging species that initiate peroxidation, (2) chelating metal ions such that they are unable to generate reactive species or decompose lipid peroxides, (3) quenching O_2 preventing formation of peroxides, (4) breaking the autoxidative chain reaction. Antioxidants are categorised as either synthetic or natural based on their source. Studies on the safety of synthetic antioxidants have been reported to have mutagenic, carcinogenic, and teratogenic effects on experimental animals.

These factors have drawn focus to the utilisation of safer edible plant resources and consumer demand for natural food ingredients has led to a large body of study on naturally occurring antioxidants. Natural antioxidants have been used in the food industry much more recently, and as a result, numerous research have been published on the subject (Lourenço *et al.*, 2019).

Throughout history, people have utilised herbs as food and medicinal. The phenolic compounds found in herbs have redox characteristics that enable them to function as metal chelators, hydrogen donors, reducing agents, and free radical quenchers in addition to acting as antioxidants. The use of plant extracts made with organic solvents as a supplement to *Ghee* has not received much attention (Pawar *et al.,* 2014). However, there are a number of problems with using these herbal extracts. Using the extracts could give the product an extremely harsh flavour. In addition, the action of extracts is less potent than that of the whole herb since during the extraction process, some small components that are essential to the activity owing to their synergistic effect may be lost. Another drawback is the existence of leftover organic solvents from the extract extraction process (Chemat *et al.*, 2019). There has been no research on using dried *Ashwagandha* in *Ghee* and even a tiny amount of extracts added to the product increased its pungency. In order to assess the potential of *Ashwagandha* as a natural antioxidant for avoiding oxidative rancidity in *Ghee*, the current study was designed.

MATERIAL AND METHODS

Chemicals and glassware :

All the chemicals and glassware used in the present study were of analytical (AR) grade and standard quality supplied by authorized dealers.

Collection and preparation of plant materials :

For the preparation of herbal *Ghee*, *Ashwagandha* root procured from the local market of Varanasi and aqueous extract was prepared from it and the herb verified from the Centre of Advanced study in Botany. Cow Cream used in the experiment was obtained from the Local Market and standardized to 40% fat using Cow skim milk. The standardized cow cream was aged at 4°C for 12 hours and then churned into butter using hand driven butter churn. Glass Bottles used in the experiment were purchased from Varanasi market. The capacity of the glass bottles was 200g.

Preperation of *Ghee* and addition of herb :

Cow milk was procured from Local Market, Varanasi. It was preheated to 45°C; cream was separated and standardized to 40% fat using Cow skim milk and then cooled to 4°C and aged overnight at this temperature. Then the aged cream was churned into butter that contained 80.00 % fat. The produced butter was melted properly and clarified at 113-115°C, followed by filtering through muslin cloth and cotton pad.

The raw Ashwagandha was dried in sunlight for a day. It was then pounded and made into coarse powder using the mortar and pestle and coarse powder (kashayachurna) was separated. The rest of the *Ashwagandha* was further pounded and made into fine powder using the mixer. 200 gram of coarse *Ashwagandha* powder was weighed and soaked in 4 litres of water overnight. The next day, the container

was placed on a gas burner and heated till the decoction was reduced to half (by 6hrs). This decoction was then filtered and kept aside. Then few liters of milk was boiled and 200g of *Ghee* was added to it along with bolus of 50 grams of fine powder (kalka churna) of *Ashwagandha* The prepared 1 litre of decoction (kashaya) was added to the container and boiled in low flame for the next 2 hours and the heating was discontinued and left to cool overnight. The heating was resumed next day morning and occasional stirring of the mixture was done. During the heating, a frothy layer appeared on the surface of the *Ghee* and the milk started curdling forming a solid consistency by around 6 hrs of continuous boiling. By 10 hrs of reduction, a cohesive mud like paste was formed at the bottom of the container, after which continuous stirring was done so as to avoid charring of the paste. Slowly by the 12th hour of stirring, the frothy layer started disappearing. The heating was continued till all the water evaporated from the *Ghee* and the *Ghee* started separating from the paste. The *Ghee* formed a clear, transparent and devoid of any froth as the preparation was nearing the end point. A small quantity of the paste was burned in fire to confirm that the entire water particle has evaporated by looking for the crackling sound when subjected to fire.

Evaluation of herbal *Ghee* :

The herbal *Ghee* samples were evaluated for their acceptability during the process of standardization. Panel members were requested to judge each sample on the basis of flavour, texture, colour and appearance, suspended solids and overall acceptability and requested to indicate their score on a 100-point score card.

Oxidative stability (Peroxide Value) :

The peroxide value of *Ghee* was determined by the method (Iodometric method) as described in IS: SP: 18 (part XI) (1981).

Radical scavenging activity (2, 2-diphenyl-1-picrylhydrazyl-DPPH Assay) :

The capacity of antioxidants to quench DPPH radical in *Ghee* was determined before and after accelerated oxidation tests (Espín *et al*., 2000). Ethyl acetate was used as a better solvent for hydrophobic compounds. The method in brief is as follows:

0.2 ml of *Ghee* sample was added to 3.8 ml of ethyl acetate to obtain 4 ml of the mixture, followed by addition of 1 ml of DPPH (6.09 ×10-5 mol/L) solution in ethyl acetate (total volume, 5 ml). After 10 min. had elapsed addition of reagents, absorbance was measured at wavelength 520nm. The reference sample used contained 1 ml of DPPH solution and 4 ml ethyl acetate. Radical-scavenging activity was expressed as percentage inhibition and was calculated using the following formula:

$$\text{Radical scavenging activity} = \frac{Ac - A}{Ac} * 100$$

Where, Ac = Absorbance of control and
A= Absorbance of sample.

Total phenolic content (TPC) :

The total phenolic content (TPC) was determined according to the method described by (Hinneburg *et al.,* 2006) with some modifications. 0.5ml of diluted sample was added to 2.5ml of 0.2N Folin-Ciocalteau reagent and placed for 5 minutes. 2ml of 75g/L of Na_2CO_3 was then added. The above solution was then kept for incubation at room temperature for 2 hours. Absorbance was measured at 760nm using 1cm cuvette UV-1800 spectrometer (Shimadzu, Japan). Gallic acid (0-10µg/ml) was used to produce standard calibration curve. The total phenolic content was expressed in µg of gallic acid equivalent (GAE)/ml of extract.

Statistical analysis :

The results of preliminary studies were expressed as mean ± standard error, Statistical significance was tested by employing analysis of variance (ANOVA).

RESULTS AND DISCUSSION

The compatibility of the herbs to be used for study was the primary factor taken into account while choosing which ones to add to *Ghee* to extend its shelf-life. *Ghee* samples were analyzed for flavor to check the compatibility of herbs in *Ghee*. Colour characteristic of *Ghee* samples was also examined by visual observation.

Peroxide value :

Peroxide value represents primary reaction products of lipid oxidation, which could be measured by their ability to liberate iodine from potassium iodide. It is considered to represent the quantity (mg) of active oxygen contained in one gram of lipid. The effect of storage period (days) on the peroxide value are presented in Table 1. It was observed that there was a significant difference ($p<0.05$) amongst peroxide value of all the two types of *Ghee*

i.e., control and *Ashwagandha Ghee*.

Our results are in agreement with the results of (Siddiq *et al.,* 2005) who reported that the addition of methanolic (80 and 100%) and acetone (80 and 100%) extracts of *Moringa oleifera* to sunflower oil significantly inhibited the development of peroxides under accelerated conditions. Among different methanolic and acetone extracts, 80 per cent methanolic extract was to be the most effective in retarding the peroxide value. The difference in results between extracts using various solvent may be attributed to hydrophilic and lipophilic nature of antioxidative compounds in herbs. In southern Indian villages, people use the fresh leaves of *Moringa 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 per cent of silica gel charcoal treated fraction of *Tulsi* leaves powder to *Ghee* was more effective than the BHA at 0.02 per cent until the peroxide value of 5 meq of peroxide oxygen was reached.

Free fatty acid content :

The effect of storage period (days) on the FFA content are presented in Table 1. It was observed that there was a significant difference ($p<0.05$) amongst FFA content of all the two types of *Ghee i.e*., control *Ghee*, and *Ashwagandha Ghee*. Batool *et al*. (2018) observed that the lipolytic changes during ripening in cheddar cheese made from buffalo milk were much slower than those in similar cheese made from cow milk. Thus, the above results are in agreement with the findings of the present study that buffalo milk *Ghee* is more resistant to lipolytic changes than cow milk *Ghee i.e.,* the increase in FFA content in cow milk *Ghee* is more (0.1797 % oleic acid) than that of buffalo milk *Ghee* (0.1512% oleic acid).

Radical-scavenging activity by DPPH assay :

Several methods have been developed for determination of the quality of *Ghee* based on physical and chemical parameters. These conventional analytical methods have many disadvantages, which was the reason to search for a new generation of rapid tests for the analysis of *Ghee*. The radical scavenging activity measurement might be the possible alternative method. Antioxidant potential was assessed by the antioxidants capacity to quench the free 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radicals. The process of inhibiting the autoxidation of lipids by antioxidants is related to the antioxidant stability to break the radical formation reaction (Sulieman *et al*., 2006). A study conducted by Pawar (2011) for evaluating the effect of herb extract (*Asparagus racemosus*/Shatavari) incorporation on storage stability of *Ghee*, the *Ghee* samples incorporated with ethanolic extract of Shatavari showed a stronger activity in quenching DPPH radicals in system before (on zero day) and after oxidation (end of 21 days) at 80±10C than the aqueous extract of the same herb.

Table 1 : Sensory evaluation of control *Ghee* and *Ashwagandha Ghee*

Parameter	Control *Ghee*	*Ashwagandha Ghee*
Flavour	9±0.15	8.8±0.20
Colour and apperance	9±0.15	8.8±0.20
Freedom from suspended solid	9.8±0.10	9.75±0.10
Texture	24.25±0.10	24.55±0.10
Overall acceptability	94.25±0.10	94±0.41

Table 2 : DPPH inhibition of control *Ghee* and *Ashwagandha Ghee*

Storage days	Control *Ghee*	*Ashwagandha Ghee*
0	20.67±0.17	38.98±0.10
5	17.87±0.10	37.65±0.10
10	13.45±0.20	35.54±0.10
15	10.65±0.13	34.78±0.10
20	8.76±0.15	33.73±0.10
25	3.56±0.14	31.54±1.64
30	2.14±0.14	30.25±1.64

Conclusion :

In conclusion, the study emphasizes the critical role of *Ghee* in Indian cuisine and highlights the challenges posed by oxidative rancidity, which deteriorates its quality and nutritional value. The oxidative degradation of *Ghee,* leading to the production of harmful compounds, underlines the importance of enhancing its storage stability. Efforts to prolong the shelf-life of ghee are explored through various means, with a particular focus on the addition of antioxidants. The distinction between synthetic and natural antioxidants is crucial, given the reported adverse effects of synthetic counterparts on experimental animals. This has led to a shift in focus towards safer, plant-based alternatives, driven by consumer demand for natural food ingredients.

The research introduces the potential use of *Ashwagandha*, a medicinal herb, as a natural antioxidant in *Ghee.* The study employs a systematic approach, from the collection and preparation of plant materials to the evaluation of herbal *Ghee* using sensory parameters and chemical analyses. The results indicate significant differences in peroxide value and free fatty acid content between control *Ghee* and *Ashwagandha*-infused *Ghee,* supporting the herb's potential as an antioxidant.Despite the challenges associated with using herbal extracts, such as flavor alterations and solvent residues, the study paves the way for exploring novel approaches to enhance food preservation naturally. The findings contribute valuable insights into the utilization of herbs in improving the quality, flavor, and shelf-life of food products, addressing the growing demand for natural and safe food ingredients in the industry.

RESULTS AND DISCUSSION

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