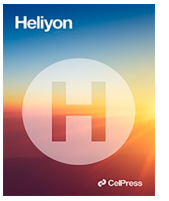
Heliyon 10 (2024) e24717

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Effects of Ajwa date seeds on the oxidative stability of butter

Ayah W. Mansour, Heba A. Sindi *

Department of Food and Nutrition, Faculty of Human Sciences and Design, King Abdul Aziz University, Jeddah, Saudi Arabia

ARTICLE INFO

Keywords:
Ajwa
Antioxidants
Butter
Date seed
Lipid oxidation
DPPH

ABSTRACT

Butter is a widely used food product. However, owing to its rich fatty acid content (saturated and unsaturated fatty acids), it is prone to lipid oxidation, which may affect the quality of butter-containing products. Because of the possible toxic properties of synthetic antioxidants, recent research has focused on the use of natural antioxidants. This study aimed to evaluate the effects of Ajwa date seeds as natural antioxidants to retard lipid oxidation in butter. Date seeds as either a powder or extract were added to butter at concentrations of 0.5 % and 1 %; 100 % butter was used as the control. The samples were stored at 60 °C for 21 d. Radical scavenging activity, peroxide value, acid value, and thiobarbituric acid value (TBA) were analysed every 7 d. This study revealed a strong relationship between storage period and oxidative stability parameters. After 21 d, butter containing date seed powder exhibited higher radical scavenging activity than date seed extract. A reduction in peroxide, acid, and TBA values was also observed in butter samples containing date seed powder. In conclusion, date seed powder increased the oxidative stability of butter. Therefore, adding date seed powder to butter-rich food products can increase their shelf-life and stability.

1. Introduction

Butter is a widely used dairy product and an important source of food lipids. Because of its appealing taste and aroma, butter plays an important role in the manufacture of food products, such as confectioneries, baked goods, ice creams, and sauces [1]. Butter is rich in milk fat components, saturated and unsaturated fatty acids, triacylglycerol, and cholesterol [2,3]; it contains approximately 61 % saturated fatty acids, 33 % unsaturated fatty acids, and 6 % other fatty acids [4]. Foods, and lipids containing unsaturated fatty acids such as butter, are more vulnerable to lipid oxidation, particularly during long periods of storage [5,6]. Numerous studies [7–9] have correlated lipid oxidation with negative health effects, such as cancer, heart disease, and early aging. There are different ways to control lipid oxidation, including reducing oxygen exposure, decreasing the degree of unsaturation of the fatty acids present, and using free radical scavenging antioxidants [10]. Among these techniques, use of antioxidants is the most common method as it retards oxidation and prolongs the shelf life of foods [9]. However, in recent years, there has been growing concern about the potential toxicity and safety of synthetic antioxidants, such as propyl gallate (PG), butylatedhydroxyanisole (BHA) and tertiary butylhydroquinone (TBHQ), applied to foods [11]. Excessive or incorrect use of such synthetic antioxidants results in carcinogenicity, cytotoxicity, oxidative stress induction, and endocrine disrupting effects [12]. Therefore, there is a constant search of natural antioxidants as alternatives to these compounds [13–15]. Many studies have explored the use of naturally sourced antioxidants to retard lipid oxidation in fats, such as butter and ghee [7,11]. Ayar et al. added methanolic extracts of sage, rosemary, and oregano to extend the shelf life of

* Corresponding author. Department of Food and Nutrition, King Abdul Aziz University, P.O. Box: (1441) 21413, Saudi Arabia.
E-mail address: hasindi@kau.edu.sa (H.A. Sindi).

https://doi.org/10.1016/j.heliyon.2024.e24717
Received 24 July 2023; Received in revised form 5 December 2023; Accepted 12 January 2024
Available online 15 January 2024

butter. Results of the study indicated that these extracts had a significant effect on butter stability, especially at the 0.05 % level [16].

Among the various sources of natural antioxidants, dates are rich in bioactive compounds, including phenolic compounds (e.g., cinnamic and coumaric acids), flavonoids (e.g., luteolin, methyl luteolin), and antioxidants [17]. These compounds are more abundant in date seeds than in date flesh [18], with seeds having approximately 18-fold more gallic acid equivalent GAE/100 g and about 4–7-fold more Trolox equivalent/g than flesh [19]. Date seeds constitute only about 10–15 % of the date fruit's weight, and they are mainly discarded or used as animal feed [20]. Date seeds are odourless with light to dark brown colouring and an astringent taste [19]. In some regions of the world, seeds are used for therapeutic purposes [19]. In addition, date seeds are used in the caffeine-free drinks in Arab countries, where powdered seeds are believed to prevent gastric upset and indigestion [19,21]. The composition of date seeds also highlights their nutritional value. The seeds contain dietary fiber (65–69 %), fat (9.9–13.5 %), protein (4.8–7.5 %), and phenolics (~4 %) [14,19], and their high-fiber content makes them suitable for the preparation of fiber-based foods and dietary supplements [22]. Total dietary fiber contents, rheological characteristics, and sensory properties of Saudi Mafrood flat breads containing 0, 5, 10, and 15 % date seed fibers were compared with control flat breads containing wheat bran. Rheological properties were similar for doughs containing coarse date seed fiber or wheat bran, but bread containing 10 % coarse date seed fiber had a higher dietary fiber content than wheat bran bread [22]. Furthermore, because of their bioactive compounds, date seeds may have important applications in the food industry as natural antioxidants. An evaluation of date seeds, therefore, could indicate the economic benefits of date cultivation and industrialisation [14].

The polyphenol content of date seeds ranges from 1864.82 to 4768.87 mg gallic acid equivalent/100 g [23]. Flavan-3-ols are the main class of polyphenols; catechins and epicatechins are the most abundant group within this class, with values ranging from 47.91 to 50.18 g/kg flavan-3-ol [24]. Date seeds also contain significant quantities of phenolic acids, such as protocatechuic, p-hydroxybenzoic, and caffeoylshikimic acid [23]. The quantity of bioactive compounds depends on factors, such as the solvent used for extraction and the date variety [25–27]. Many solvents, such as water, ethanol, acetone, n-hexane, chloroform, and n-butanol, have been used to prepare date seed extracts. According to Thouri et al. [28], the most efficient solvents for extracting polyphenolic compounds from date seeds are water and methanol. They result in extracts with the highest antioxidant activity and strong inhibition of thiobarbituric acid (TBA)-reactive substances. Similarly, date variety is an important factor that can affect the quantity of antioxidants in date seeds. Ahmed et al. [29] compared the phenolic profiles of date seeds from different types of dates (Hallawi, Aseel, and Ajwa) and found that the Ajwa variety of date produced seeds with the maximum phenolic content detected. Therefore, Ajwa date seeds may be a good source of natural antioxidants for use in butter-rich food products.

In this study, we aimed to determine the antioxidant effects of Ajwa cultivar seeds on the oxidative stability of butter over 21 d at high temperatures. While previous studies have examined the antioxidant properties of various natural substances, this research specifically focuses on the potential impact of Ajwa date seeds on the oxidative stability of butter. Incorporation of Ajwa date seeds as a natural antioxidant in butter represents a novel approach to enhancing food product shelf life and nutritional value. We hypothesised that the inclusion of Ajwa date seeds in butter would significantly improve its oxidative stability as measured by reduced lipid oxidation and enhanced resistance to rancidity, thereby demonstrating its potential for use as a natural antioxidant for food preservation. There are few studies on the integral use of Ajwa date seeds as natural antioxidants. In addition, little information is available on the use of date seeds as a natural preservative in fats and oils. To our knowledge, this is the first study to specifically evaluate the use of Ajwa date seeds as natural antioxidants for retarding lipid oxidation in butter.

2. Material and methods

2.1. Materials

Chemicals, such as potassium hydroxide (KOH), sodium thiosulfate (Na2S2O3.5H2O), TBA, and 2,2-diphenyl-1-picrylhydrazyl (DPPH), were obtained from Fisher Scientific (USA) and Sigma-Aldrich (Germany). Unsalted butter was purchased from a local market in Jeddah, Kingdom of Saudi Arabia (KSA). Coarsely ground Ajwa date seeds were purchased from Al-Ansar Date and Sweets Company, Al-Madinah, KSA.

2.2. Methods

2.2.1. Preparation of samples

Date seeds were ground and passed through 1–2 mm screens. The aqueous extract was prepared by dissolving 50 g of date seed powder in 1 L of distilled water. The mixture was heated on a hot plate at 100 °C until it reached a brownish colour (~5 min) [30]. The extract was filtered using Whatman filter paper no. 2 and stored at 4 °C. The date seed extract was added as an aqueous solution to the butter samples. The following four samples were prepared: butter with 0.5 % date seed powder (P_1), butter with 1 % date seed powder (P_2), butter with 0.5 % date seed extract (E_1), and butter with 1 % date seed extract (E_2). All samples were compared to 100 % butter (control). Each sample was homogenised using a homogeniser mixer for 1 min at 3,000 rpm and stored at 60 °C for 21 d [31]. The samples were analysed after 7, 14, and 21 d for radical scavenging activity (RSA) using a DPPH assay and peroxide, acid, and TBA values. The samples were centrifuged at 4,000 rpm for 3 min before each analysis.

2.2.2. RSA

To prepare the DPPH solution, 0.004 g of DPPH was mixed with 15 mL ethanol. The mixture was then transferred into a 50-mL volumetric flask, wrapped with aluminium foil, and ethanol was added gradually until the solution reached 50 mL. Antioxidant

activity was determined using the DPPH radical scavenging method proposed by Blois [32], with 0.2 mL of a butter sample added to a test tube, followed by 3.8 mL of ethyl acetate added as a solvent for hydrophobic compounds, and then 1 mL of DPPH solution (total volume 5 mL). Subsequently, the samples were stored in the dark for 30 min. Absorbances were measured at a 517-nm wavelength. The reference sample was 1 mL of DPPH solution and 4 mL of ethyl acetate. RSA was expressed as percentage inhibition and calculated using the following formula.

$$\%\ \text{radical scavenging activity} = \frac{(control\ OD\ -\ Sample\ OD)}{control\ OD} \times 100$$

2.2.3. Peroxide value

The primary oxidation products were determined according to the method recommended by the Association of Official Analytical Chemists [33].

Into a 250-mL conical flask, 5 g of a sample was added. Then, 30 mL of acetic acid chloroform solution (3 acid: 2 water) was added to the sample and vortexed until dissolved. Saturated potassium iodide (KI) solution (0.5 mL, 75 g of KI/50 ml water) was added, and the mixture was held for a minute in the dark with occasional shaking. After this, 30 mL of water was introduced, and the standard solution was slowly titrated with (0.01 N) $Na_2S_2O_3$ and shaken vigorously until the yellow colour disappeared. Then, 0.5 mL of 1 % starch solution was added as an indicator and titrated again against the 0.01 N $Na_2S_2O_3$. A blank without the butter sample was run in parallel. Peroxide values were calculated using this equation:

$$\text{Peroxide value} = \frac{(\text{Titre} \times 0.01\ \text{N} \times 1000)}{\text{W}}$$

Where, Titre = mL of $Na_2S_2O_3$ used (blank corrected), W = sample weight.

2.2.4. Acid value

The acid value is a measure of free fatty acids. Acid values were determined according to the method described by the Food Safety and Standard Authority of India [34].

Ten grams of a sample was placed in a 250-mL conical flask, and then 50 mL of ethanol and 1 mL of phenolphthalein indicator solution were added. Next, the sample was boiled for 5 min. While the sample was hot, it was titrated immediately against 0.1 N KOH solution, shaken vigorously until a pink colour appeared, and then left to rest for about 30 s. A blank was prepared using the same steps without the addition of butter. Acid values were calculated using the following equation:

$$\text{Acid value} = \frac{(\text{v1}\ -\ \text{v0}) \times 0.1\ \text{N} \times 56.1}{\text{w}}$$

where V1 = sample volume, V0 = blank volume, N = normality of KOH, W = sample weight.

2.2.5. TBA

TBA values were used as an assessment of the final stages of lipid oxidation in the butter samples, based on methods described by Patton and Kurtz [35] and Low [36]. Initially, 0.1 g of butter sample was accurately weighed into a 15-mL stoppered centrifuge tube. Then, 1 mL of Trichloroacetic acid (TCA-20 %) and 2 mL of TBA reagent (0.02 M) were added. After this, the contents were incubated in a boiling water bath for 15 min. The tubes were cooled under running tap water, and then 1 mL of glacial acetic acid and 2 mL of chloroform were added. The contents were mixed well using a vortex mixer and centrifuged at 3,000 rpm for 4 min. After centrifugation, two clearly separated layers had formed. A blank without butter was prepared simultaneously. The optical density of the supernatant layer was measured at 532 nm using a spectrophotometer (APEL-PD303-UV). The TBA values calculated and expressed as mg malonaldehyde (MDA)/Kg sample [36].

2.2.6. Statistical analyses

All collected data were analysed using the Statistical Package for the Social Sciences for Windows, version 23 (IBM Corp., Armonk, NY, USA). Three replications of each parameter measurement were performed. Data are presented as the mean ± standard deviation. One-way analysis of variance, followed by Tukey's honest significant difference testing, was used to determine the significance of relationships between different groups.

3. Results and discussion

3.1. RSA

The most common method used to study antioxidant activity is the DPPH radical-scavenging assay [37]. This method results in the formation of stable free radicals, which can be detected using a common spectrophotometric technique [38]. This study revealed that there was no significant difference in RSA among butter samples at day 0 (baseline) (Fig. 1).

After 7 d of storage, P_1 (36.24 %) showed higher RSA than P_2 (25.13 %). This result concurred with that of Abid et al. [39], who determined the effect of tomato processing by-product (TPB) on the oxidation on traditional Tunisian butter (TTB) during 60 d of storage at 4 °C. They stated that both raw TTB and highly enriched TTB (800 mg of TPB extract/kg of TTB) displayed lower antioxidant

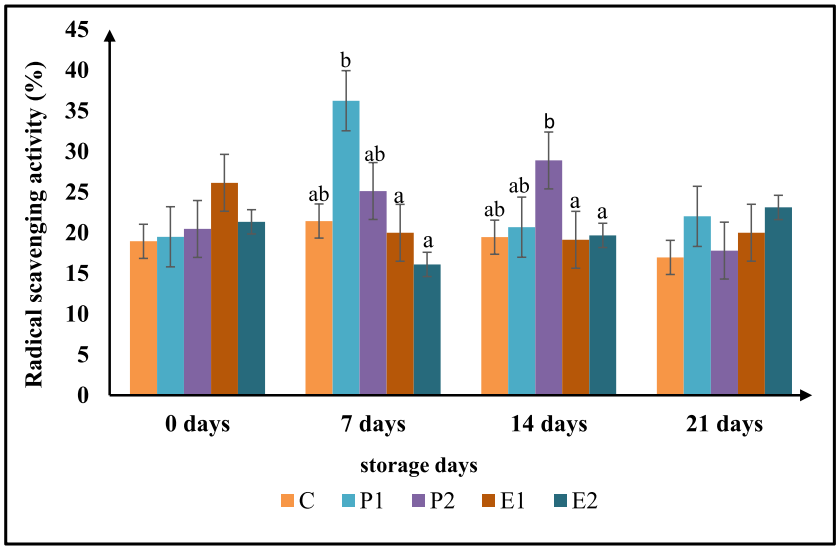


Fig. 1. Radical scavenging activity (RSA) of samples at 0, 7, 14, and 21 d of storage determined by 2,2-diphenyl-1-picrylhydrazyl assay
C = control, P1 = butter with date seed powder (0.5 %), P2 = butter with date seed powder (1 %), E1 = butter with date seed extract (0.5 %), and E2 = butter with date seed extract (1 %).

activity than 400 mg of TPB extract/kg. High concentrations of antioxidants have pro-oxidant activity [14]. In addition, according to Sayas-Barberá et al., fibre in combination with bioactive compounds may reduce the effect of antioxidant activity [14]. The activity of phenolic compounds in food can be influenced by chemical composition, environmental conditions, interactions with lipids, the presence of other active substances, the ability to scavenge radicals, and other factors [14].

After 7 and 14 d of storage, P_1 and P_2 showed higher RSA levels than E_1. These differences were significant ($p < 0.05$). This result was in agreement with the findings of Roy et al. [40] that rice bran oil treated with mulberry powder (0.05 %) showed higher RSA than rice bran oil treated with 200 ppm mulberry extract after accelerated oxidation at 100 °C for 5 d and heat treatment at 180 °C for 1 h. High RSA levels in powder is related to heat effects, which can liberate antioxidant compounds during storage [41].

After 21 d of storage, P_1 and P_2 seemed to have lower RSA levels than that after storage for either 7 or 14 d. This confirmed the findings of Asha et al. [31], who studied the effect of orange peel extract (1 %) on ghee samples during 21 d of storage at 60 °C. They found that RSA in all ghee samples decreased during the storage period, which was attributable to antioxidant compounds that quenched radicals produced in the oxidation process during the storage period.

The low RSA levels in E_1 and E_2 compared to those in P_1 may reflect the effects of water on antioxidant activity. According to Amorati et al. [42] and Choe and Min [43], the effectiveness of antioxidants in scavenging free radicals from food is positively correlated with the weak bond dissociation energy between oxygen and phenolic hydrogen. The bond dissociation energy of the O–H bonds in phenolic antioxidants is high in polar solvents. Thus, polar solvents, such as water, decrease the RSA of antioxidants.

3.2. Peroxide value

Peroxide values were determined using a classical method for quantitation of hydroperoxides and used as an index for the primary stages of lipid oxidation [44]. The peroxide values in this study (Table 1) demonstrated a significant positive relationship between storage time and peroxide concentration ($p < 0.05$). This finding supports those of Saba et al. [45] and Makhlouf et al. [46]. Saba et al.

Table 1
Effects of date seeds (powder and extract) on peroxide values of butter during 21 d of storage.

Storage time	7 d	14 d	21 d
Sample	Peroxide value (meq/kg)	Peroxide value (meq/kg)	Peroxide value (meq/kg)
C	0.90 ± 0.10^{aA}	1.66 ± 0.05^{aB}	2.70 ± 0.10^{aC}
P1	0.73 ± 0.05^{aA}	1.43 ± 0.05^{bB}	2.40 ± 0.05^{bC}
P2	0.46 ± 0.05^{bA}	1.40 ± 0.10^{bB}	2.43 ± 0.05^{bC}
E1	0.93 ± 0.15^{aA}	1.70 ± 0.00^{aB}	3.26 ± 0.05^{cC}
E2	0.73 ± 0.05^{aA}	1.80 ± 0.10^{aB}	4.33 ± 0.05^{dC}

One-way analysis of variance (ANOVA), followed by Tukey's honest significant difference (HSD) testing, was used to evaluate statistical significance, N = 3 (mean ± standard deviation [SD]). Different lowercase letters in the same column imply a significant difference at $p < 0.05$. Different capital letters in the same row imply a significant difference at $p < 0.05$. C = control, P1 = butter with date seed powder (0.5%), P2 = butter with date seed powder (1%), E1 = butter with date seed extract (0.5%), and E2 = butter with date seed extract (1%).

[45] studied the effect of storage time (4 months) and different temperatures (4 °C and room temperature) on the properties of shea butter. Increasing the time of storage at a high temperature led to thermal hydrolysis of triglycerides, which released peroxide into the shea butter samples. Similarly, Makhlouf et al. [46] found that peroxide values of acorn oil samples extracted by different methods increased gradually with a storage time over a 180-d period. In our study, after 7 d, P_2 had the lowest peroxide value (0.46 meq/kg) of all samples ($p < 0.05$). After 14 and 21 d of storage, butter containing date seed powder (P_1 and P_2) had the lowest peroxide values of all samples at these time points ($p < 0.05$). Peroxide values decreased with the additive because of phenolic compounds, which can donate hydrogen to alkyl peroxy radicals, in the date seeds [43].

These results concur with the findings of Emami et al. [47] and Roy et al. [40]. Emami et al. [46] found that butter samples fortified with hazelnut powder at three concentrations (10, 20, and 30 %) had lower peroxide values than the control (0 %) at storage times of 0, 2, and 4 weeks at 4 °C. In addition, Roy et al. [40] found that adding 0.05 % mulberry powder effectively decreased the peroxide value of rice bran oil compared to that of the control (0 %) after heat treatment (180 °C/h).

The high peroxide values of E_1 and E_2 may be attributed to the type of solvent used. According to Al Ghezi et al. [18], the phenolic content in water extracts of date seeds was lower than that in organic extracts. Furthermore, addition of the water extract increased the water content in butter, making it highly vulnerable to oxidation. This explanation is consistent with that of Rege et al. [48], who found that an imbalance between water content, fat molecules, and antioxidant compounds increased oxidation.

3.3. Acid value

In measuring the content of free fatty acids (FFAs) formed after hydrolytic degradation of lipid molecules during the primary stages of lipid oxidation, the acid value is an important index indicating the degree of rancidity in hydrolysed fat [49]. Table 2 shows that acid content gradually increased through the storage period. Increasing the storage time at high temperatures can cause gradual thermal hydrolysis of triglycerides, which releases peroxides and FFA [50,51]. In this study, there were no significant differences in the acid values of all samples and the control at 7 d of storage. At 14 d of storage, P_2 had the lowest acid concentration of all samples at that time point (0.64 mg/g). This result agreed with that of Mehdizadeh et al. [52], who studied the effect of walnut kernel extract on acid values of traditional butter. They found that samples treated with a hydroalcohol extract (0.5, 0.1, and 0.05 %) of walnut kernel septum membranes showed lower levels of FFA than the control after 90 d of storage. A reduction in the acid value of P_2 is likely related to the high concentration of phenolic compounds and antioxidant activity in the sample [31]. In contrast, the highest acid concentration (0.70 mg/g), relative to that in the control and P_2, was observed in E_1 ($p < 0.05$). The increasing acid values in E_1 and E_2 were due to the use of water extracts and high-temperature (60 °C) butter storage, which together promote the hydrolysis and oxidation of triglycerides and increase the FFA content [47,53]. Adding water, as described above, might affect the balance between water content, fat molecules, and antioxidant compounds [48].

At the end of the storage period, P_1 (0.77 mg/g), E_1 (0.79 mg/g), and E_2 (0.77 mg/g) had significantly higher acid values than the control (0.73 mg/g). This result confirmed the findings of Emami et al. [47] that showed butter samples treated with hazelnut powder at different concentrations (10, 20, and 30 %) had higher acid values than the control (0 %) after 0, 2, and 4 weeks of storage at 4 °C. The study showed that this increase in hydrolytic rancidity arose from the inherent lipolytic activity of hazelnuts.

3.4. Thiobarbituric acid values

Peroxides and FFA are oxidised during the final stages of lipid oxidation to form secondary and tertiary oxidation products, such as malondialdehyde [54]. The TBA method was used to analyse this oxidation. As illustrated in Fig. 2, after 7 and 14 d of storage, the lowest TBA values were observed in P_2. These results agree with the findings of Sayas-Barberá et al. [14], who studied the effects of date seed powder (1.5, 3, and 6 %) on lipid oxidation in meat burgers. They found that after 10 d of storage, samples treated with date seed powder (1.5 and 3 %) showed lower TBA values than the control. This decrease in TBA values was due to the antioxidant activity of the date seeds.

After 7 and 14 d, E1 exhibited higher TBA values than the control. This result contrasted with the findings of Najgebauer-Lejk et al. [55], who studied the storage stability of butter made from sour cream with the addition of 2 % dried herbs (sage or rosemary). They

Table 2
Effect of date seeds (powder and extract) on acid values of butter during 21 d of storage.

Storage	7 d	14 d	21 d
Samples	Acid value (mg/g)	Acid value (mg/g)	Acid value (mg/g)
C	0.34 ± 0.01^{aA}	0.67 ± 0.00^{abB}	0.73 ± 0.00^{aC}
P1	0.32 ± 0.01^{aA}	0.69 ± 0.01^{bcB}	0.77 ± 0.01^{bC}
P2	0.31 ± 0.00^{aA}	0.64 ± 0.00^{aB}	0.72 ± 0.01^{aC}
E1	0.32 ± 0.01^{aA}	0.70 ± 0.01^{cB}	0.79 ± 0.00^{bC}
E2	0.32 ± 0.01^{aA}	0.69 ± 0.02^{bcB}	0.77 ± 0.02^{bC}

One-way ANOVA, followed by Tukey's HSD testing, was used to evaluate statistical significance, N = 3 (mean ± SD). Different lowercase letters in the same column imply significant differences at $p < 0.05$. Different capital letters in the same row imply significant differences at $p < 0.05$. C = control, P1 = butter with date seed powder (0.5 %), P2 = butter with date seed powder (1 %), E1 = butter with date seed extract (0.5 %), and E2 = butter with date seed extract (1 %).

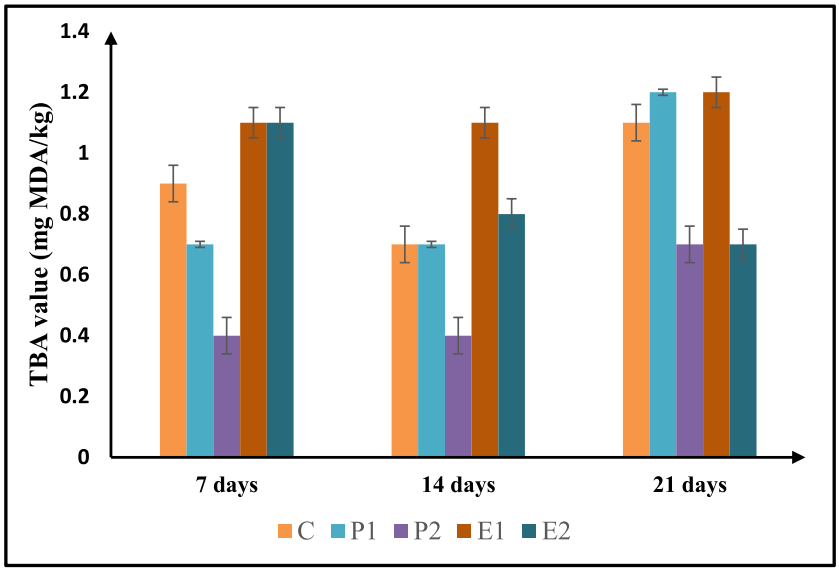


Fig. 2. Thiobarbituric acid (TBA) value of butter samples during 21 d of storage
C = control, P1 = butter with date seed powder (0.5 %), P2 = butter with date seed powder (1 %), E1 = butter with date seed extract (0.5 %), and E2 = butter with date seed extract (1 %).

found that sage and rosemary butter exhibited much lower levels of TBA than natural butter after 5 months of storage at 4 °C. The increase in TBA observed in our study may be attributed to several factors, such as chemical changes, colour compounds in the date seed extract, and the interaction of malondialdehyde with multiple compounds, rather than the development of secondary lipid oxidation products alone [31].

The highest TBA values for the control, P_1, and P_2 were recorded after 21 d of storage. Ozturk et al. [56] studied the effects of a-Tocopherol at 50 and 100 ppm as a natural antioxidant on butter during 6 months of storage at 4 °C and −20 °C. They reported that TBA increased gradually over the storage period.

The TBA values of samples were convergent, with no significant differences among them ($p > 0.05$). There are certain limitations to using the TBA test to evaluate the oxidative state of foods and biological systems because of their chemical complexity [44]. In such cases, application of high-performance liquid chromatography (HPLC) offers better specificity and sensitivity in MDA determination based on its analysis of MDA-TBA complexes [57]. Moreover, peroxides and FFA possibly need more time to convert into secondary and tertiary oxidation products, such as malondialdehyde, which is the main marker during the final stages of lipid peroxidation [58].

3.4.1. Limitations

The limited time available for the study led to a short storage time used in experiments.

4. Conclusions

In the current study, Ajwa date seeds were assessed as natural antioxidants that might retard lipid oxidation in butter. The high antioxidant levels in date seeds contribute to the oxidative stability of butter. The optimal antioxidant percentage to provide resistance to peroxide and FFA development was 1 % in powder form. Adding powdered date seeds significantly retarded lipid oxidation as compared to adding water extract, based on peroxide and acid values. These results confirmed the hypothesis that inclusion of Ajwa date seeds in butter can significantly improve its oxidative stability. Antioxidants in date seeds were effective in inhibiting the initial stages of lipid peroxidation. Differences in TBA values between samples were not significant; accurate methods, such as HPLC, should be used to determine TBA values in a follow-up study. The results of this study may help the butter industry increase the stability of food products using Ajwa date seeds. Additional studies are needed to evaluate the long-term (>21 d) effects of date seeds on the oxidative stability of butter to resemble real shelf-life processes. Further studies should examine the sensory and physiochemical qualities of butter with Ajwa date seeds.

Funding

This research was not supported by any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CRediT authorship contribution statement

Ayah W. Mansour: Conceptualization, Investigation, Methodology, Resources, Writing – original draft. **Heba A. Sindi:** Formal analysis, Resources, Supervision, Validation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] S. Cheng, W. Li, S. Wu, Y. Ge, C. Wang, S. Xie, L.Z. Cheong, Functional butter for reduction of consumption risk and improvement of nutrition, Grain Oil Sci. Technol. (2023), https://doi.org/10.1016/j.gaost.2023.09.001.

[2] R.V. Rios, M.D.F. Pessanha, P.F.d. Almeida, C.L. Viana, S.C.d.S. Lannes, Application of fats in some food products, Food Sci. Technol. 34 (2014) 3–15, https://doi.org/10.1590/S0101-20612014000100001.

[3] D. Mudgil, S. Barak, Development of low sodium table butter via partial replacement of sodium chloride with potassium chloride, Biointerface Res. Appl. Chemistry 10 (2020) 6112–6118, https://doi.org/10.33263/BRIAC105.61126118.

[4] F.D. Gunstone, Fatty Acid and Lipid Chemistry, Springer, 2012.

[5] N. Akbarieh, P. Sadighara, N. Shariatifar, E. Molaee-Aghaee, R. Aslani, Lipid peroxidation in sheep and cow traditional butter obtaind from southern Tehran, Iran, J food safe & hy 8 (4) (2022) 237–242, https://doi.org/10.18502/jfsh.v8i4.11956.

[6] J.H. Lee, Changes in flavor compounds and quality parameters of goat cream butter during extended refrigerated storage, Int. J. Food Prop. 23 (2020) 306–318, https://doi.org/10.1080/10942912.2020.1720716.

[7] N. Pawar, S. Arora, R.R. Bijoy, B.K. Wadhwa, The effects of Asparagus racemosus (shatavari) extract on oxidative stability of ghee, in relation to added natural and synthetic antioxidants, Int. J. Dairy Technol. 65 (2012) 293–299, https://doi.org/10.1111/j.1471-0307.2011.00816.x.

[8] S.A. Vieira, G. Zhang, E.A. Decker, Biological implications of lipid oxidation products, J. Am. Oil Chem. Soc. 94 (2017) 339–351, https://doi.org/10.1007/s11746-017-2958-2.

[9] X.-F. Leong, Lipid oxidation products on inflammation-mediated hypertension and atherosclerosis: a mini review, Front. Nutr. 710 (2021), https://doi.org/10.3389/fnut.2021.717740.

[10] D. Wang, H. Xiao, X. Lyu, H. Chen, F. Wei, Lipid oxidation in food science and nutritional health: a comprehensive review, Oil Crop Sci (2023), https://doi.org/10.1016/j.ocsci.2023.02.002.

[11] C. Vanlallawmzuali, N. Pushpa, Production of natural antioxidant rich food products (bread, tofu & curd)–an alternate to synthetic food products, Ann. Romanian Soc. 25 (2021) 5683–5693.

[12] X. Xu, A. Liu, S. Hu, I. Ares, M.R. Martínez-Larrañaga, X. Wang, M.A. Martínez, Synthetic phenolic antioxidants: metabolism, hazards and mechanism of action, Food Chem. 353 (2021) 129488, https://doi.org/10.1016/j.foodchem.2021.129488.

[13] S. Yassari, E. Yasari, Effects of extracts of Thompson orange peels on the stability of canola oil, Intl. J. Agric. Crop Sci. 5 (2013) 410–420.

[14] E. Sayas-Barberá, A.M. Martín-Sánchez, S. Cherif, J. Ben-Abda, J.Á. Pérez-Álvarez, Effect of date (*Phoenix dactylifera* L.) pits on the shelf life of beef burgers, Foods 9 (2020) 102, https://doi.org/10.3390/foods9010102.

[15] M. Viana da Silva, M.R.C. Santos, I.R. Alves Silva, E.B. Macedo Viana, D.A. Dos Anjos, I.A. Santos, N.G. Barbosa de Lima, C. Wobeto, N. Jorge, S.C.D.S. Lannes, Synthetic and natural antioxidants used in the oxidative stability of edible oils: an overview, Food Rev. Int. 38 (2022) 349–372, https://doi.org/10.1080/87559129.2020.1869775.

[16] A. Ayar, M. Özcan, A. Akgul, N. Akin, Butter stability as affected by extracts of sage, rosemary and oregano, J. Food Lipids 8 (2001) 15–25, https://doi.org/10.3390/foods12030471.

[17] K. Jdaini, F. Alla, F. Mansouri, A. Parmar, M.A. Elhoumaizi, Optimizing the extraction of phenolic antioxidants from data palm fruit by simplex-centroid solvent mixture design, Heliyon 9 (1) (2023) e12738, https://doi.org/10.1016/j.heliyon.2022.e12738.

[18] N.A.S. Al Ghezi, A.E.B.H.J. Al-Mossawi, A.K.J. Al-Rikabi, Antioxidants activity of date seed extraction of some date varieties, Med. Leg. Update 20 (2020) 922–928, https://doi.org/10.37506/v20/i1/2020/mlu/194714.

[19] A.F. Ahmed, J.H. Al-Qahtani, H.M. Al-Yousef, M.S. Al-Said, A.E. Ashour, M. Al-Sohaibani, S. Rafatullah, Proanthocyanidin-rich date seed extract protects against chemically induced hepatorenal toxicity, J. Med. Food 18 (3) (2015) 280–289, https://doi.org/10.1089/jmf.2014.3157.

[20] K.L. Alharbi, J. Raman, H.J. Shin, Date fruit and seed in nutricosmetics, Cosmetics 8 (3) (2021) 59, https://doi.org/10.3390/cosmetics8030059.

[21] M.A. Zakeri, M.H. Bagheripour, M. Iriti, M. Dehghan, Portal vein thrombosis after the consumption of date seed powder: a case study, Case Rep. Med. 2021 (2021) 1–5, https://doi.org/10.1155/2021/6668722.

[22] M.B.H. Najafi, Date seeds: a novel and inexpensive source of dietary fiber, International conference on food engineering and biotechnology (2011) 326.

[23] S. Hilary, J. Kizhakkayil, U. Souka, F. Al-Meqbaali, W. Ibrahim, C. Platat, In-vitro investigation of polyphenol-rich date (*Phoenix dactylifera* L.) seed extract bioactivity, Front. Nutr. 8 (2021) 667514, https://doi.org/10.3389/fnut.2021.667514.

[24] H. Habib, A. Othman, S. Al-Marzooqi, A. Al-Bawardi, J.Y. Pathan, S. Hilary, U. Souka, S. Al-Hammadi, W. Ibrahim, C. Platat, The antioxidant activity of date seed: preliminary results of a preclinical in vivo study, Emir. J. Food Agric. 822–832 (2017), https://doi.org/10.9755/ejfa.2017.v29.i11.1477.

[25] S. Khalid, A. Ahmad, M. Kaleem, Antioxidant activity and phenolic contents of ajwa date and their effect on lipo-protein profile, Funct. Foods Health Dis. 7 (2017) 396–410, https://doi.org/10.31989/ffhd.v7i6.337.

[26] M.H. Rathi, A.-L. Molan, M.H. Ismail, The impact of the extracting solvent and the cultivar on the determination of total phenolic contents and anti-radical activities of extracts from roasted date-seeds of two date cultivars cultivated in Iraq, Plant Arch 18 (2018) 830–834.

[27] A.T. Idowu, O.O. Igiehon, A.E. Adekoya, S. Idowu, Dates palm fruits: a review of their nutritional components, bioactivities and functional food applications, AIMS Agric. Food. 5 (2020) 734–755, https://doi.org/10.3934/agrfood.2020.4.734.

[28] A. Thouri, H. Chahdoura, A. El Arem, A.O. Hichri, R.B. Hassin, L. Achour, Effect of solvents extraction on phytochemical components and biological activities of Tunisian date seeds (var. Korkobbi and Arechti), BMC Compl. Alternative Med. 17 (2017) 248, https://doi.org/10.1186/s12906-017-1751-y.

[29] A. Ahmed, M.U. Arshad, F. Saeed, R.S. Ahmed, S.A.S. Chatha, Nutritional probing and HPLC profiling of roasted date pit powder, Pakistan J. Nutr. 15 (2016) 229–237.

[30] S.H. Orabi, S.M. Shawky, Effect of date palm (Phoenix dactylifera) seeds extracts on hematological, biochemical parameters and some fertility indices in male rats, Int. J. Sci. Basic Appl. Res. 17 (2014) 137–147.

[31] A. Asha, M. Manjunatha, R.M. Rekha, B. Surendranath, P. Heartwin, J. Rao, E. Magdaline, C. Sinha, Antioxidant activities of orange peel extract in ghee (butter oil) stored at different storage temperatures, J. Food Sci. Technol. 52 (2015) 8220–8227, https://doi.org/10.1007/s13197-015-1911-3.

[32] M.S. Blois, Antioxidant determinations by the use of a stable free radical, Nature 181 (1958) 1199–1200, https://doi.org/10.1038/1811199a0.

[33] Aoac, Peroxide value of butter oils (Titrationmethod), in: Official Methods of Analysis, Association of Official Analytical Chemists Inc., Gaithersburg, USA, 2000. No. 965.33.

[34] FSSAI, Manual of methods of analysis of foods: oils and fats, in: Food Safety and Standard Authority of India, 2015. New Delhi.

[35] S. Patton, G.W. Kurtz, 2-Thiobarbituric acid as a reagent for detecting milk fat oxidation, J. Dairy Sci. 34 (1951) 669–674, https://doi.org/10.3168/jds.S0022-0302(51)91763-8.
[36] L.K. Low, C.S. Ng, Determination of thiobarbituric acid (TBA) number, in: H. Hasegawa (Ed.), Laboratory Manual on Analytical Methods and Procedures for Fish and Fish Products, Marine Fisheries Research Department, Southeast Asian Fisheries Development Center, Singapore, 1987.
[37] İ. Gulcin, S.H. Alwasel, DPPH radical scavenging assay, Processes 11 (8) (2023) 2248, https://doi.org/10.3390/pr11082248.
[38] J. Ismael, E. Dessalegn, W.M. Fereja, In vitro antioxidant and antibacterial activity of leaf extracts of *Measa lanceolata*, Int. J. Food Prop. 24 (2021) 702–712, https://doi.org/10.1080/10942912.2021.1917608.
[39] Y. Abid, S. Azabou, M. Jridi, I. Khemakhem, M. Bouaziz, H. Attia, Storage stability of traditional Tunisian butter enriched with antioxidant extract from tomato processing by-products, Food Chem. 233 (2017) 476–482, https://doi.org/10.1016/j.foodchem.2017.04.125.
[40] L.G. Roy, S. Arabshahi-Delouee, A. Urooj, Antioxidant efficacy of mulberry (Morus indica L.) leaves extract and powder in edible oil, Int. J. Food Prop. 13 (2010) 1–9, https://doi.org/10.1080/10942910802120139.
[41] K. Sharma, E.Y. Ko, A.D. Assefa, S. Ha, S.H. Nile, E.T. Lee, S.W. Park, Temperature-dependent studies on the total phenolics, flavonoids, antioxidant activities, and sugar content in six onion varieties, J. Food Drug Anal. 23 (2015) 243–252, https://doi.org/10.1016/j.jfda.2014.10.005.
[42] R. Amorati, A. Cavalli, M.G. Fumo, M. Masetti, S. Menichetti, C. Pagliuca, G.F. Pedulli, C. Viglianisi, Kinetic and thermochemical study of the antioxidant activity of sulfur-containing analogues of vitamin E, Chem. Eur J. 13 (2007) 8223–8230, https://doi.org/10.1002/chem.200700309.
[43] E. Choe, D.B. Min, Mechanisms of antioxidants in the oxidation of Compr. Rev, Food Sci. Food Saf. 8 (2009) 345–358, https://doi.org/10.1111/j.1541-4337.2009.00085.x.
[44] C.C. Akoh, Food Lipids: Chemistry, Nutrition, and Biotechnology, CRC press, 2017, https://doi.org/10.1201/9781315151854.
[45] A.M. Saba, D.G. Tsado, J.O. Okafor, Determination of the effect of storage time and condition on the properties of shea butter, J. Chem. Eng. Process Technol. 9 (2018) 382, https://doi.org/10.4172/2157-7048.1000382.
[46] F.Z. Makhlouf, G. Squeo, G. Difonzo, M. Faccia, A. Pasqualone, C. Summo, M. Barkat, F. Caponio, Effects of storage on the oxidative stability of acorn oils extracted from three different Quercus species, J. Sci. Food Agric. 101 (2021) 131–138, https://doi.org/10.1002/jsfa.10623.
[47] S. Emami, S. Azadmard-Damirchi, J. Hesari, S.H. Peighambardoust, Y. Ramezani, M. Nemati, M. Esmaili, S.A. Rafat, Production of butter incorporated with hazelnut powder, J. Agric. Sci. Technol. 16 (2014) 1623–1632.
[48] S.A. Rege, S.A. Momin, D.N. Bhowmick, A.A. Pratap, Stabilization of emulsion and butter like products containing essential fatty acids using kalonji seeds extract and curcuminoids, J. Oleo Sci. 61 (2012) 11–16, https://doi.org/10.5650/jos.61.11.
[49] A. Zeb, F. Ullah, A simple spectrophotometric method for the determination of thiobarbituric acid reactive substances in fried fast foods, J. Anal. Methods Chem. (2016) 9412767, https://doi.org/10.1155/2016/9412767, 2016.
[50] P.K. Pidatala, D. Bellmer, W. McGlynn, Oxidative stability of a new peanut butter bite product, Int. J. Food Sci. 2021 (2021) 5528315, https://doi.org/10.1155/2021/5528315.
[51] W. Qingling, J. Guofeng, W. Ning, G. Xin, J. Yongguo, M. Meihu, Lipolysis and oxidation of lipids during egg storage at different temperatures, Czech J. Food Sci. 35 (2017) 229–235, https://doi.org/10.17221/174/2016-CJFS.
[52] T. Mehdizadeh, N. Mohammadipour, A.M. Langroodi, M. Raeisi, Effect of walnut kernel septum membranes hydroalcoholic extract on the shelf life of traditional butter, Heliyon 5 (3) (2019), https://doi.org/10.1016/j.heliyon.2019.e01296.
[53] M.E. Di Pietro, A. Mannu, A. Mele, NMR determination of free fatty acids in vegetable oils, Processes 8 (2020) 410, https://doi.org/10.3390/pr8040410.
[54] E.D.N.S. Abeyrathne, K. Nam, D.U. Ahn, Analytical methods for lipid oxidation and antioxidant capacity in food systems, Antioxidants 10 (2021) 1587, https://doi.org/10.3390/antiox10101587.
[55] D. Najgebauer-Lejko, T. Grega, M. Sady, J. Domagała, The quality and storage stability of butter made from sour cream with addition of dried sage and rosemary, Biotechnol. Anim. Husb. 25 (5–6-2) (2009) 753–761.
[56] S. Ozturk, S. Cakmakci, The effect of antioxidants on butter in relation to storage temperature and duration, Eur. J. Lipid Sci. Technol. 108 (11) (2006) 951–959, https://doi.org/10.1002/ejlt.200600089.
[57] A. Papastergiadis, E. Mubiru, H. Van Langenhove, B. De Meulenaer, Malondialdehyde measurement in oxidized foods: evaluation of the spectrophotometric thiobarbituric acid reactive substances (TBARS) test in various foods, J. Agric. Food Chem. 60 (38) (2012) 9589–9594, https://doi.org/10.1021/jf302451c.
[58] A. Ayala, M.F. Muñoz, S. Argüelles, Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal, Oxid. Med. Cell. Longev. (2014) 360438, https://doi.org/10.1155/2014/360438, 2014.