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Research Article

Evaluation of antioxidant activity of common culinary spices in ghee

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Abstract

The study was planned to evaluate the antioxidant properties of twelve common culinary spices (black pepper, cardamom, cinnamon, clove, coriander, cumin, fennel, fenugreek, ginger, mustard, nutmeg, and turmeric) in retarding deterioration of ghee by oxidation. The total phenolic content and DPPH assay of spices varied widely among the spices. The highest total phenolic content (4148.82 mg GAE/ 100 g) and DPPH radical scavenging activity (84.26% inhibition) was found in clove. The spices were evaluated for their antioxidant activity in ghee. Ghee samples added with spices individually at the rate of 0.5 per cent (w/w) and control were stored at elevated temperature (80±2°C) to accelerate the oxidation. Addition of spices retarded oxidative deterioration of ghee. Ghee added with clove, ginger, nutmeg and turmeric showed significantly lower peroxides development as compared with control ghee. The antioxidant activity of ghee samples was reduced with the time of storage. The highest stability of antioxidant in ghee during storage was found with clove which was followed by nutmeg, ginger and turmeric.

Keywords: Antioxidant, Ghee**,** Peroxide, Phenolics, Spices

Highlights

- Ghee is vulnerable to oxidative deterioration under ambient storage conditions.
- Spices contain variety of bioactive substances including antioxidants.
- Spices differed in phenolic content and antioxidant activity by DPPH assay.
- Addition of clove, ginger, nutmeg and turmeric showed lower peroxides development in ghee.
- Stability of antioxidant during storage of ghee samples was clove> nutmeg> ginger> turmeric.

INTRODUCTION

Ghee is considered as an excellent medium for cooking. It has very excellent nutritive, medicinal and therapeutic properties. Oxidative deterioration of ghee is autocatalytic andmake the ghee unpalatable (Rangappa & Achaya, 1974). The stability against oxidative deterioration in turn determines the storage stability and is of paramount importance from economic viewpoints. Research endeavours are made to extend the shelf-life of ghee by various approaches. Common approach is addition of antioxidants. The mere mention of natural antioxidants brings about an association with spices and herbs, because they are the most important targets in the search for natural antioxidants from the point of view of safety. Spices in addition to contributing taste and aroma to foods, contain variety of bioactive substances including antioxidants. The phenolic content in spices act as antioxidant due to their redox properties, allowing them to act as free radical quenchers and metal chelators. Many spices are known to exhibit antioxidant activity in food lipids (Pokorny & Korczak, 2001; Barak & Mudgil, 2023).

Some studies have evaluated the effect of incorporating plants and plant extracts in ghee on its oxidative stability (Gandhi & Lal, 2015; Lodh et al., 2018). Some limited work has been reported for utilization of spice extracts and oleoresins in ghee (Patel et al., 2013; Patel et al., 2014; Shende et al., 2014). Whole spices are chemically complex, containing numerous components. The major components determine the biological properties of the spices. However, studies conducted on some spices have shown that minor components have a critical part to play in biological activity, probably by producing a synergistic effect between major components of whole spices (Burt, 2004). The advantage of extracting antioxidative principles from spices using edible oil or fat is a promising approach due to its simplicity and safety, as no organic solvents are used for the extraction (Pokorny & Korczak, 2001). Therefore, the study was contemplated with a view to evaluate the potential of ground for retarding oxidative rancidity in ghee.

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MATERIALS AND METHODS

Glassware and chemicals: Analytical grade (AR) chemicals were used in the study. Chemicals and glassware were supplied by authorized dealers.

Spices: All the twelve dried spices including, black pepper, cardamom, cinnamon, clove, coriander seeds, cumin seeds, fennel, fenugreek seeds, ginger, mustard seeds, nutmeg and turmeric were obtained at local market in Anand city, Gujarat, India. The spices were inspected for any visible dirt and damaged parts were removed. They were ground in an electric grinder into fine powder (screening through standard sieve of 36 mesh size) and stored in air tight containers at refrigeration temperature.

Total phenolics and antioxidant activity of spices: Total phenolics and antioxidant activity of individual spice powder was determined using Folin-ciocalteu's reagent according to the procedure described by Singleton and Rossi (1965). The radical-scavenging activity of individual spice powder was determined as the ability to scavenge 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radicals (Brand-Williams et al., 1995).

Preparation of ghee and addition of spice powder: White butter for ghee preparation was procured from a commercial dairy plant. Ghee was prepared by creamery butter method. The butter was taken in a in a stainless steel vessel (pan) and clarified (115°C for no hold) into ghee with continuous stirring. The clarified fat was allowed to cool at room temperature until it attained a temperature of 80°C and filtered through six folds of muslin cloth in dry glass beaker. Ghee sample as prepared above was divided into thirteen portions of 100 g each. To portion 1 nothing was added which served as control. To the remaining twelve portions, above listed spices were added individually at the rate of 0.5 per cent (w/w) respectively. The mixtures of ghee and spices were thoroughly mixed and allowed to stand at 80°C for 30 min. The treated ghee was filtered using muslin cloth. The samples of ghee were stored in incubator at 80°±2°C for 21 days (accelerated condition). Oxidative changes taking place in ghee were monitored by analyzing the ghee samples for peroxide value, radical scavenging activity (DPPH assay) when fresh and after an interval of three days. Simultaneously, the samples were also monitored for changes in flavour score by sensory evaluation using 9-point hedonic scale.

Determination of peroxide value: The peroxide value of ghee was determined by the iodometric method as given in IS: SP: 18 (part XI) Bureau of Indian Standards [BIS] (1981).

Statistical analysis: The data were analyzed using Analysis of Variance (ANOVA) at the 5% level of significance. The data obtained for each of the attributes under study were subjected to statistical analysis in completely randomized design.

RESULTS

Total phenolics and antioxidant activity of spices: Total phenolics and antioxidant activity of spices are given in Fig. 1.

The phenolic content of spices varied widely among the spices. The highest total phenolic (mg GAE/ 100 g) content was found in clove (4148.82) which was followed by cinnamon (2190.39), nutmeg (1143.80), turmeric (931.61), ginger (778.92), cumin (717.28), fennel (646.31), black pepper (568.07), coriander (530.89), mustard (487.17), fenugreek (295.77), cardamom (163.87), respectively.

Total phenolics and antioxidant activity of spices varied widely among spices. The highest was found in clove (84.26%) which was followed by cinnamon (71.39%), nutmeg (64.56%), turmeric (58.44%), ginger (45.03 %), cumin (43.46%), fennel (37.39 %), coriander (36.09%), black pepper (35.03%), mustard (25.59%), fenugreek (22.53%) and cardamom (13.03%), respectively.

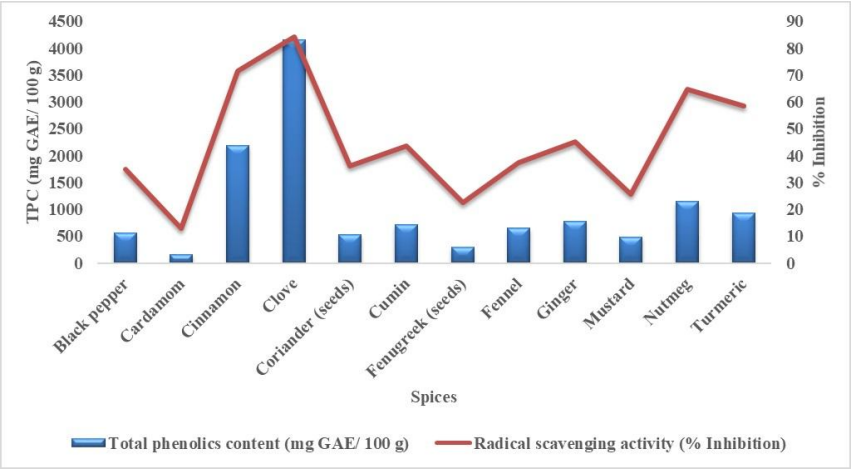

Fig. 1. Total phenolic content and radical scavenging activity of spices

Table 1. Changes in flavour score of ghee during accelerated storage (80±2°C)

Sr. No.	Spice added	Storage period (days)							
		0	3	6	9	12	15	18	21
		Flavour Score (9-point hedonic scale)							
1	Black pepper	8.23	7.75	7.12	6.06	5.17	3.33	2.51	2.00
2	Cardamom	8.00	7.17	6.50	5.07	3.40	2.67	2.28	1.30
3	Cinnamon	7.30	6.62	6.20	5.57	4.60	4.03	3.17	2.13
4	Clove	8.03	7.74	7.23	6.62	6.10	5.63	3.45	2.48
5	Coriander	7.63	7.03	6.20	5.52	5.00	3.87	2.70	1.63
6	Cumin	8.00	7.40	6.67	6.00	5.43	4.05	3.13	2.40
7	Fenugreek	7.23	6.70	6.13	5.38	4.27	3.25	2.00	1.31
8	Fennel	8.10	7.42	6.73	6.25	5.27	3.83	2.53	1.40
9	Ginger	8.80	8.22	7.73	7.17	6.50	4.42	3.73	3.05
10	Mustard	7.63	6.90	6.17	5.64	4.50	3.03	2.12	1.43
11	Nutmeg	8.30	7.92	7.43	6.80	6.42	6.02	4.80	3.42
12	Turmeric	8.10	7.70	7.22	6.60	6.12	5.30	4.27	3.10
13	Control ghee	9.00	7.93	6.38	5.14	4.21	3.40	2.33	1.07

Source of variation	Storage period(P)	Treatment (T)	Interaction (P x T)
CD (0.05)	0.14	0.17	0.49

Table 2. Changes in peroxide value of ghee during accelerated storage (80±2°C)

Sr. No.	Spice added	Storage period (days)							
		0	3	6	9	12	15	18	21
		Peroxide value (meq of O_2 per kg fat)							
1	Black pepper	0	1.43	2.44	4.52	5.62	13.56	17.26	23.26
2	Cardamom	0	1.76	2.83	4.00	8.31	14.61	21.25	28.11
3	Cinnamon	0	1.89	2.41	3.96	5.38	9.27	13.32	21.67
4	Clove	0	0.94	1.95	3.10	3.94	6.07	8.49	14.39
5	Coriander	0	1.97	2.37	4.26	6.63	11.01	18.48	26.68
6	Cumin	0	1.57	2.28	4.10	6.38	12.81	18.80	24.80
7	Fenugreek	0	1.37	2.30	3.16	7.83	14.83	19.20	25.69
8	Fennel	0	1.97	3.21	5.17	6.85	15.34	20.50	26.04
9	Ginger	0	1.20	2.14	2.61	4.09	7.85	14.37	16.48
10	Mustard	0	1.98	2.89	4.25	7.97	15.59	21.74	28.03
11	Nutmeg	0	0.93	1.51	2.45	3.35	6.90	9.53	14.47
12	Turmeric	0	1.13	1.54	2.33	3.48	8.14	11.72	16.55
13	Control ghee	0	1.95	4.28	6.44	11.56	14.73	20.33	29.46

Source of variation	Storage period(P)	Treatment (T)	Interaction (P x T)
CD (0.05)	0.39	0.50	1.40

Effect of spices on changes in flavour score of ghee during storage at 80±2°C: The changes in flavour score of ghee samples (control and treated with spice powder) during accelerated storage (80±2°C) are presented in Table1.

On zero day (when samples were fresh) there was a significant difference between flavour scores of control ghee and the samples of ghee containing spices. During storage the flavour score of control sample, ghee samples added with cardamom, cinnamon, coriander, fenugreek and mustard declined at a greater rate and reached below unacceptable level (<6) on 9th day of the storage. Flavour scores of the ghee samples added with black pepper, clove, cumin, fennel, ginger, nutmeg

and turmeric remained higher compared to that of the control sample. The flavour score of ghee samples added with clove, ginger, nutmeg and turmeric were above the acceptable level on 12th day of storage. On 15th day of storage ghee sample added with nutmeg was having acceptable flavour score.

Effect of spices on changes in peroxide value of ghee during storage at 80±2°C: The changes in peroxide value of ghee samples (control and treated with spice powder) during accelerated storage (80±2°C) are presented in Table 2.

The statistical analysis of data revealed that spices and storage period have significant (P<0.05) effect on changes in peroxide value of ghee during storage. It is observed that on zero day control ghee and ghee samples incorporated with spices showed zero peroxide value. During storage peroxide value increased in all samples of ghee. On 12th day of storage control ghee sample had peroxide value of 11.56 meq/kg fat while ghee samples incorporated with clove, ginger, nutmeg and turmeric had peroxide value 3.94, 4.09, 3.35 and 3.48 meq O_2/kg fat respectively which was less than 5 meq/kg fat. As per BIS (1981), the quality of ghee is considered fair, when the peroxide value is between 4 and 5 meq O_2/kg fat and is acceptable.

Effect of spices on changes in radical scavenging activity of ghee during storage at 80±2°C: The changes in radical scavenging activity of ghee samples (control and treated with spice powder) during accelerated storage (80±2°C) are presented in Table 3.

On zero day (before oxidation) the radical scavenging activity of ghee incorporated with spices (black pepper 37.71%; cardamom 28.58%; cinnamon 38.41%; clove 46.31%; coriander 34.64%; cumin 35.48%; fenugreek 29.53%; fennel 31.56%; ginger 40.39%; mustard 32.70%; nutmeg 42.90% and turmeric 40.28%) were significantly higher (P<0.05) than those with control ghee sample (28.16%). The antioxidant potential of ghee samples was reduced with the time of storage. On 12th day of storage, radical scavenging activity of ghee samples incorporated with the clove (35.55%), ginger (26.47%), nutmeg (29.34%) and turmeric (26.33%) were significantly higher (P<0.05) than those with control ghee sample (13.63%) and other spices. The highest stability of antioxidant during storage of ghee samples was found with clove which was followed by nutmeg, ginger and turmeric.

DISCUSSION

Total phenolic content and radical scavenging activity of spices: The total phenolic content and radical scavenging activity of spice powder obtained in this study corroborated with the reported values in literature. The phenolic content of spices varied widely among the spices. Phenolic substances have been shown to be responsible for the antioxidant activity of plant materials (Rice-Evans et al., 1996).

Table 3. Changes in radical scavenging activity of ghee during accelerated storage (80±2°C)

Sr. No.	Spice added	Storage period (days)							
		0	3	6	9	12	15	18	21
		Radical Scavenging Activity (% Inhibition)							
1	Black pepper	37.71	35.51	31.74	30.21	23.71	15.60	11.69	5.13
2	Cardamom	28.58	25.53	20.54	18.47	13.63	11.32	5.35	2.47
3	Cinnamon	38.41	34.42	30.50	26.75	20.53	14.67	9.18	4.83
4	Clove	46.31	42.76	40.46	37.75	35.55	28.77	21.40	13.69
5	Coriander	34.64	32.26	28.88	25.59	19.24	13.67	8.56	4.42
6	Cumin	35.48	33.43	30.17	28.42	22.24	15.59	9.52	5.54
7	Fenugreek	29.53	25.44	21.58	18.57	13.33	8.41	4.40	2.87
8	Fennel	31.56	28.52	25.54	22.32	20.22	14.92	8.72	3.71
9	Ginger	40.39	36.64	34.13	30.59	26.47	20.62	13.52	10.28
10	Mustard	32.70	28.89	23.95	21.84	16.57	10.43	4.64	3.46
11	Nutmeg	42.90	39.91	35.36	31.57	29.34	22.35	16.44	13.54
12	Turmeric	40.28	36.67	33.64	30.53	26.33	17.54	12.57	9.82
13	Control ghee	28.16	25.75	22.57	20.47	13.63	8.45	3.37	2.52

Source of variation	Storage period(P)	Treatment (T)	Interaction (P x T)
CD (0.05)	0.64	0.82	2.32

In a study by Hinneburg et al. (2006) hydro distilled extracts from fennel, cumin, cardamom, and ginger were assessed for their total phenol content. The mean total phenol content (mg GA/g extract) for fennel, cumin, cardamom and ginger were 30.3, 37.4, 24.2 and 23.5, respectively. Wojdyło et al. (2007) analysed total phenolic content of 32 spices extracts.It ranged from 0.07 to 15.2 mg GAE/100 g dry weight. Major phenolic acids identified in analyzed species were caffeic, p-coumaric, ferulic and neochlorogenic. Gupta (2013) did comparative analysis of nine different spices for their total phenolic content and antioxidant activity. The total phenolic content showed the following order; maximum for cloves>cinnamon>ajwoin>star anise>cumin > black pepper>fennel>coriander>black cumin.

Patel et al. (2013) reported mean phenolic content of oleoresin and steam distillled extract of coriander to be 21.93 and 54.50 mg caffeic acid equivalents/g extract, respectively. Shende et al. (2014) reported higher levels of phenolics in steam distilled clove extract (256.5 mg of GAE/g) than its oleoresin (177.1 mg of GAE/g). The difference in phenolic content could be ascribed to the presence of hydrophilic and lipophilic antioxidant compounds in extracts, methods of extraction, and difference in solubility.

The DPPH radical scavenging activity (% inhibition) varied widely among spices. The antioxidant activity (DPPH assay) of green tea was the strongest, followed in descending order by black tea, clove, sweet black pepper, ginger, black pepper, fenugreek and cardamom (Khalaf et al., 2008).

Deepa et al. (2013) found a significant linear correlation between the concentration of phenolic compounds and the antioxidant activity of extracts from cardamom, coriander and dried bay leaves. Patel et al. (2013) observed that the radical-scavenging activity (DPPH assay) exhibited by the steam distilled coriander extract was significantly higher than the coriander oleoresin. Shende et al. (2014) reported that the radical scavenging activity of steam distilled clove extract was higher than its oleoresin counterpart. The difference in radical scavenging activity of steam distilled clove extract and clove oleoresin might be due to high phenolic content of steam distilled clove extract than its oleoresin.

It could be inferred from our results that the higher antioxidant activity of the spices could be attributed to the higher content of phenolics.

Effect of spices on changes in flavour score, peroxide value and radical scavenging activity of ghee during storage at 80±2°C: Control ghee (0 day) was judged to be superior in terms of flavour score. This may be due the reason that the characteristic flavour of ghee was masked by the flavour of added spices. Spices impart aroma and taste to food preparations and sometimes mask undesirable odours. Volatile oils give the aroma, and oleoresins impart the taste. Aroma compounds play a significant role in the production of flavourants. They are classified by functional groups, e.g. alcohols, aldehydes, amines, esters, ethers, ketones, terpenes, thiols and other miscellaneous compounds. In spices, the volatile oils constitute these components (Parthasarthy et al., 2008).

From changes in peroxide value, it can be inferred that addition of spices exerts resistance towards the oxidative deterioration of ghee. Ghee incorporated with clove, ginger, nutmeg and turmeric showed significantly lower peroxides development throughout 21 days of storage at 80±2°C as compared with control ghee.

The aqueous and ethanolic extracts of *Asparagus racemosus* (shatavari) significantly retarded deterioration of ghee relative to the control (without addition of synthetic or natural herb extract), as observed in peroxide value (8.3, 13.2 and 25.8 mmol O_2/kg fat respectively) after 21 days of accelerated storage study (Pawar et al., 2012). Patel et al. (2013) observed that the coriander extracts (steam distilled and oleoresin) significantly inhibited the peroxide development throughout the 21 days of storage at 80°C as compared to the control. Seven percent by weight of ethanolic extract of *T. arjuna* Wight &Arn bark was effective in controlling the auto-oxidation of ghee during storage (Parmar et al., 2013). Betel leaves had significant ability to enhance the antioxidant potential of ghee in terms of peroxide value (Kapadiya &Aparnathi, 2018). Ghee incorporated with orange peel extract showed stronger activity in quenching DPPH radicals and least development of peroxide value than ghee incorporated with BHA and control (Asha et al., 2015). Maiza et al. (2020) found that the ghee samples added with clove and rosemary had significantly lower peroxide values compared to the control.

Conclusion

Changes in flavour score, peroxide value and antioxidant potential of ghee during accelerated storage study at 80±2°C had conclusively suggested that clove, ginger, nutmeg and turmeric were better in retaining the flavour of ghee by retarding oxidative deterioration. The better performance of the above four

spices found in present study may be attributed to their higher content of antioxidant principles.

Conflict of interest: Authors have no conflict of interest in this study.

Author's contributions: AKJ: Involved in investigation, data generation, preparing original draft; KDA: Engaged in conceptualization, data curation, supervision; SCP, AIS: Engaged in analytical help, editing the manuscript.

Data availability statement: Data will be provided on the request to corresponding author.

Ethical approval: Not required for this study.

Use of artificial intelligence tools: AI tools are not used in the study.

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