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Antioxidant activities of lemon and mandarin peel extracts as natural preservatives in ghee (butter oil) stored at different storage temperatures

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Abstract

Using natural preservatives in ghee and fat-rich products has great economic importance as it can minimize adverse effects on human health and improve the marketing prospects of products. The present study aimed to use lemon (*Citrus lemon* L.) and mandarin (*Citrus reticulata* L.) peel extracts as natural preservatives instead of synthetic antioxidants of butylated hydroxyanisole (BHA) to retard the oxidation of ghee during accelerated storage at various temperatures. Antioxidant activities of lemon peel extracts (LPE), mandarin peel extracts (MPE) and BHA in ghee samples stored at different storage temperatures (T1: 5±2°C, T2: 25±2°C, T3: 60±2°C) were evaluated during the storage period (0, 20 and 40 days). The peroxide value (PV), free fatty acids (FFA), and antioxidant activity of the ghee samples were analyzed and compared with control sample (without additives). The PV and FFA content were significantly higher in the control sample than in the ghee samples treated with BHA, LPE, and MPE at all storage temperatures and during the storage period. All treatments led to a reduction in the PV and FFA content, but LPE had the most significant effect, followed by BHA and MPE, respectively. Antioxidant activity was evaluated by the 3-ethyl benzthiazoline-6-sulphonic acid (ABTS) assay; LPE had the highest antioxidant activity, followed by MPE. Compared to the control sample: BHA, LPE and MPE led to a higher reduction in the PV and FFA content of ghee samples at all storage temperatures and periods owing to their antioxidant activities. Results of incorporated samples showed that using LPE (0.1%) prevented the development of PV and FFA content of ghee samples less than using BHA (0.2%). The present study suggests that LPE (0.1%) could be used as a good natural antioxidant preservative to reduce oxidative deterioration of ghee and fat-rich products.

1. Introduction

Ghee (clarified butter fat) is a very important and expensive product that has been consumed all over the world since ancient times and is added to food for various purposes. Ghee has a distinct flavor that makes it different from other fat-rich dairy products (Lodh *et al.*, 2018; Wani *et al.*, 2022). It is known by different names in different regions, such as *maslee* and *samna* in the Middle East, *meshho* in Aramea, *samuli* in Uganda and *samin* in Sudan (Wani *et al.*, 2022). Ghee has a unique flavor that distinguishes it from other fat-rich dairy products. It is a kind of anhydrous milk fat that plays a significant role in the Egyptian diet. In Egypt, ghee is commonly prepared from domestic water buffalo or dairy cow's milk (Ali *et al.*, 2020). The Codex Alimentarius (2018) describes ghee as a product made exclusively from milk, cream, or butter using various methods which remove non-fat particles and water and have a particularly developed physical structure and flavor. Oxidation of ghee occurs during storage, and the degree of degradation is mostly determined by the storage temperature (Pawar *et al.*, 2012; Gandhi *et al.*, 2013; Shende *et al.*, 2014).

Oxidation of ghee leads to the formation of free radicals and undesirable oxidants, which leads to the development of undesirable flavors and tastes and reduces the shelf life of the products (Ahmed *et al.*, 2016). Adding antioxidants to oils and fats significantly reduces fat oxidation (Hertog *et al.*, 1993). The commonly used commercial antioxidants in ghee are butylated hydroxyanisole (BHA), propyl gallate (PG), tertiary butylhydroquinone (TBHQ), and butylhydroxytoluene (BHT), which are used to extend the shelf life of foods (Patra *et al.*, 2022). Most countries have limited the use of synthetic antioxidants in food

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items because they have toxic effects on human health and the environment. For example, synthetic antioxidants, such as BHT and BHA, have been linked to liver damage and cancer (Grice, 1988). In addition, long-term use of synthetic antioxidants may have side effects even in small quantities; therefore, there is a need for antioxidants without side effects (Erickson *et al.*, 2022).

In this specific context, many studies have focused on using plants (such as herbs, vegetables, fruits, seeds, and other plants) because they contain antioxidants such as phenols, flavonoids, vitamin E, vitamin C, and carotenoids (Abdel-Ghany, 2018). Herbs (Kapadiya and Aparnathi, 2018; Baburao *et al.*, 2020), curcumin (yellow pigment found in turmeric) (Lodh *et al.*, 2018), garden cress and jojoba oils (Taha *et al.*, 2022), and extracts of taro (*Colocasia esculenta*) peels (Abdel-Ghany, 2018) have been used as natural antioxidant compounds in ghee (Yassari and Yasari, 2013). The identification and use of natural antioxidants for increasing the functionality and shelf life of ghee have been challenging (Scartezzini and Speroni, 2000).

Citrus peel is a byproduct of the fruit juice industry and has antioxidant properties. Therefore, it has the potential to improve the safety, quality, and nutritional value of edible oils (Suri *et al.*, 2022). Citrus peel is cheap and available globally, and may find extensive use in medicine, food, and perfume industries as a vital source of natural antioxidants (Al-Qassabi *et al.*, 2018). The present study aimed to use lemon (*Citrus lemon L.*) and mandarin (*Citrus reticulata L.*) peel extracts as natural antioxidants to retard the oxidation of ghee during accelerated storage at various temperatures. The antioxidant activities of lemon and mandarin peel extracts in ghee were compared to those of the synthetic antioxidant BHA in ghee.

2. Materials and methods

2.1 Materials

Freshly prepared milk cream was obtained from an experimental dairy plant of the Assiut government. Ghee was prepared using the creamery butter method by clarifying white butter at 120°C, according to Patel *et al.* (2013). The synthetic antioxidant BHA was obtained from HiMedia (USA)**.** Lemon (*Citrus lemon* L.) and mandarin (*Citrus reticulata* L.) peel extracts were purchased from Cairo (Egypt).

2.2 Storage of ghee

The ghee samples included the control, ghee incorporated with BHA (0.02%), lemon peel extract (LPE, 1.0%), and mandarin peel extract (MPE, 1.0%) stored at three temperatures (T1: 5±2, T2: 25±2°C, T3: 60±2°C) for 40 days. The samples were analyzed for peroxide value (PV) and free fatty acid (FFA) content. The antioxidant activity by ABTS assay of the ghee samples was analyzed at 0, 20 and 40 days.

2.3 Determination of total phenolic content

The TPC was determined according to the Folin-Ciocalteu procedure described by Zilic *et al.* (2012). Briefly, 50 µL of the extract was pipetted into a test tube and adjusted to 3.5 mL using distilled water. Then, 250 µL of Folin–Ciocalteu reagent was added. After 5 mins, the mixture was neutralized with 1.25 mL of 20% sodium carbonate (Na_2CO_3) solution. Absorbance was measured at 725 nm against a solvent blank after 40 mins of incubation in the dark. Total phenolic content was determined using a calibration curve prepared with chlorogenic acid and expressed as milligrams of chlorogenic acid equivalent (mg CAE) per gram of sample.

2.4 Free fatty acids

The percentage of free fatty acids (FFA %) was determined in triplicate using the AOCS Official technique Ca 5a-40 (2003).75 mL of hot neutralized 95% ethanol and 2 mL of 1% phenolphthalein indicator solution were added to a well-mixed ghee sample (7.0±0.05 g) correctly weighed into a 250 mL Erlenmeyer flask. Heating 75 mL of 95% ethanol with 2 mL of 1% phenolphthalein indicator solution to incipient boiling formed the hot neutralized 95% ethanol. The ethanol was neutralized by adding 0.25 N sodium hydroxide solution until a faint permanent pink color appeared. The ghee samples were then titrated against 0.25 N sodium hydroxide until the appearance of the first permanent pink color of the same intensity as that of the neutralized ethanol before the addition of the sample. The permanent pink color persisted for at least 30 seconds during titration. The Free Fatty Acids content (FFA %) and acid number were calculated (Equation 1):

$$\text{FFA \%(as oleic fatty acid)} = \frac{\text{alkali volume (mL)} \times \text{alkali normality} \times 28.2}{\text{sample weight (g)}} \quad (1)$$

2.5 Peroxide value

The value of peroxide was determined using AOCS Official Method Cd 8-53. (2003). In a conical flask, five grams of ghee samples were weighed, and 30 mL of a 3:2 solvent combination of glacial acetic acid chloroform was added to the ghee samples. After adding 0.5 mL saturated potassium iodide (KI) solution and allowing it to stand for 1 min, 30 mL distilled water was added and titrated with 0.01 N sodium thiosulfate solution using starch indicator until the yellow color was discharged. Along with the ghee samples, a blank was made. The following peroxide value was calculated (Equation 2):

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$$\text{Peroxide value} = \frac{10 \times (v_1 - v_2)}{m} \quad (2)$$

Where V_1 volume of $Na_2S_2O_3$ for determination of test sample in mL, V_2 volume of $Na_2S_2O_3$ for determination of blank solution in mL and m is mass of test portion in g (5 g).

2.6 Determination of antioxidant activity by ABTS

For the ABTS assay, the stock solutions of $ABTS^{\bullet+}$ reagent were prepared according to (Hwang and Do Thi, 2014) by reacting equal quantities of a 7 mM aqueous solution of $ABTS^{\bullet+}$ with 2.45 mM potassium persulfate for 16 hrs at ambient temperature in the dark. The working solution was then prepared by diluting 1 mL $ABTS^{\bullet+}$ solution with 60 mL of ethanol: water (50:50 v/v) to obtain an absorbance of 1.0±0.02 units at 734 nm using the spectrophotometer. Extracts (50 µL) were allowed to react with 4.95 mL of $ABTS^{\bullet+}$ solution for 1 hr in the dark. Absorbance was then measured at 734 nm. A standard curve was prepared using the Trolox software. The results were expressed as milligrams of Trolox equivalent (mg TE) per gram of sample.

2.7 Sensory evaluation

All ghee samples prepared in the laboratory were evaluated for sensory characteristics (flavor and color) on a 9-point hedonic scale by ten trained panelists according to Aydın *et al.* (2021)

2.8 Statistical analysis

Data obtained from the analysis of the samples were statistically analyzed by analysis of variance (ANOVA) using SPSS statistical software program version 13 (SPSS Inc., USA). Statistical significance was set at $p < 0.05$.

3. Results and discussion

3.1 Sensory evaluation

The sensory attributes of ghee, such as color and flavor, are important for consumer acceptance. In the first sensory investigation, the color and flavor of the ghee samples infused with 0.25%, 0.5%, 1.0%, 2.0% and 3.0% LPE and MPE were assessed and compared with the control (ghee without any additives). The sensory attributes of the ghee samples were not within the acceptable ranges to include 0.25%, 0.5%, 2.0% and 3.0% LPE and MPE (data not shown). Therefore, based on the initial sensory evaluation, 1.0% LPE and MPE were selected and evaluated further. The color and flavor scores of ghee with 1.0% LPE and MPE were compared with the control. The mean values of ghee color and flavor scores with 1% MPE, 1% LPE, DHA, and control are shown in Table 1. Control and 0.2% DHA samples had high color and flavor scores from the sensory panel (more than 8; 8 = like very much). Among treated samples, the ghee sample with 1.0% MPE had high color and flavor scores, while the flavor score of the ghee sample with 1.0% LPE was 8 (like very much).

Table 1. Sensory evaluation of ghee incorporated with 1.0% lemon peel extract (LPE), mandarin peel extract (MPE), and 0.2% DHA and control.

Treatment	Color	Flavor
Control	8.5	8.5
DHA 0.2%	8.5	8.4
LPE 1.0%	8.0	8.1
MPE 1.0%	8.3	8.3

3.1 Peroxide formation in ghee samples stored at different temperatures

The primary oxidation of oils and fats can be monitored using the peroxide value (PV) determined by titrating iodine released from potassium iodide (Chatha *et al.*, 2006; Anwar *et al.*, 2007). It indicates the presence of active oxygen (mg) in 1 g of oils and fats. Table 2 shows the rate of peroxide development in control and incorporated ghee samples at (T1: 5±2, T2: 25±2°C, T3: 60±2°C), and during the storage period of 40 days. Peroxide formation was not observed in any of the study samples at T1, which could be due to the presence of antioxidant components, and the stability of these components at low temperatures gradually increased during the storage period. There was a significant ($P < 0.05$) development of peroxides in all samples with an increase in temperature at T3, and this clearly appeared at the end of the storage period (40 days) (Figure 1). The LPA samples recorded the lowest percentage of PV followed by BHA after 40 days of storage compared to the rest of the samples. The temperature degree has the greatest effect of PV development in ghee samples than

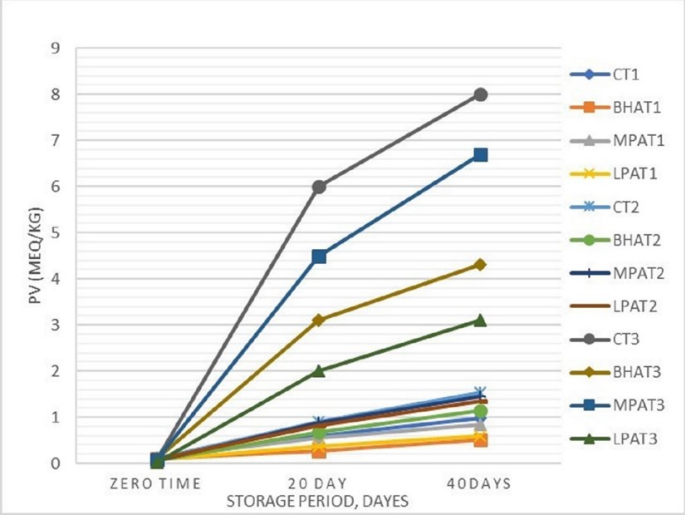

Figure 1. Peroxidase value (PV) of ghee samples stored at different temperatures. C: control, T1: 5±2°C, T2: 25±2°C, T3: 60±2°C, BHA: butylated hydroxyanisole, MPA: mandarin peel extract, LPA: lemon peel extract.

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Table 2. ANOVA indicating peroxidase value (PV) and free fatty acid (FFA) content of ghee samples stored at different storage temperatures.

Source	PV				FFA			
	df	MS	F	S/NS	df	MS	F	S/NS
Storage days(D)	2	36.3	3.25	*	2	0.516	3.477	*
Treatment (Tr)	2	51.6	4.62	*	2	0.108	726.5	*
Temperature (t)	3	4.4	394	*	3	0.023	158.2	*
D*Tr	4	13.9	1.25	*	4	0.024	162.02	*
D*t	6	1.12	100.65	*	6	0.005	34.77	*
D*Tr*t	12	0.772	69.18	*	12	0.003	17.216	*

*p < 0.05; S: significant, NS: Not significant.

treatments and storage period. LPA samples showed lower PV than BHA, MPA, and control at all temperatures during the storage period (Table 2), which could be due to the lemon peels having the highest total phenol content and antioxidant activity (Al-Qassabi *et al.*, 2018).

3.2 Free fatty acids in ghee samples stored at different temperatures

Cleavage and oxidation of double bonds and hydrolysis lead to the formation of FFAs (Paul *et al.*, 1997). The current results showed a significant ($p < 0.05$) increase in FFA content in all study samples. The lowest increase was observed in the ghee sample incorporated with LPE at higher temperatures during the storage period (Figure 2). The FFA content significantly ($p < 0.05$) increased at temperatures of 25°C and 60°C in all samples and was a higher control sample compared with ghee samples incorporated with LPE, MPE, and BHA. The samples incorporated with LPE had the lowest FFA content. Table 2 shows that treatment had a significant effect on FFA content, unlike the storage period and temperature. The FFA content reduced significantly ($p < 0.05$) in the order of ghee samples incorporated with LPE, followed by those with MPE, and then ghee incorporated with BHA. The reduced formation of FFA in ghee incorporated with LPE and MPE might be due to their antioxidant components, such as phenolic compounds, which agrees with the results of Shende *et al.* (2014) and Suri *et al.* (2022).

3.3 Total phenol content of peel extract

The total phenol content (TPC) varied considerably from one citrus type to another. Phenolic antioxidants are products of secondary metabolism in plants and are good sources of natural antioxidants in the human diet (Chun *et al.*, 2005). The TPC of LPE and MPE was determined. TPC was 1.76 and 2.83 mg GAE/mL of LPE and MPE, respectively.

3.4 Antioxidant activity by ABTS

The antioxidant activity of the ghee samples was evaluated using the ABTS assay. The experimental results for the antioxidant activity of the ghee samples are shown in Figure 3. The antioxidant activity of the control samples at all storage temperatures and during the storage period was significantly lower than that of the treated samples, which might be due to the absence of antioxidant components. The samples incorporated with LPE recorded the highest antioxidant activity at the beginning of the storage period, which decreased gradually with an increase in storage temperature and during the storage period. The samples incorporated with LPE had the highest antioxidant activity compared to the other samples at all storage temperatures and storage periods (Figure 3). These results are consistent with

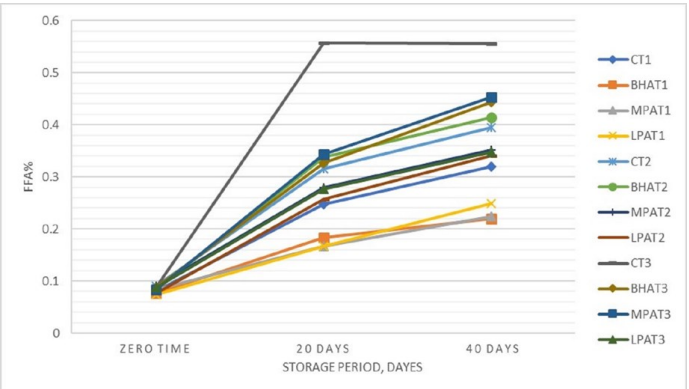

Figure 2. Free fatty acid (FFA) content of ghee samples stored at different temperatures. C: control, T1: 5±2°C, T2: 25±2°C, T3: 60±2°C, BHA: butylated hydroxyanisole, MPA: mandarin peel extract, LPA: lemon peel extract.

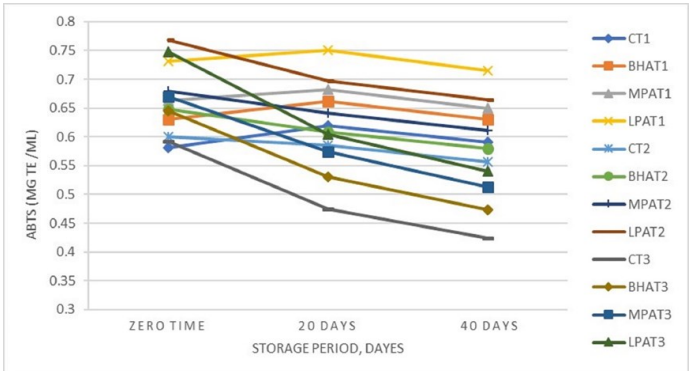

Figure 3. Antioxidant activity (by ABTS) of ghee samples stored at different temperatures. C: control, T1: 5±2°C, T2: 25±2°C, T3: 60±2°C, BHA: butylated hydroxyanisole, MPA: mandarin peel extract, LPA: lemon peel extract.

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previous results reported by (Pawar *et al.*, 2012). that mentioned the ability of LPE to prevent the formation of FFA. This led to a better retention of quality in ghee incorporated with LPE at different temperatures and storage, periods, compared to those incorporated with MPE and BHA. Table 3 shows the highly significant ($p < 0.05$) effect of the treatment, followed by storage temperature and storage period.

Table 3. ANOVA indicating the antioxidant activity (by ABTS) ghee samples storage at different storage temperatures.

Source	ABTS (mg TE/mL)			
	df	MS	F	Sig.
Storage days (D)	2	0.048	113.89	0
Treatment (Tr)	2	0.065	154.68	0
Temperature (t)	3	0.05	119.55	0
D*Tr	4	0.19	44.34	0
D*t	6	0.001	1.591	0.178
D*Tr*t	12	0.001	0.65	0.785

4. Conclusion

In this antioxidant study involving LPE, MPE, and BHA, the PV and FFA in ghee at different storage temperatures (T1: 5±2°C, T2: 25±2°C; T3: 60±2°C) and storage periods (0, 20 and 40 days) were evaluated. Storage temperature had a more significant effect on the development of PV and FFA in the ghee samples than the storage period. In addition, treatment with LPE significantly reduced the PV and FFA content in ghee samples compared with MPE and BHA. The antioxidant activity of the incorporated ghee samples and control, estimated by ABTS, and the effect of LPE on the development of PV and FFA could be due to the higher antioxidant activity of LPE than that of the other treatments at all storage temperatures and during the storage period. Results of incorporated samples showed that using LPE (0.1%) prevented development of PV and FFA content of ghee samples less than using BHA (0.2%). The present study suggests that LPA (0.1%) could be used as a good natural antioxidant preservative to reduce oxidative deterioration of ghee or fat-rich products.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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