

TOCOPHEROLS: THEIR ROLE IN STABILIZING ANHYDROUS BUTTER-FAT (*Ghee*) AND BUTTER COOKIES

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ABSTRACT

Two different concentrations of mixed Tocopherols known commercially as Covi-OxT50 (Covi 50) and Covi-OxT70 (Covi 70) together with a single concentration of a synthetic antioxidant known as Tenox 25 (T25) were added to butter and anhydrous butter fat (ABF) *Ghee*. Butter cookies made from the aforementioned butter were prepared and stored at tropical room temperature conditions (37-45°C and 95% RH) for 4 months. Chemical and sensory tests were performed for quality testing. The oxidative stability of ABF was performed by determining the conjugated dienes (CD), acid value (AV), free fatty acids (FFA) and peroxide value (PV) at regular intervals (2 weeks) under accelerated storage conditions (63°C for 6 weeks).

Results of the oxidative rancidity indicated that the addition of Covi 70 and T 25 to the ABF significantly ($P<0.05$) retarded the oxidative rancidity as measured by the aforementioned parameters. No significant effects were detected in most of the tests between Covi 70 and T25 which emphasize the outstanding effect of Covi 70 in retarding the onset of rancidity and stabilizing the ABF under the experimental conditions.

Results of the chemical analysis indicated the presence of a significant ($P<0.05$) drop in both the moisture content and the pH values and a significant rise ($P<0.05$) in the TBA values of the stored cookies as a function of time. Both Covi 50 and Covi 70 exhibited a significant ($P<0.05$) effect in maintaining the aforementioned parameters as compared to the control and the synthetic antioxidant. The sensory evaluation results showed that higher scores for the taste, odour and overall acceptance were given to the Covi 70- containing cookies as compared to the control and the other added antioxidants.

Keywords : mixed tocopherols, synthetic antioxidants, anhydrous butter fat, oxidative rancidity, storage.

INTRODUCTION

Anhydrous butter fat (ABF) is produced at home and cottage levels in many Middle Eastern countries. A number of factors thus contribute to the keeping quality of ABF, which include: the source and history of the butter before processing, the method of manufacture and the storage conditions (Singh *et al.*, 1979, Basson, 1981, Hamzawi, 1990).

Oxidative changes of butter fat are one of the most important factors limiting the keeping quality of fat-rich dairy products and affects such products in numerous ways. Most noticeable are loss of flavour and aroma and subsequent development of rancidity (IFI, 1992, Shahidi, 1996).

It is often possible to add synthetic antioxidants to milk fat to improve its oxidative stability during storage. Both butylated hydroxyanisole (BHA) and butylated hydroxyt-

oluene (BHT) are still the most widely used antioxidants. Their relatively low prices may restrain the use of naturally derived antioxidants (IFI, 1992, Loliger & Wille, 1993). The use of natural antioxidants has received special attention because of the worldwide trend to avoid or reduce the use of synthetic food additives. These synthetic compounds have been considered potentially toxic if consumed routinely (Frankel, 1996, Auroma, 1998). The most important natural antioxidants are tocopherols, they are the most widely distributed and most extensively tested for food use (Buck & Edwards, 1997, Neff *et al.*, 2003).

Tocopherols claim about 50% of the Japanese market and 2% of the US antioxidant market (IFI, 1992). Tocopherols are effective antioxidants in most food products deficient in natural antioxidants such as animal fat, waxes and butter fat (Giese, 1996, Berry, 2003). Alpha (α) tocopherol has considerable

antioxidant properties but less than gamma (γ) and delta (δ) tocopherols (Buck & Edwards, 1997, Berry, 2004).

The antioxidative effects of tocopherols and ascorbyl palmitate (AP) were investigated in various fats and oils. Results showed that a distinct antioxidative action was obtained and remained stable in lard at tocopherol concentrations up to 2500 ppm and decreased above this concentration. Increased stability of the fat was also observed due to high concentration of γ - and δ -isomers obtained from a natural soybean oil extract versus synthetic α -tocopherol. The addition of ascorbyl palmitate to tocopherols produced an additional antioxidative effect in lard depending on tocopherol concentration (Timmerman & Adams, 1989). Meanwhile, Hamzawi (1990) studied the combined antioxidative effect of phospholipids and α -tocopherol on the stability of butter fat. Results indicated that the addition of phospholipids to butter fat increased the effectiveness of the antioxidant properties of α -tocopherol. The effectiveness of some natural antioxidants (mixed tocopherols, extract of rosemary, ascorbic acid and ascorbyl palmitate) was compared against that of four major synthetic antioxidants (BHA, BHT, TBHQ and propyl gallate) in various fats and oils. Results showed that TBHQ was highly effective in lard and was more effective in combination with BHA. Tocopherols were also effective as the other used synthetic antioxidants and showed superior permanence and resistance to volatility compared with synthetic antioxidants in long term frying tests and during storage (IFI, 1992, Dougherty, 1993, Warner, 2003).

The objectives of this work were: (1) Study the storage stability of buffalo's ABF containing two concentrations of mixed tocopherols namely Covi-OxT50 and Covi-OxT70 versus a type of synthetic antioxidant (Tenox 25) as compared to a control during accelerated storage conditions at 63°C for 6 weeks. (2) Chemical and sensory evaluation of a type of cookie product made from butter with the aforementioned added antioxidants concentrations together with a control sample were studied. Samples were stored at tropical conditions (37-45°C and 95% relative humidity typical to Jeddah City in Saudi Arabia) for four months.

MATERIALS AND METHODS

Materials

Butter samples: Five kilograms of buffalo's butter were purchased from vendors at a farm near Cairo. The butter sample was split into two subsamples. Half was kept as a butter while the other half was thermally heated in the traditional method without added salt to transform it to anhydrous butter fat, (ABF) *ghee*. Samples were stored frozen before mixing with the antioxidants at -18°C for 2 weeks.

Antioxidants: Two types of natural tocopherols namely Covi-Ox T50 (Covi 50) and Covi-Ox T70 (Covi 70) were purchased from Henkel Corporation, IL, USA. Both are clear brownish red viscous oils containing mixed tocopherols concentrate. The following values (expressed as a percent of the total tocopherols) are typical: d- α 14%, d- β 1%, d- γ 62% and d- δ 23%. Each gram of Covi 50 contains 500mg of total tocopherols, including a minimum of 400mg of non- α -tocopherols. Each gram of Covi 70 contains 700 mg of total tocopherols, including a minimum of 560 mg of non- α -tocopherols. Both types were applied at 0.02% of the total fat content (Dougherty, 1988). A synthetic type of antioxidants namely Tenox 25 (T25) was purchased from Eastman Chemical Company, TN, USA. It is a viscous golden brown oil containing 10% butylated hydroxy toluene (BHT) and 10% tertiary butyl hydroquinone (TBHQ) and 3% citric acid. It was applied at 0.02% (200ppm) based on fat weight according to FDA regulations.

Methods

Application of antioxidants: The antioxidants used in the present study were applied to the fat according to the direct method described by Eastman (1996) by heating the butter and/or unhydrous butter fat to 60°C (140°F) and agitating sufficiently to dissolve the antioxidant. The gentle agitation was continued for an additional 20 min. to ensure uniform distribution.

Cookies preparation: The following ingredients were used in the formulation of cookies as g/100g of the total ingredients: butter 29%, icing sugar 10.1%, flour 50.7%, eggs 7.6%, salt 0.3%, baking powder 0.3% and vanilla 0.1%. Cookies were prepared as described by Smith (1972). Cookie's product with the added concentrations of antioxidants

as mentioned previously were put in plastic plates and wrapped with polyethylene cover, stored for 4 months at room temperature which ranged between 37-45°C, typical to Jeddah City in Saudi Arabia, and evaluated monthly for chemical and sensory tests.

Determination of oxidative rancidity in the anhydrous butter fat (ABF): The ABF treated with the added levels of tocopherols, synthetic antioxidant and the control sample were subjected to an accelerated aging test where samples were stored separately in clear capped glass jars, put in a forced draft oven adjusted at a constant temperature of 63°C for 6 weeks. Samples were withdrawn at 2 weeks interval for chemical evaluation. Oxidative rancidity was evaluated by the measurement of conjugated dienes (CD) as described by IUPAC (1978). Absorbance was measured at wave length of 234 nm using a spectrophotometer (Ultraspec, 2000, Pharmacia Biotech, USA). The hydrolytic rancidity was evaluated by determining both the acid value (AV) and the free fatty acid (FFA) content of the ABF following the IUPAC (1979), results were expressed as g oleic acid Kg⁻¹. The peroxide value (PV) was determined according to AOCS official methods (1986) by titration with standard sodium thiosulphate solution and calculated as mEq/O₂kg sample.

Quality tests of cookies made from butter: The moisture content of the cookies was monitored monthly using an electric oven at 105±0.5°C for 2-3 hrs until constant weight (AOAC, 1984). The pH was performed in 10g sample thoroughly mixed with 90ml distilled water and the pH measured by an ATC pH meter G353, UK. The oxidative rancidity of the stored cookies was evaluated by the thiobarbituric acid number (TBA) as described by Tarladgis *et al.* (1960). The TBA number was calculated as mg malonaldehyde/kg sample.

Sensory evaluation: The cookies were evaluated for sensory attributes every one month interval for 4 months by means of ten panelists from the Department of Food and Nutrition (King Abdulaziz University, Jeddah, KSA), where an evaluation sheet was distributed for the ranking test. The attributes tested were colour, odour, taste, texture and overall acceptance. Scores were distributed on a five point scale ranging between 1 and 5 where 5 stands for maximum acceptance and

1 for maximum rejection due to negative distinct changes in one or more of the tested attributes. Tap water and salt crackers were introduced between each treatment (Moskowitz, 1985).

Statistical analysis :

The general linear model procedure (GLA) was used to perform analysis of variance (ANOVA) between time × treatment using the Statistical Analysis System (SAS, 1985). Duncan's Multiple Range Test was performed to determine differences among means.

RESULTS AND DISCUSSION

Results presented in Table (1) showed the presence of a significant (P<0.05) increase in the values of the conjugated dienes (CD) of the ABF stored at accelerated temperature conditions as a function of time which was significantly obvious at the fourth week of storage. The application of both natural and synthetic antioxidants significantly (P<0.05) decreased the CD values as compared to the control. As a matter of fact, Covi 50 successfully achieved the lowest value in this respect as compared to the control and was not significantly different from the other antioxidants used in the present study. It was well documented that the CD increase is proportional to the uptake of oxygen and the formation of peroxides in the early stages of oxidation (Gray, 1978).

It was also clear from Table (1) that both the acid value and the FFA values showed a significant increase (P<0.05) as a function of time. Both Covi 70 and T 25 had a significant effect in reducing hydrolysis as compared to the control and Covi 50, but were not significantly different from each other emphasizing the role of the natural antioxidant Covi 70 in this respect. These results are in accordance to Thakar *et al.* (1984), Iskandar *et al.* (1985) and Warner (2003) who reported the effect of some natural plant antioxidants in controlling hydrolytic rancidity in cow's ABF during storage.

Data in Table (1) also show the peroxide values of ABF during the accelerated storage conditions. Results showed a significant gradual rise in PV's with time reaching its maximum (18.82 mEqO₂/kg) at the end of the storage period. The addition of both natural and synthetic antioxidants greatly restricted the development

Table 1: Effect of different added levels of natural and synthetic antioxidants on the stability of anhydrous butter fat stored at 63°C for 6 weeks

Tested parameter	Storage time (weeks)	Treatments				Mean (\bar{x}) time
		Control	Covi 50	Covi 70	T25	
CD	0	4.00	3.96	4.05	3.98	3.99 ^a
	2	3.90	4.08	4.01	4.09	4.02 ^a
	4	4.64	4.16	4.32	4.27	4.35 ^b
	6	5.33	4.43	4.73	5.17	4.91 ^c
	\bar{x} treatment	4.46 ^a	4.15 ^b	4.27 ^{ab}	4.37 ^{ab}	
FFA	0	0.58	0.48	0.54	0.51	0.52 ^a
	2	0.78	0.55	0.51	0.52	0.59 ^b
	4	0.96	0.55	0.53	0.53	0.64 ^c
	6	1.42	0.86	0.59	0.56	0.85 ^d
	\bar{x} treatment	0.93 ^a	0.60 ^b	0.54 ^c	0.53 ^c	
AV	0	1.15	0.95	1.08	1.01	1.04 ^a
	2	1.53	1.10	1.04	1.03	1.17 ^b
	4	1.92	1.10	1.06	1.06	1.28 ^c
	6	2.84	1.70	1.25	1.23	1.75 ^d
	\bar{x} treatment	1.86 ^a	1.21 ^b	1.10 ^c	1.08 ^c	
PV	0	3.20	3.60	3.00	2.60	3.10 ^a
	2	4.26	4.80	4.13	3.66	4.21 ^b
	4	9.66	8.36	8.43	3.73	7.54 ^c
	6	28.60	21.83	10.36	14.50	18.82 ^d
	\bar{x} treatment	11.43 ^a	9.64 ^b	6.48 ^c	6.12 ^c	

CD : Conjugated Dienes – absorbance at 234 nm.

FFA : Free Fatty Acids (g oleic acid Kg⁻¹).

AV : Acid Value (g oleic acid Kg⁻¹).

PV : Peroxide Value (mEqO₂/kg sample).

Means in a column not sharing the same superscript are significantly different at P<0.05.

of PV's as compared to the control. Both Covi 70 and T 25 exhibited the best capability in preventing the onset of oxidation and prolonging the induction period in the stored ABF and were not differently significant from each other, thus emphasizing the effect of the natural antioxidant Covi 70 in this respect. The previous results were in accordance to Eastman (1993), Giese (1996) and Berry (2003) who confirmed that tocopherols are more thermally stable and may be used in high concentration in stabilizing food systems that are deficient in natural antioxidants and subject to high temperature whether during frying and/or baking. It was also mentioned by Loliger (1991) that after 2hr of heat exposure more than 50% of the tocopherols still exist in the fatty material in comparison to 10% only in case of using BHA. He also mentioned that even adding 50 ppm tocopherols to lard equally

stabilized lard when adding to it 200 ppm synthetic antioxidant.

Data in Table (2) showed the presence of a significant (P<0.05) drop in the moisture content of the cookies as a function of time. The mean moisture content at the beginning of the storage time was 8.91% and lowered to 6.05% at the end of the storage period. This drop in moisture may be explained due to the equilibrium relative humidity (ERH) of bakery products which make them liable to loss of moisture than gaining it (Almasi, 1979). The addition of natural and synthetic antioxidants greatly maintained the stability of the moisture in the cookies containing Covi 70 and T 25 as compared to the control.

Data in Table (2) also indicated the presence of a significant (P<0.05) drop in the pH

Table 2: Chemical evaluation of butter cookies with different added levels of natural and synthetic antioxidants stored at tropical conditions for 4 months

Tested parameters	Storage time (months)	Treatments				Mean (\bar{x}) time
		Control	Covi 50	Covi 70	T25	
Moisture	0	7.51	8.08	10.44	9.62	8.91 ^a
	1	7.08	8.05	8.06	9.42	8.15 ^b
	2	7.05	7.85	7.93	8.35	7.80 ^b
	3	7.00	6.63	6.59	6.24	6.61 ^c
	4	6.13	5.75	6.40	5.94	6.05 ^c
	\bar{x} treatment	6.95 ^a	7.27 ^b	7.88 ^c	7.91 ^c	
pH	0	5.48	5.53	5.50	5.54	5.51 ^a
	1	4.73	4.94	5.38	5.20	5.06 ^b
	2	6.98	4.35	5.26	4.85	4.61 ^c
	3	3.23	3.76	5.14	4.51	4.16 ^d
	4	2.48	3.70	5.02	4.16	3.84 ^c
	\bar{x} treatment	3.98 ^a	4.45 ^b	5.26 ^c	4.58 ^d	
TBA*	0	0.179	0.125	0.191	0.177	0.168 ^a
	1	0.424	0.183	0.216	0.250	0.268 ^b
	2	0.448	0.327	0.267	0.280	0.330 ^c
	3	0.541	0.242	0.300	0.325	0.356 ^d
	4	0.750	0.247	0.325	0.585	0.476 ^c
	\bar{x} treatment	0.468 ^a	0.225 ^b	0.259 ^c	0.327 ^d	

* TBA : Thiobarbituric acid expressed as mg malonaldehyde / kg sample.

Means in a column not sharing the same superscript are significantly different at $P < 0.05$.

of cookies as a function of time. The pH value of the cookies at the beginning of the experiment was 5.51 then dropped to 3.84 at the end of the storage period indicating the progression of hydrolysis which is more liable in low moisture products (Allen & Hamilton, 1989). The application of the added antioxidants significantly ($P < 0.05$) protected the drop in the pH of cookies where the natural antioxidant Covi 70 was more pronounced in this respect as compared to the control and the other antioxidants used in the present study.

The TBA number of the stored cookies (Table 2) exhibited a significant rise ($P < 0.05$) with the elapse of time. The application of both natural and synthetic antioxidants greatly minimized the increase in TBA number where Covi 50 was the most effective in this respect as compared to the control and the synthetic antioxidant (T 25). It is worth mentioning that high temperature, temperature fluctuations and high relative humidity cause the movement and migration of fats

causing rancidity problems (Manley, 1996). It was also emphasized by Acker (1962) and Amr (1991) that the low water content of some products may initiate the free-radical activation.

As it can be seen from Table (3), the addition of different concentrations of natural and synthetic antioxidants significantly ($P < 0.05$) enhanced the odour and taste attributes of the stored cookies as they were highly accepted by the panelists up to the end of the storage time as compared to the control samples. As a matter of fact, cookies containing Covi 70 were highly scored for odour, taste and the overall acceptance as compared to the control and the other added antioxidants. The addition of the different antioxidants had no significant effects on the colour and texture of the cookies. It was confirmed by Hamzawi (1990) and Amr (1991) that oxidation affects negatively the quality of food products in several manner, the most distinct of all is the loss of flavour and appearance of rancidity. These facts also attributed to the nature of

Table 3: Effect of different added levels of natural and synthetic antioxidants on the sensory attributes of butter cookies stored at tropical conditions for 4 months

Sensory attribute	Storage time (months)	Treatments				Mean (\bar{x}) time
		Control	Covi 50	Covi 70	T25	
Colour	0	4.30	3.75	4.30	4.30	4.16 ^a
	1	4.40	4.80	4.50	4.20	4.47 ^a
	2	3.90	3.70	3.40	4.20	3.80 ^a
	3	3.90	3.40	3.50	3.40	3.55 ^a
	4	3.30	3.60	4.30	3.50	4.42 ^a
	\bar{x} treatment		3.96 ^a	3.85 ^a	4.00 ^a	4.52 ^a
Odour	0	3.00	3.70	3.90	4.10	3.67 ^a
	1	3.20	4.10	4.10	3.80	3.80 ^a
	2	3.70	3.90	3.70	3.70	3.75 ^a
	3	1.80	2.85	3.10	2.80	2.63 ^b
	4	1.95	3.20	3.60	3.40	3.03 ^b
	\bar{x} treatment		2.73 ^a	3.55 ^b	3.68 ^b	3.56 ^b
Taste	0	3.40	3.70	4.10	4.50	3.92 ^a
	1	3.50	4.20	4.10	3.80	3.90 ^a
	2	3.20	4.00	3.50	3.60	3.57 ^a
	3	2.00	3.20	3.00	2.90	2.77 ^b
	4	1.95	1.95	3.60	2.90	2.60 ^b
	\bar{x} treatment		2.81 ^a	3.41 ^b	3.66 ^b	3.54 ^b
Texture	0	4.10	4.00	4.20	4.30	4.15 ^a
	1	4.00	3.70	4.10	3.80	3.90 ^{ab}
	2	3.70	4.20	4.0	4.10	4.00 ^a
	3	3.30	3.80	3.60	3.30	3.50 ^b
	4	3.50	3.50	4.20	3.70	3.72 ^{ab}
	\bar{x} treatment		3.72 ^a	3.84 ^a	4.02 ^a	3.84 ^a
Overall acceptance	0	3.50	3.90	4.10	4.30	3.95 ^a
	1	3.20	4.00	4.05	3.80	3.76 ^a
	2	3.10	3.90	3.60	4.00	3.65 ^a
	3	1.60	3.30	3.10	2.80	2.70 ^b
	4	2.00	3.00	3.70	2.90	2.90 ^b
	\bar{x} treatment		2.68 ^a	3.62 ^b	3.71 ^b	3.56 ^b

Means in a column not sharing the same superscript are significantly different at $P < 0.05$.

fatty acids comprised in the milk butter where butyric acid is the most abundant together with those fatty acids of low molecular weight and in less manner those unsaturated ones. Such a variety of fatty acids is liable to hydrolysis and autoxidation under fluctuated storage conditions causing the appearance of unacceptable flavours usually described as cheesy-off flavour. Thus, the aforementioned results of the sensory evaluation showed that the application of all types of antioxidants greatly prevented the appearance of off-flavours where the nat-

ural antioxidant Covi 70 showed a pronounced role in this respect.

It may be concluded that the highly concentrated natural antioxidant Covi 70 was more pronounced in stabilizing the fat material experimented under the accelerated high temperature conditions and in the cookies stored at room temperature at tropical conditions. Covi 70 was equally effective in most of the cases as the powerful synthetic antioxidant Tenox 25 emphasizing the capability of Covi 70 in stabilizing the onset of rancidity in both ABF and butter-cookies.

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التوكوفيرولات: دورها في ثبات الزبد منزوع الماء (السمن) وأقراص الكعك المصنعة من الزبد (كوكيز)

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تم إضافة تركيزات مختلفة من خليط التوكوفيرولات المعروفة تجارياً باسم (Covi 50) (Covi-OxT50) والـ (Covi 70) (Covi-OxT70) ونوع من مضادات الأكسدة الصناعية المعروفة تجارياً باسم (T 25) (Tenox 25) إلى كل من الزبد والزبد منزوع الماء (السمن). تم تحضير كوكيز من الزبد المعامل بالتوكوفيرولات المختلفة حيث خزن على درجة حرارة الغرفة تحت ظروف استوائية (37-45°م) لمدة 4 أشهر. كما تم إجراء الاختبارات الكيماوية والحسية لتقييم جودة المنتج تحت الظروف السابقة.

تم إجراء اختبارات الثبات التأكسدي على السمن وذلك بتقدير كل من قيمة الروابط المزدوجة المتبادلة (CD) وقيمة الحامض (AV) والأحماض الدهنية الحرة (FFA) وقيمة البيروكسيد (PV) وذلك بطريقة منتظمة كل أسبوعين تحت ظروف تجريبية معجلة من درجة الحرارة (63°م لمدة 6 أسابيع).

أظهرت نتائج التزنخ التأكسدي أن إضافة كل من الـ (Covi 70) والـ (T 25) للسمن قد أدى إلى تحفيز معنوي ($P < 0.05$) في ثبات الدهن تحت الظروف المعجلة للتجربة. كما لوحظ وجود فروق غير معنوية بين كل من تأثير الـ (Covi 70) والـ (T 25) في معظم الاختبارات مما يؤكد التأثير المحفز الإيجابي للـ (Covi 70) كمضاد أكسدة طبيعي في تأخير ظروف التزنخ وتثبيت السمن تحت ظروف التجربة.

كما أسفرت نتائج التحليل الكيماوي عن وجود انخفاض معنوي ($P < 0.05$) في كل من المحتوى الرطوبي وقيمة الـ pH للكوكيز المخزن مقابل وجود ارتفاع معنوي ($P < 0.05$) لقيم التزنخ التأكسدي مقاساً باختبار حامض الثيوباربيتوريك (TBA) مع مرور وقت التخزين. كما أظهرت النتائج وجود ثبات معنوي ($P < 0.05$) لتأثير كل من الـ (Covi 50) والـ (Covi 70) على الاختبارات الكيماوية السابقة مقارنةً بالكонтроل وبمضاد الأكسدة الصناعي. كما لوحظ من نتائج الاختبار الحسي أن الكعك المحتوي على الـ (Covi 70) لاقى أعلى التقديرات من قبل المحكمين للطعم والرائحة والتقبل العام مقارنةً بالكонтроل ومضادات الأكسدة الأخرى المستخدمة في الدراسة.