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A comparative study on the effectiveness of *Plectranthus amboinicus* dried leaf powder and essential oil extract on controlling oxidation in Vechur ghee

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Abstract
In this study the potential ability of Dried leaf powder and essential oil extract of *Plectranthus amboinicus* to enhance the oxidative stability of Vechur Ghee was verified. Vechur ghee was prepared using direct cream method. *Plectranthus amboinicus* essential oil was extracted using hydro-distillation method. The dried leaf powder was prepared by drying the leaves at 60 ℃ and then grinding the dried leaves to a fine powder. Essential oil and dried leaf powder was added to the ghee after ghee preparation during the cooling down stage when the temperature reached 40 ℃ at the 0.25, 0.5 and 0.75 per cent levels. Accelerated shelf life study was carried out by storing the milk at 80±1 ℃ for 15 days. Sensory analysis and DPPH assay was carried out at 5days intervals over the storage period of 15 days. The overall acceptability was highest for the control followed by dried leaf powder added ghee and finally essential oil added ghee. In the study it was observed that ghee samples added with essential oil had significant improvement in the oxidative stability while ghee samples added with dried leaf powder was seen to have lowered oxidative stability.

Keywords: Vechur ghee, DPPH assay, *Plectranthus amboinicus*, cow ghee, essential oil

Introduction
Ghee is one of the most common food ingredient found in Indian homes. Ghee has been a part of Indian cuisine for generations and is used widely in the preparations of various food items. Apart from its culinary importance ghee is also a major constituent of many ayurvedic medicines especially ghee made from indigenous species like Vechur. But ghee if not stored properly is one of the easiest dairy products to get spoiled. Oxidative stability is a critical factor influencing the shelf life and overall quality of ghee. Lipid oxidation initiated by the presence of oxygen and catalyzed by trace metal ions, enzymes, and light, forms free radicals and peroxides. The propagation of lipid oxidation not only causes the development of off-flavors but also results in the degradation of essential fatty acids, vitamins, and other bioactive compounds present in ghee, thereby compromising its nutritional value. Additionally, the formation of secondary oxidative products such as aldehydes and ketones can produce potentially toxic and harmful compounds, making it crucial to address the issue of oxidative instability in ghee.
In this study essential oil extract and dried leaf powder of *Plectranthus amboinicus*, one of the least explored plants despite having evident antioxidant activity, has been used to enhance the oxidative stability of ghee.

Materials and Methods
The Vechur milk used for ghee preparation was procured from Vechur conservation Unit, KVASU, Mannuthy, Thrissur. Ghee was prepared using direct cream method as described by De (2001) [8].

Extraction of essential oil from Panikkurkka leaves
Distillation was done in order to separate oil content using hydro distillation technique. The leaves of panikkurkka was dried in hot air oven at 55 ℃ for 24 hours and crushed in a mixer. 60 gram samples were put inside a round bottom flask equipped with Clevenger apparatus.

Afterwards water was added until the ratio of water to sample is 6:1. The flask was then placed on top of a heating mantle. Distillation was done for 6 hours or until the oil content inside distilled water had run out, or considered done marked by clear distillate water.

The essential oil was purified using a centrifuge at a spin speed of 10,000 rpm for 10 min, and *P. amboinicus* essential oil was stored in an amber bottle in a refrigerator at 4 ºC until required for further analysis (Satongrod and Wanna, 2020) [1].

Preparation of powder from Panikkurkka leaves

Panikkurkka leaves were cleaned thoroughly in tap water and removed extraneous matter and other leaves. The leaves thus cleaned were dried in hot air oven at 55 ℃ for 24 hours and crushed in a mixer to fine powder.

Ghee was prepared following the direct cream method by heating cream at 120±2 ℃. The ghee thus obtained was divided into 7 portions as mentioned in the table below.

Table 1: Levels and form of plant extract added to Vechur ghee

Vechur ghee							
	Control ghee	Herbal ghee					
		Essential oil			Panikkurkka leaf powder		
Level of plant extract	0	0.25%	0.50%	0.75%	0.25%	0.50%	0.75%
Sample number	1	2	3	4	5	6	7

The optimal level and form of the extract addition were selected based on the sensory characteristics (9 points hedonic scale) and oxidative stability of ghee. Oxidative stability was assessed by measuring the peroxide value, thiobarbituric acid value, Free fatty acid value and the antioxidant activity by DPPH assay. The sensory analysis and oxidative stability were assessed during accelerated storage at 80 °C±1 °C at regular intervals of 0, 5, 10, and 15 days.

All samples of ghee made in the laboratory were evaluated for their sensory characteristics on a 9-point hedonic scale (ranging from 9=like extremely to 1= dislike extremely) by a panel of 9 experienced judges consisted of faculty, technical staff and doctoral students. The Each judge evaluated the ghee for overall acceptability using the 9-point hedonic scale.

DPPH Assay

DPPH assay was carried out to determine the effectiveness of leaf extract and dried leaf powder on enhancing the oxidative stability of ghee.

The capacity of antioxidants to quench DPPH radical in ghee was determined by the method of Espin *et al.* (2000) [3]. Ethyl acetate was used as a better solvent for hydrophobic compounds.

Preparation of DPPH reagent

2.4 mg of 2, 2-Diphenyl-1-picrylhydrazyl was weighed accurately in a 50 ml glass beaker and 25ml of ethyl acetate was added to it and mixed well. The solution obtained was kept at refrigerated temperature (4 °C) overnight with slow and continuous stirring. This was then transferred to a 100 ml volumetric flask and volume was made up to the mark using ethyl acetate.

Procedure

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 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. Blank used was ethyl acetate. Radical-scavenging activity was expressed as percentage inhibition and was calculated using the following formula:

$$\text{Radical scavenging activity} = \frac{\text{OD of Reference} - \text{OD of sample}}{\text{OD of reference}} \times 100$$

Result and Discussion

The assessment of antioxidant capability was conducted using the DPPH assay and quantified as the percentage of radical scavenging activity (%RSA).

Table 2: Change in antioxidant activity of Samples during storage at 80±1 ℃

Sample			Zeroth day	5th day	10th day	15th day	F value
1	Control		44.90±0.604^{Aa}	34.03±0.018^{Ab}	30.65±0.064^{Ac}	27.22±0.085^{Ad}	18353.31**
2	Dried Leaf Powder	0.25%	51.38±0.409^{Ba}	45.03±0.017^{Bb}	37.86±0.041^{Bc}	29.42±0.170^{Bd}	4905.432**
3		0.50%	56.81±0.545^{Ca}	47.16±0.094^{Cb}	35.12±0.046^{Cc}	25.45±0.155^{Cd}	6862.36**
4		0.75%	65.90±0.081^{Da}	43.46±0.266^{Db}	33.40±0.040^{Dc}	22.45±0.187^{Dd}	12531.76**
5	Essential Oil	0.25%	76.15±1.43^{Ea}	66.32±0.188^{Eb}	46.81±0.033^{Ec}	35.12±0.319^{Ed}	2108.26**
6		0.50%	82.88±0.09^{Fa}	71.26±0.154^{Fb}	54.46±0.055^{Fc}	48.83±0.023^{Fd}	33008.13**
7		0.75%	88.62±0.095^{Ga}	80.06±0.039^{Gb}	66.45±0.064^{Gc}	62.32±0.110^{Gd}	29342.824**
F value			2977.46**	14457.67**	66302.84**	7057.63**	

Mean±SE, n=4

a, b: means within columns with different lowercase superscripts are significantly different (p<0.05) from each other

A, B: means within rows with different uppercase superscripts are significantly different (p<0.05) from each other

**-significant at one percent level (p<0.01)

*- significant at one percent level (p<0.05)

^{ns}-not significant

From the table 2. It can be concluded that both the leaf powder and the essential oil increases the antioxidant activity significantly (p<0.01) of Vechur Ghee. But over storage antioxidant activity found decrease in all samples. Over storage and increasing concentration, antioxidant activity of ghee added with leaves powder was found to decrease at a

higher rate than control. In case of essential oil, antioxidant activity increases with increase in concentration, and decreases with storage but in a slower rate than control.

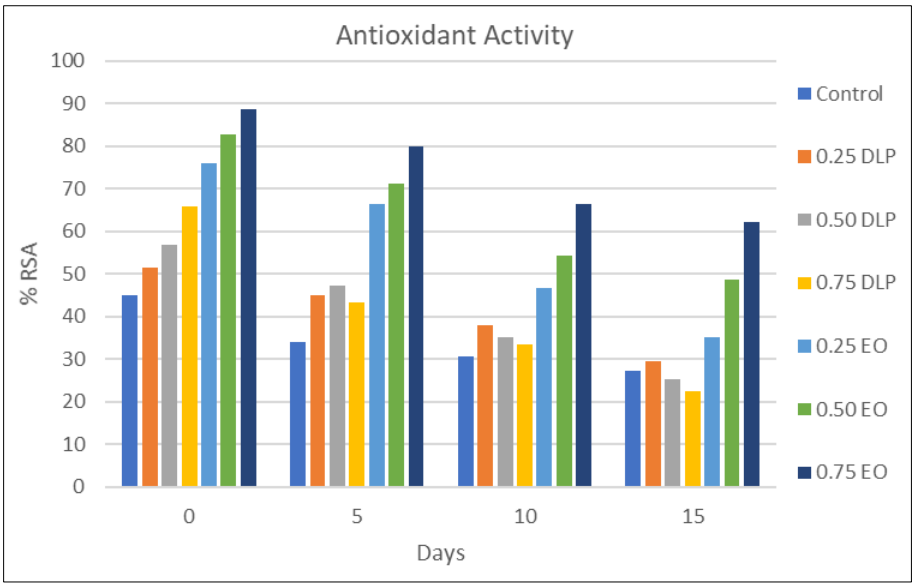

Fig 1: Antioxidant Activity

Table 3: Change in sensory score (overall acceptability) of Samples during storage at 80±1 ℃

Sample			0th day	5th day	10th day	15th day	
1	Control		9.00±0.00^{Aa}	9.00±0.00^{Aa}	8.50±0.29^{Aa}	8.50±0.29^{Aa}	2.00^{ns}
2	Dried Leaf Powder	0.25%	9.00±0.00^{Aa}	8.5±0.29^{ABa}	8.25±0.25 ^{Aa}	7.75±0.48^{Aba}	2.9^{ns}
3		0.50%	9.00±0.00^{Aa}	8.75±0.25^{Aa}	7.75±0.48 ^{Aab}	6.75±0.48^{BCb}	8.12**
4		0.75%	9.00±0.00^{Aa}	8.75±0.25^{Ab}	7.25±0.75 ^{Aab}	5.75±0.48^{Cb}	10.6**
5	Essential Oil	0.25%	8.25±0.25^{Aa}	7.75±0.48^{ABCa}	8.50±0.29 ^{Aa}	8.00±0.00^{ABa}	1.11^{ns}
6		0.50%	6.00±0.58^{A}	7.00±0.41^{BC}	7.50±0.50 ^{A}	7.00±0.00^{ABC}	2.11^{ns}
7		0.75%	5.25±0.48^{Aa}	6.25±0.48^{Ca}	7.00±0.71 ^{Aa}	6.00±0.00^{Ca}	2.17^{ns}
			29.100*	9.100**	1.459^{ns}	9.622**	

Mean±SE, n=4
a, b: means within columns with different lowercase superscripts are significantly different (p<0.05) from each other
A, B: means within rows with different uppercase superscripts are significantly different (p<0.05) from each other
**-significant at one percent level (p<0.01)
*- significant at one percent level (p<0.05)
^{ns}- not significant

From table 3, it can be seen that there is significant difference between the overall acceptability of ghee samples. During storage there is no significant difference between acceptability of samples at 10th day of storage. On storage at 80 ℃ Change in overall acceptability is non-significant for control, ghee added with essential oil and ghee added with 0.25% DLP. Overall acceptability decreases with ghee added with 0.50% and 0.75% DLP.

Conclusion

For ghee with added dried leaf powder, overall acceptability score slightly decrease with increase in concentration and storage period during storage. An opposite result was observed in case of essential oil.

The study indicates that the antioxidant activity of ghee is increased by the powder but the activity is not stable in accelerated shelflife study. Phenolics and polyphenolics, two active substances found in plants, are recognised to have antioxidant properties. Since all antioxidants are redox agents, they have the potential to speed up lipid peroxidation by acting as pro-oxidants. (Ling *et al.*, 2010) [4]. During storage at 80 ℃ there may be some changes to the antioxidants that converted them to prooxidant. It indicates that the leaf powder is not a good option to extend the shelflife of the ghee.

Essential oil added ghee with concentrations 0.25%, 0.50% and 0.75% showed antioxidant activities of 76.15±1.43, 82.88±0.09 and 88.62±0.095% RSA respectively. Essential oil added Vechur ghee showed antioxidant activities significantly greater than the control ghee and ghee added with leaf powder in same concentrations. Throughout the storage period, the ghee with essential oil has significantly greater antioxidant activity than the control ghee. In Ghee added with leaf powder, antioxidant activity is increases with increase in concentration of essential oil. This study supports the findings of Manjamalai and Grace (2012) [5], Bezerra *et al.* (2017) [7] and Hosseinzadeh *et al.* (2023) [6] which shows that there is significant and stable antioxidant activity in *P. amboinicus* essential oil.

From the study, it can be concluded that the essential oil is more effective in enhancing the oxidative stability in vechur ghee, moreover it was also observed that dried leaf powder had a negative impact on the oxidative stability of ghee.

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