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Jui Lodh
Phd Scholar, DT Division,
NDRI, Karnal, Haryana, India

Kaushik Khamrui
Principal Scientist, DT Division,
NDRI, Karnal, Haryana, India

Correspondence
Jui Lodh
Phd Scholar, DT Division,
NDRI, Karnal, Haryana, India

Evaluation of physico-chemical changes in Curcumin fortified buffalo Ghee during storage at 30±1˚C

Jui Lodh and Kaushik Khamrui

Abstract
Ghee is one of the most widely used dairy products in Indian household. Shelf life of ghee therefore is a major concern for consumers who consume ghee throughout the year. Shelf life of ghee is extended by using synthetic antioxidants. One of the natural compound having antioxidative activity is Curcumin which can be added to ghee to improve its shelf life. In the present study, Ghee was incorporated with curcumin and the samples were stored at 30±1 ºC for 30 days. It was observed that the ghee samples in which curcumin were incorporated, they showed better resistance against development of peroxides, thiobarbituric acid (TBA) and Free Fatty Acid (FFA) than the ghee incorporated with Butylated Hydroxy Anisole. Ghee incorporated with curcumin showed stronger antioxidant activity in quenching free radicals.

Keywords: Ghee, curcumin, storage study, FFA, TBA

1. Introduction
Ghee is one of the most widely used dairy products of India with about 30-35% of the milk annually produced in India is converted into ghee. However, high fat containing product like ghee loses its popularity as they are often being implicated in atherosclerosis, CHD due to cholesterol content and composition of fatty acids. Further, ghee when stored at room temperature, undergoes oxidative deterioration [1, 2, 3] which not only effect the sensory and nutritive value of ghee but also affecting the suitability for consumption, eventually reducing shelf-life of the product [4, 2, 5, 6]. Numerous scientific evidences correlating oxidized lipids with negative health implications [7] like cancer, heart disease and early aging in consumers who consume oxidized oils and fats. The use of antioxidants is the most appropriate way to prevent lipids oxidation and to protect fats [8]. Synthetic antioxidants such as butylated hydroxyl anisole (BHA), propyl gallate and tertiary butyl hydroquinone (TBHQ) are often used in ghee to prevent oxidative deterioration. Scientific studies have shown that application of synthetic antioxidants in foods may cause liver damage and cancer [9]. However, complications resulting from the prolong use of these compounds in man cannot be ignored [8]. It is therefore necessary to provide a natural antioxidant that is acceptable in all respects including health issues. This opens up a new avenue for dairy researchers to introduce naturally occurring antioxidants. Curcumin comprise one such category of naturally occurring antioxidants that have thus emerged.
Numerous herbs have been known to retard lipid oxidation in foods during storage [3]. Curcumin (diferuloylmethane), a fat soluble bioactive, yellow pigment present in Indian spice turmeric (*Curcuma longa* L.), known for its numerous functional attributes e.g., antiinflammatory, antioxidant, hypotensive, hypocholesteremic, antidiabetic, anti- bacterial, antiviral, etc. [8]. Although turmeric has been used in Ayurvedic medicine since ancient times in various medical applications, research on use of curcumin as a natural antioxidant in ghee was not reported in the available scientific literature. Therefore, the present study was carried out to investigate the antioxidant activities of curcumin in buffalo ghee stored at 30±1 ˚C and the antioxidant activities of curcumin in ghee were compared with the antioxidant activities of ghee incorporated with BHA (@200 ppm).

Materials and methods
Procurement of Raw materials
Curcumin and all other chemicals were obtained from Himedia and were of analytical grade.

Fresh, good quality buffalo milk was obtained from the experimental dairy plant of the institute, ICAR-NDRI, Karnal. The synthetic antioxidant Butylated hydroxyanisole (BHA) was obtained from HI MEDIA®, Mumbai, India.

Preparation of ghee and addition of antioxidants

The effect of curcumin content (160-350 ppm), heating/clarification temperature (110-120 ˚C) and duration of heating (16-22 min) on sensorial and antioxidative property of curcumin fortified *ghee* was studied using Response Surface Methodology (RSM) in a three factor five level Central Composite Rotatable Design (CCRD). A total 20 experiments were carried out as per the design matrix suggested by RSM in randomized manner and the obtained data were fitted in polynomial quadratic model. Fortification with 350 ppm curcumin and clarification at 115˚C for 17.89 (≈20) min were found to be optimum with a desirability quotient of 0.941 based on maximum sensory scores and *in vitro* antioxidative activity by DPPH method with minimum conjugated diene value. Optimized curcumin fortified buffalo *ghee* was prepared and evaluated for all the responses which were found to be very close with the predicted scores.

Ghee prepared without any kind of antioxidants (natural or synthetic) served as control. *Ghee* fortified with permitted synthetic antioxidant, BHA @200 ppm was prepared for comparison with control and curcumin fortified ghee.

Storage study of ghee

Ghee samples were stored in hot air oven at 30±1ºC and analysed at 30 days intervals for peroxide value, free fatty acid (FFA) content, thiobarbituric acid value (TBA) and radical scavenging activity by DPPH assay.

Peroxide value: Peroxide value of ghee samples were determined by the method as described in IS: 3508 [9].

Free fatty acids: Free fatty acid levels of ghee samples were determined by the method as described in IS: 3508 [9].

Thiobarbituric acid (TBA) value: TBA value of ghee samples were determined by the method of Patton and Kurtz [10].

Radical Scavenging Activity by DPPH Method: The radical-scavenging activity of clove extracts (steam distilled and oleoresin) and BHA were determined according to the procedure of Blois [11] with a minor modification, by using ethanol instead of methanol during sample preparation.

Statistical Analysis

All determinations were carried out in triplicate and data was subjected to analysis of variance. In the experiments, one way and two way analysis of variance (ANOVA) with a subsequent difference ($P > 0.05$) in the mean values was conducted as described by Snedecor and Cochran [12].

Results and discussion

Effect of addition of curcumin on Peroxide Value of ghee during storage

Results depicted in Fig. 1(a), revealed that the initial peroxide value (0.0) did not increase upto two months for all the ghee samples during storage at 30˚C. After that peroxide value in all the ghee samples was significantly ($p<0.05$) increased with the progression of storage but the extent was less in curcumin fortified buffalo ghee and buffalo ghee added with synthetic antioxidant BHA than control buffalo ghee. Initially peroxide content between curcumin fortified buffalo ghee and buffalo ghee added with synthetic antioxidant BHA were not noticeable, it became significant ($p<0.05$) after four month of storage. After seven month of storage, there was a sharp rise in peroxide content {Fig. 1(a)} in all the ghee samples. The formation of peroxide was significantly higher in control than in the ghee incorporated with curcumin and BHA at 30±1°C. Resistant of curcumin in peroxide development was comparable to BHA in buffalo ghee during the all storage periods initially but increased latter suggesting a greater stability of curcumin than BHA, which could be due to presence of more antioxidant compounds such as phenolic compound curcumin which is in agreement with results reported by Asha *et al*. [13].

Higher resistance towards the development of peroxides may be attributed to the presence of polyphenolic compound curcumin whose antioxidant potential have already been extensively reviewed by many workers [14-16]. Thermal stablility [17] and stability of curcumin in acidic condition [18] might also have helped in retention of curcumin induced antioxidant activity during storage.

Further, these data suggest the superiority of curcumin, the principle compound of turmeric over synthetic antioxidant BHA, because of their long term effectiveness and stability. All antioxidants remain effective over a specific period of time, and with the passage of time their effectiveness decreases and they finally become ineffective [19]. Such antioxidants end or at least interrupt oil and fat deterioration in the early stages and thus delay the onset of the reaction and are found to be efficient only upto a specific period. It may be hypothesized that phenolic antioxidants inhibit lipid peroxidation at the cost of their own life and thus decompose and deteriorate

Effect of addition of curcumin on Free Fatty Acid of ghee during storage

FFAs measure hydrolytic rancidity of fat and oils. Results presented in Fig. 1(b), indicated that *ghee* samples containing curcumin and BHA showed higher resistance against the development of FFA compared to control. It was also observed that initially there was no significant ($p<0.05$) difference in FFA content in all four *ghee* samples. The FFA content was marginally increased during the first four months of storage for CFG, BG and three months for CG. Highest FFA development was occurred in CG while lowest in CFG.

Curcumin addition @ 350 ppm gave better protection against hydrolysis than control during the entire storage while provided similar protection like BHA. Control sample always showed lower FFA which might be due to the absence of antioxidant in *ghee*. Similar trend was observed by Patel [20] in *ghee* added with curry and leaves @1% concentration during 147 days of storage at 30 ˚C whereas Stark *et al*. [21] observed a higher FFA values in butter oil stored at 30˚C, reported that the initial FFA content was 0.24 which increased to 0.35 after 90 days of storage. Similar trends of rise in the FFA content of stored *ghee* were also observed by other workers [22]. Jain [23] observed a 100% increase in FFA in *ghee* after 150 days of storage, who reported that the variation in FFA content might be due to condition of handling of milk and cream, excessive souring of cream and uncontrolled clarification temperature.

It can be concluded that curcumin showed slightly better resistance against the development of FFA compared to BHA. Phenolics present in curcumin are thought to be responsible

for this. Generally, the antiradical and antioxidant activities of plant extracts are associated to theirs phenolic content. The two phenolic groups of curcumin are attached to different unadjacent benzene rings that give the two OHs the mobility to work freely without hindrance [24]. Similar conclusion was drawn by Patel and Rajorhia [25] found that maximum permitted level of BHA and BHT (@0.02%) was less effective than curry leaves and beetle leaves by a margin of about 10% in preventing FFA development in *ghee* during storage at 30˚C. Naaz and Prakash, [26] also reported that turmeric was effecting in lowering free fatty acid formation in *ghee* upto 90 days of storage.

Effect of addition of curcumin on TBA Value of ghee during storage

The TBA test is used frequently to assess the oxidative state of a variety of food systems, despite its limitations, such as lack of specificity and sensitivity [27]. Despite its limitations, the TBA value provides an excellent means for evaluating lipid oxidation in foods, especially on a comparative basis [28]. The effect of addition of curcumin on the development of thiobarbituric acid reactive substances in buffalo *ghee* was assessed and results are presented in Fig. 1 (c). TBA value marginally increased after the first four months of storage for the samples CFG, BG, and two months for CG. The increase was significant towards the end of storage ($p < 0.05$). Highest was observed in CG while lowest in CFG. TBA measures the formation of secondary oxidation products i.e. aldehydes or carbonyls, which may contribute to off-flavour of oxidized fat products.

Results clearly indicated that curcumin fortification was better to prevent malonaldehyde formation during the entire storage period. Our result is consistent with Asha *et al*. [29], who reported that orange peel extract was more effective in reducing the increase in TBARS in buffalo *ghee* than BHA and control at lower temperatures like 6±2 as well as 32±2˚C while BHA was more effective in reducing the increase in TBA than orange peel extract at higher temperature (60 °C). Generally, TBA value has been shown to be useful quality index for assessment of oxidative rancidity in oils and fats. However, TBARS values of oils and fats vary with their profile of unsaturated fatly acids [30]. Kumar and Bector [31] noted a strong relationship between peroxide value, TBA value free fatty acid and flavour of *ghee* during storage.

Effect of addition of curcumin on Radical-Scavenging Activity of ghee during storage

Antioxidant potential of the samples were assessed by the ability of antioxidants to quench DPPH free radicals. Inhibition process of the auto-oxidation in lipids by antioxidants is linked to the ability of antioxidants to break the radical formation reaction [32]. Consequently, the antioxidant potential can also be estimated in systems in which the radicals are generated by chemical means [33].

It was observed that before oxidation, the radical scavenging activity of control *ghee* samples were much lower than buffalo *ghee* samples added with BHA @ 200 ppm and curcumin added (@350 ppm) buffalo *ghee* samples throughout the storage. Thus suggesting a significant ($P<0.05$) potential of curcumin on quenching DPPH free radicals than the synthetic antioxidant BHA. Further, it was also noticed that DPPH radical scavenging activity was significantly ($P<0.05$) decreased with the progression of storage period irrespective of treatment. Throughout the storage a sharp decreasing trend in radical scavenging activity was observed. In contrast to this, highest radical scavenging activity was observed in case of CFG and lowest was for CG.

Thus it can be inferred that curcumin had strong ability in quenching DPPH free radicals in ghee before and after oxidation. Higher percentage of DPPH scavenging activity may be attributed to the high reducing power and higher total phenolic contents present in curcumin. The antioxidant and radical scavenging activities are closely related to polyphenols (Reddy and Lokesh, 1994). In DPPH assay, the antioxidants were able to reduce DPPH to yellow coloured diphenyl picrylhydrazone [34]. The method based on the reduction of DPPH in alcoholic solution in the presence of hydrogen donating antioxidant due to formation of the non-radical form DPPH-H in the reaction. DPPH is usually used as a reagent to evaluate free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. Further, curcuminoids are reported to have better stability and heating does not affect the concentration of individual curcuminoids. This characteristic renders them as one of the highly recommended sources of functional food component. The improved antioxidant activity of buffalo *ghee* was due to the incorporation of phenolic compounds including curcumin, which had been shown to possess strong antioxidant activity [35]. Tuba and Ilhami [36] found the total antioxidant activity of curcumin, BHA, BHT, a-tocopherol and trolox as determined by the ferric thiocyanate method in the linoleic acid system, demonstrating that curcumin had a marked antioxidant effect in linoleic acid emulsion. At similar concentrations, the hydrogen peroxide scavenging effect of curcumin and four standard compounds decreased in the order of curcumin > trolox > BHT > BHA a-tocopherol (Tuba and Ilhami, 2008). Similar conclusions were drawn by the Asha *et al*. [13], who reported that *ghee* incorporated with orange peel extract showed maximum potential to quench the DPPH radicals than control and *ghee* incorporated with BHA throughout storage period at 32 ± 2°C. Radical scavenging activity was decreased in all the samples as antioxidant compounds utilized in the oxidation process to quench radical as the storage period progressed.

Conclusion

Effect of storage temperature on development of peroxides and thiobarbituric acid (TBA) in ghee was significantly higher than the effect of treatment (curcumin and BHA) and storage period while treatment had more significant effect on the change in free fatty acids (FFA) and radical scavenging activity as compared to storage temperature and storage period. Ghee incorporated with curcumin was found to be better resistance against development of peroxides, thiobarbituric acid (TBA) and FFA than the ghee incorporated with BHA. Ghee incorporated with curcumin showed stronger activity in quenching DPPH radicals. The study revealed that curcumin could be a good natural source of antioxidants which can be used in fat rich food products like ghee to retard oxidative deterioration during storage (30±1˚C).

References

1. Shende S, Patel S, Arora S, Sharma V. Oxidative stability of ghee incorporated with clove extracts and BHA at elevated temperatures. Internatl. Jr. of Food Prop. 2014; 17(7):1599-1611.
2. Gandhi K, Arora S, Nilkanth P, Kumar A. Effect of Vidarikand (extracts) on oxidative stability of ghee, A comparative study. Res. and Rev.: Jr. of Dairy Sci. and Technol. 2013; 2(1):1-11.

3. Pawar N, Arora S, Bijoy RR, Wadhwa BK. The effects of Asparagus racemosus (shatavari) extract on oxidative stability of ghee, in relation to added natural and synthetic antioxidants. Internatl. Jr of Dairy Technol. 2012; 65(2):293-299.
4. Choe E, Min DB. Mechanisms and factors for lipid oxidation. Compre. Rev. of Food Sci. 2006; 5:169-186.
5. Pukalskas A, vanBeek TA, deWaard P. Development of a triple hyphenated HPLC–radical scavenging detection–DAD–SPE–NMR system for the rapid identification of antioxidants in complex plant extracts. Jr.of Chromato. 2005; 1074(1):81-88.
6. Yeh JY, Hsieh LH, Wu KT, Tsai CF. Antioxidant properties and antioxidant compounds of various extracts from the edible basidiomycete Grifola frondosa (Maitake). Molec. 2011; 16(4):3197-3211.
7. Yassari S, Yasari E. Effects of extracts of Thompson orange peels on the stability of canola oil. Internatl. Jr. of Agri. and Crop Sci. 2013; 5(4):410-415.
8. Naik SR, Thakare VN, Patil SR. Protective effect of Curcumin on experimentally induced inflammation, hepatotoxicity and cardiotoxicity in rats, Evidence of its antioxidant property. Experimental and Toxicologic Path. 2010; 63(5):419-431.
9. IS: 3508. Method of sampling and test for ghee. Bureau of Indian Standards. New Delhi.1966.
10. Patton S, Kurtz GW. 2-Thiobarbituric acid as a reagent for detecting milk fat oxidation. Jr. of Dairy Sci. 1951; 34:669-674.
11. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature, 1958; 181:1199-1200.
12. Snedecor GW, Cochran WG. Statistical Method, 8th Ed.; Affiliated East-West Press: New Delhi, 1994.
13. Asha A, Manjunatha M, Rekha RM, Surendranath B, Heartwin P, Rao J *et al*. Antioxidant activities of orange peel extract in ghee (butter oil) stored at different storage temperatures. Jr. Food Sci.and Techno. 2015; 52(12):8220-8227.
14. Reddy ACP, Lokesh BR. Studies on spice principles as antioxidants in the inhibition of lipid peroxidation of rat liver microsomes. Mol. Cell Biochem. 1992; 111:117-124.
15. Unnikrishnan MK, Rao MNA. Curcumin inhibits nitrogen dioxide induced oxidation of hemoglobin. Molecular and Cellular Biochem. 1995; 146(1):35-37.
16. Sreejayan N, Rao MN. Nitric oxide scavenging by curcuminoids. Jr.of Pharmacy and Pharmacol. 1997; 49:105-107.
17. Zebib B, Mouloungui Z, Noirot V. Stabilization of curcumin by complexation with divalent cations in glycerol/water system. Bioinorg. Chem. and Appli. 2010; 4:292-300.
18. Kumavat SD, Chaudhari YS, Borole P, Mishra P, Shenghani K, Duvvuri P. Degradation studies of curcumin. Intern. Jr. of Pharmaceutical. Rev. and Res. 2013; 3(2):50-55.
19. Landrault N, Poucheret P, Ravel P, Gasc F, Cros G, Teissedre PL. Antioxidant capacities and phenolics levels of French wines from different varieties and vintages. Jr. of Agri. and Food Chem. 2001; 49(7):3341-3348.
20. Patel RS. Antioxidative Role of Betel and Curry Leaves in Ghee. M.Tech. Thesis. Submitted to National Dairy Institute (Deemed University). Karnal.1977.
21. Stark W, Urbach G, Hamilton JS. Volatile compounds in butter oil, V. The quantitative estimation of phenol, o-methoxyphenol, m-and p-cresol, indole and skatole by cold-finger molecular distillation. Jr. of Dairy Res. 1976; 43(03):479-489.
22. Achaya KT. Ghee, vanaspati and special fats in India. In Lipid Technologies and Applications, Gunstone FD and Padley FB (eds), Marcel Dekker Inc., New York, 1997, 369-390.
23. Jain MK. Note on the flavour changes in ghee on storage, their sensory and chemical assessment. Indian Jr.of Ani. Sci. 1973; 43(1):67-72.
24. Borra SK, Gurumurthy P, Mahendra J. Antioxidant and free radical scavenging activity of curcumin determined by using different *in vitro* and ex *vivo* models. Jr. of Medicinal Plants Res. 2013; 7(36):2680-2690.
25. Patel RS, Rajorhia GS. Anti-oxidative role of curry (Murrya koenigi) and betel (Piper betel) leaves in ghee. Jr. of Food Sci. and Technol. 1979; 16(4):158-160.
26. Naaz T, Prakash J. Storage stability of ghee with added spices. Indian Jr.of Nutri. and Diet. 2000; 37(1):20-27.
27. de las Heras A, Schoch A, Gibis M, Fischer A. Comparison of methods for determining malondialdehyde in dry sausage by HPLC and the classic TBA test. Europ. Food Res. and Technol. 2003; 217(2):180-184.
28. Shahidi F, Zhong Y. Lipid oxidation, measurement methods. Bailey's industrial oil and fat Prod. 2005, 235-241.
29. Pohle WD, Gregory RL, Van GB. Relationship of peroxide value and thiobarbituric acid value to development of undesirable flavor characteristics in fats. Jr. of Ameri. Oil Chemists Soc. 1964; 41(10):649-650.
30. Kumar A, Bector BS. A comparative study on the determination of oxidative rancidity in ghee by different methods. Asian Jr. of Dairy Res. 1985; 4(1):23-28.
31. Sulieman AER, EL-makhzangy ATTYA, Ramadan MF. Antiradical performance and physicochemical characteristics of vegetable oils upon frying of french fries, a preliminary comparative study. Jr. of Food Lip. 2006; 13(3):259-276.
32. Parmar P, Khamrui K, Devaraja HC, Singh RRB. The effects of alcoholic extract of Arjuna (Terminalia arjuna Wight Arn.) bark on stability of clarified butterfat. Jr.of Medi. Plants Res. 2013; 4(35):2545-2550.
33. Frankel EN, Meyer AS. The problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants. Jr. Sci. of Food and Agri. 2000; 80(13):1925-1941.
34. Oyaizu M. Studies on products of browning reaction--antioxidative activities of products of browning reaction prepared from glucosamine. Japanese Jr. of Nutri. 1986; 44:307-315.
35. Jayaprakasha GK, Negi PS, Anandharamakrishnan C, Sakariah KK. Chemical composition of turmeric oil – a byproduct from turmeric oleorsin industry and its inhibitory activity against different fungi. Z. Naturforsch. 2001; 56:40-44.
36. Tuba AK, İlhami GS. Antioxidant and radical scavenging properties of curcumin. Chemico-biological Interact. 2008; 174(1):27-37.