Use of Some Essential Oils as Natural Preservatives for Butter

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Thyme and cumin essential oils were used in the present study in an attempt to prevent butter deterioration during storage at room temperature. Butter oxidation and lipolysis were followed by measuring the acid, peroxide and TBA values. Lipolytic activity and total microbial and lipolytic bacterial counts were also measured. During butter storage, very little change in the peroxide and TBA values were found while a gradual increase in the acid value was noticed. The addition of cumin and thyme oils at 200 ppm to butter caused very little increase in the acid value. The data for lipolytic bacterial counts were in general agreement with the acid values. Thyme and cumin essential oils showed a greater anti-hydrolytic effect and act as superior preservatives compared to BHT.

Autooxidation and lipolysis are responsible for off-flavors in lipid-containing food products. Butter deteriorates by oxidative rancidity from the reaction with atmospheric oxygen and hydrolytic reactions catalyzed by lipases from food or from microorganisms (1). Antioxidants are widely used in many foods to prevent fat rancidity. It has been found that the addition of BHT and BHA at concentrations ranging from 50-500 ppm in ghee or butter retarded the development of both fatty acids and peroxides during storage (2-5). A combination of propyl gallate (100 ppm) and dodecyl gallate (500 ppm) was effective as an antioxidant in butter, as was propyl gallate at 200 ppm (6,7). There has been some discussion recently of the undesirable use of synthetic antioxidants, since BHA had toxic and carcinogenic effects (8). Also, BHT caused changes in rat thyroids, stimulation of DNA synthesis and induction of enzymes (9). Consequently, there is a need for other types of antioxidants. The present work has been conducted to study the effect of some naturally occurring essential oils as preservatives for butter.

MATERIALS AND METHODS

Source of milk. Fresh cow's milk was obtained from the Experimental Station Herd, Faculty of Agriculture, Cairo University, Giza, Egypt.

Milk processing. Milk was separated into cream and skim milk using an Alfa-Laval separator (Alfa-Laval, Sweden). Cream was churned to obtain butter.

Butylated hydroxy toluene (BHT) and essential oils. Crystalline BHT was obtained from Sigma Chemical Company (St. Louis, MO). The fruits and leaves of cumin (*Cuminum cyminum*, L.) and thyme (*Thymus vulgarius*, L.) plants were collected from the Pharmacy Farm, Cairo University, Giza, Egypt. The plant materials, cut into small pieces (ca. 100 g), were placed in a flask (2 L) together with double distilled water (1.5 L). A steam distillation continuous extraction head was attached to the flask. After steam distillation the oil was isolated and dried over anhydrous sodium sulfate.

Authentic volatile compounds. A set of 24 standard materials with a stated purity of 99% by GLC was obtained from Dragoco company (Holzminden, West Germany). The standard materials were: cyclic terpenes (α-pinene, B-pinene, camphene, limonene, γ-terpinene, terpinolene and phellendrene); aliphatic hydrocarbon (myrecene); aromatic hydrocarbon (p-cymene); sesquiterpene (caryophyllene); phenol ethers (eugenol, thymol and methyl chavicol); cyclic terpene ketones (carvone, dihydrocarvone, and thujone); aromatic aldehydes (cumin aldehyde); aliphatic alcohols (linalool and geraniol); cyclic terpene alcohols (t-carvol, α-terpineol and borneol) and terpene esters (linalyl acetate and terpinyl acetate).

Identification and determination of essential oil composition. The essential oils were analyzed by a GCV Pye Unicam gas chromatograph equipped with dual flame ionization detectors. The chromatograph was fitted with a coiled glass column (1.5 m × 4 mm) packed with Diatomite C (100-120 mesh) and coated with 10% PEGA. The oven temperature was programmed at 4°C/min from 60°C to 180°C and was held at 180°C for 15 min. Detector and injector temperatures were 220°C and 300°C, respectively. Gas flow rates for N_2, H_2 and air were 30, 33 and 330 ml/min, respectively. Peak identification was performed by comparing the relative retention times of each peak with those of known compounds. Also, the essential oils were mixed with their major compounds and injected into GLC to verify the peak identity. The relative retention times for thymol and cumin aldehyde are given a value of 1.00, depending on essential oil origin. The peak areas were measured by triangulation, and percentage of each oil component was calculated as the ratio of the peak area to the total chromatographic area. All samples were analyzed in triplicate and the values agreed within 2%. Mean values are presented in the text.

Oxidation systems. Butter packaged in sterilized glass bottles was thoroughly mixed with BHT (200 ppm), cumin (200 ppm) and thyme (200 ppm) oils and stored at room temperature. Samples were removed periodically and subjected to chemical and microbiological analyses.

Chemical analyses. Hydrolytic and oxidative rancidity of butter were followed by determining the acid value, peroxide number and thiobarbituric acid (TBA) value. To get the acid value, a known weight of butter fat (5 g) was dissolved in a neutralized alcohol (50 ml) and titrated with KOH (0.1 N) (10). For the peroxide number, a known weight of butter fat (2 g) was dissolved in a mixture of $CH_3COOH:CHCl_3$ (3:2, v/v), and saturated solution of KI (1 ml) was then added. The liberated iodine was titrated with sodium thiosulfate solution (0.1. N) in the presence of starch as an indicator (10). The TBA test was performed by adding H_2O (8 ml), TBA solution (6 ml, 0.025 mM) and trichloroacetic acid (3 ml) to the butter fat (0.5 g). After heating the mixture (20 min), the interfering materials were extracted three times with ether and discarded. The aqueous phase was completed with distilled water to a known volume (25 ml) and the absorbance of this solution was recorded at 532 nm (11). All chemical determinations were conducted in triplicate and the results are presented as average values.

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Determination of lipolytic activity. The lipolytic activity of butter was determined and is defined as the number of moles of free fatty acids (as oleic acid) neutralized by NaOH (12).

Microbiological analysis. The counts of total bacteria and lipolytic bacteria were carried out with melted butter (1 ml) diluted with appropriate volumes of sterilized saline solution and thoroughly mixed with a suitable medium. Treptone-glucose-yeast-agar medium was used for total bacterial count (13). Treptone-glucose-yeast-sterilized ghee medium was used for lipolytic bacteria (14). The plates containing the media and butter were incubated at 32°C for four days in all cases.

Statistical analysis. The calculated Δ-values for all chemical values, lipolytic activity and counts of total bacteria and lipolytic bacteria were statistically analyzed using a split design (15).

RESULTS AND DISCUSSION

Consumers all over the world are becoming increasingly conscious of the nutritional value and the safety of their food and its ingredients. At the same time, there is an increased preference for natural foods and food ingredients which are generally believed to be safer, healthier and less subject to hazards than foods containing artificial food additives. Cumin and thyme oils were demonstrated to have a positive and effective inhibitory effect on synthetic media containing bacteria, yeast and fungi (16). These microorganisms are known to be responsible for food deterioration. Consequently, the essential oils under study were added to butter in an attempt to study their effect on preventing lipid oxidation and hydrolysis. It has been reported that the minimum inhibitory concentration (MIC) required to prevent certain types of microorganisms was 200 ppm of these essential oils (16). Hence, the essential oils were added to the natural medium (butter) at 200 ppm, which is similar to MIC. The levels of essential oils added to butter are beyond the concentration of antioxidants added in industry to food products (1). An experiment was conducted using BHT at 200 ppm along with other experiments in order to compare the antioxidative ability of the essential oils towards butter rancidity. The experimental period was terminated (18 days) when an objectionable odor was obviously noticed with the control sample.

Table 1 shows the peroxide and TBA values for the systems consisting of butter containing BHT, cumin and thyme oils. The results show that the changes in the peroxide values during the first 15 days were very low in all cases. This means that very little autooxidation had taken place. On the eighteenth day of the storage, the peroxide values were remarkably increased, and the autooxidation process commmenced. Concerning TBA values, no obvious change occurred in any cases throughout the entire experimental period. This would indicate that the secondary products, such as aldehydes and ketones, had not yet been formed.

It is worth mentioning that the statistical analysis indicated that there were significant differences between TBA values and various storage periods for each system under study. However, the differences in TBA values occurred within the second decimal points. In our experience these changes are meaningless.

Table 2 and Figure 1 show the acid values and Δ-acid values for the systems under study. Without addition of BHT or essential oils (control), the acid value of butter gradually increased with the storage time. The acid values for butter containing BHT were significantly lower than that of the control. The addition of cumin and thyme oils at 200 ppm to butter possessed a more significant lowering effect on the acid values than did BHT. Therefore, both systems were more effective than BHT in preventing butter hydrolysis. We also observed that butter containing thyme oil had a lower Δ-acid value than butter containing cumin oil. The effectiveness of added materials in protecting butter, based on their abilities to slow down the acid value rise, followed this sequence: thyme > cumin > BHT > control.

Table 3 illustrates the lipase activity of all systems under study determined at the end of the experiment.

TABLE 1

Influence of BHT and Some Essential Oils on the Peroxide (PV) and TBA Values of Butter Stored at Room Temperature

Storage period	Butter (control)				Butter + BHT (200 ppm)				Butter + Cumin oil (200 ppm)				Butter + Thyme oil (200 ppm)			
(day)	PV	Δ-PV	TBA	ΔTBA	PV	Δ-PV	TBA	ΔTBA	PV	Δ-PV	TBA	ΔTBA	PV	Δ-PV	TBA	ΔTBA
0	2.3	0.0	0.01	0.00	2.2	0.0	0.02	0.00	2.2	0.0	0.02	0.00	2.5	0.0	0.01	0.00
3	2.5	0.2	0.02	0.01	2.3	0.1	0.03	0.01	2.2	0.0	0.03	0.01	2.7	0.2	0.03	0.02
6	2.5	0.2	0.02	0.01	2.6	0.4	0.03	0.01	2.4	0.2	0.03	0.01	2.7	0.2	0.03	0.02
9	2.4	0.1	0.02	0.01	2.8	0.6	0.03	0.01	2.8	0.6	0.04	0.02	2.8	0.3	0.05	0.04
12	2.5	0.2	0.06	0.05	2.9	0.7	0.06	0.04	3.2	1.0	0.06	0.04	3.1	0.6	0.05	0.04
15	3.4	1.1	0.06	0.05	3.1	0.9	0.07	0.05	3.8	1.6	0.04	0.02	3.4	0.9	0.07	0.06
18	5.9	3.6	0.07	0.06	6.0	3.8	0.08	0.06	7.1	4.9	0.05	0.03	5.5	3.0	0.05	0.04

Peroxide value is expressed as milliequiv. peroxide/kg fat.

TBA value refers to the absorbance of the coloured product formed at 532 nm.

L.S.D. (0.05) values for Δ-P.V. between treatments and various experimental periods within each treatment were 0.081 and 0.192, respectively.

L.S.D. (0.05) values for Δ-TBA-test between treatments and various experimental periods within each treatment were 0.0033 and 0.007, respectively.

TABLE 2

Effect of BHT and Some Essential Oils on the Acid values (A.V.) of Butter Stored at Room Temperature

Storage period	Butter (control)		Butter + BHT (200 ppm)		Butter + Cumin oil (200 ppm)		Butter + Thyme oil (200 ppm)	
(day)	A.V.	Δ-A.V.	A.V.	Δ-A.V.	A.V.	Δ-A.V.	A.V.	Δ-A.V.
0	0.5	0.0	0.7	0.0	0.5	0.0	0.9	0.0
3	1.4	0.9	1.5	0.8	1.3	0.8	1.4	0.5
6	2.5	2.0	2.4	1.7	1.6	1.1	1.5	0.6
9	4.0	3.5	3.2	2.5	2.2	1.7	2.0	1.1
12	4.9	4.4	3.9	3.2	3.0	2.5	3.0	2.1
15	6.3	5.8	4.5	3.8	3.8	3.3	4.0	3.1
18	7.4	6.9	5.6	4.9	4.1	3.6	4.6	3.7

Acid value is expressed as milligrams of KOH required to neutralize 1 g fat.

L.S.D. (0.05) values for Δ-A.V. between treatments and various experimental periods within each treatment were 0.131 and 0.351, respectively.

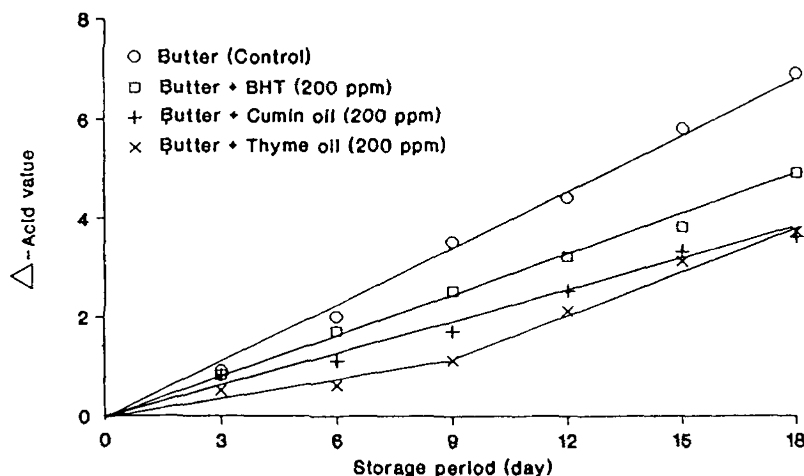

FIG. 1. Changes in the acid values of butter stored at room temperature without and with added BHT and essential oils.

Their effectiveness to inhibit the lipolytic activity in the butter can be ranked in the following order: cumin = thyme ≥ BHT > control. Statistical analysis revealed that BHT and thyme oil added to butter had the same lowering effect on lipase activity. However, the values for lipase activity show that cumin and thyme oils act as a more superior preservative for butter than BHT. Table 3 lists the total bacterial and lipolytic bacterial counts measured at the end of the storage period. It is obvious that the added materials to butter significantly lowered both total bacteria and lipolytic bacterial counts. However, the degree of effectiveness was largely dependent upon added materials. For instance, the systems containing cumin and thyme oils were more effective than BHT in lowering the total and lipolytic bacterial counts. Statistical analysis revealed that the trend towards decreasing the lipolytic bacterial counts was in accordance with the trend for lipase activity.

The volatile substances of these essential oils were qualitatively and quantitatively determined by gas-liquid chromatography (Table 4). The most abundant compounds in cumin and thyme oils were cumin aldehyde (55.7%) and thymol (42.7%), respectively. It seems that there is a relationship between inhibitory effect on the growth of microorganisms and the chemical composition of the tested essential oils. Generally, the extent of the inhibitory effect of the oils can be attributed to the presence of an aromatic nucleus containing a polar functional group. The wide spread use of phenols and related compounds as disinfectants is well established. Thymol oil had a higher inhibitory action than cumin oil, which might be due to the presence of phenolic OH groups. It is well known that the OH group is much more reactive and easily forms hydrogen bonds with the active sites of the hydrolytic enzymes.

The changes in peroxide values and TBA values for all systems during the various experimental periods were small compared to the changes in the acid values. Hence, these results indicate that the main cause of butter spoilage is hydrolytic rancidity and not oxidative rancidity. The essential oils studied showed a more powerful antihydrolytic effect than BHT. The essential oils can be obtained from materials which are widely cultivated, inexpensive and safe. Therefore, the authors recommended the essential oils derived from thyme and cumin should be used to extend the shelf life of butter.

TABLE 3

Influence of BHT and Some Essential Oils on the Lipase Activity and Bacterial Counts in Butter at the Eighteenth Day of Storge

Lipid system	Lipase activity	Total bacterial count × 10^4	Lipolytic bacterial count × 10^3
Butter (control)	0.61[c]	280 [a]	418.3[c]
Butter + BHT (200 ppm)	0.45[b]	180.5[b]	207.0[b]
Butter + Cumin oil (200 ppm)	0.32[a]	57.0[c]	153.6[a]
Butter + Thyme oil (200 ppm)	0.39[a,b]	104.5[d]	156.0[a]

L.S.D. (0.05) values for lipase activity, total bacterial counts and lipolytic bacterial counts were 0.11, 37.53 and 45.41, respectively.
[a,b,c,d]The values within a column followed by the same letter are not significantly different by L.S.D. test.

TABLE 4

Chemical Composition of Cumin and Thyme Essential Oils

	Cumin oil		Thyme oil	
Component	RRT[a]	%	RRT[a]	%
α-Pinene	0.07	0.3	0.08	1.1
β-Pinene	0.11	20.6	0.10	0.3
Camaphene	0.17	0.4	—	—
Limonene	0.24	5.4	0.20	0.3
γ-Terpinene	0.28	0.2	0.13	0.1
Terpinolene	0.37	12.0	—	—
Phelleridrene	—	—	0.17	1.5
p-Cymene	0.34	4.0	0.27	36.0
Caryophyllene	0.78	0.6	—	—
Thymol	—	—	1.00	42.7
Thujone	—	—	0.41	0.2
Cumin aldehyde	1.00	55.7	—	—
Borneol	—	—	0.52	0.7
Linalyl acetate	—	—	0.19	1.0
Terpinyl acetate	—	—	0.13	0.1
Unidentified compounds	—	0.8	—	16.0

[a]RRT refers to the relative retention time for the major compound for each essential oil which is given a value of 1.00.

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