Journal of Medicinally Active Plants

Volume 1 | Issue 2

June 2012

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Recommended Citation
Nahm, Hee Seung, Hector Rodolfo Juliani, James E. Simon. 2012. "Effects of Selected Synthetic and Natural Antioxidants on the Oxidative Stability of Shea Butter (Vitellaria paradoxa subsp. paradoxa)," *Journal of Medicinally Active Plants* 1(2):69-75.
DOI: https://doi.org/10.7275/R5BR8Q4R
Available at: http://scholarworks.umass.edu/jmap/vol1/iss2/5

Effects of Selected Synthetic and Natural Antioxidants on the Oxidative Stability of Shea Butter (*Vitellaria paradoxa* subsp. *paradoxa*)

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Manuscript received: December 15, 2011

Keywords: BHT, gallic acid, linoleic acid, natural preservatives, plant fat, oleic acid, oxidation, rosmarinic acid.

ABSTRACT

Shea butter is a plant fat extracted from kernels of shea nuts, the seeds of shea trees (*Vitellaria paradoxa*). The extracted fat, which has a long history of use in sub-Saharan Africa for medicinal, culinary, and other applications, serves as cocoa butter equivalents (CBEs) in the manufacture of chocolate and is an ingredient for cosmetics in the international market. Since shea butter contains relatively high levels of unsaturated fatty acids (more than 50 %), oxidation can occur during extraction and during post-harvest processing and storage. This study investigated the protective effects of synthetic butylated hydroxytoluene (BHT) and selected natural antioxidants (rosmarinic acid and gallic acid) on shea butter against oxidation. Each antioxidant (0.02%) was added to shea butter and the mixtures were placed at 90°C for 0, 72, and 144 h to accelerate oxidation. Measures of oxidation indicated the shea butter was protected from oxidation by addition of the antioxidants. The addition of antioxidants to shea butter produced no significant changes in the parameters used to measure oxidation (e.g. peroxide values, conjugated dienes and TBARS), as well as in the levels of major fatty acids. The natural antioxidants were almost as effective as the synthetic antioxidant.

INTRODUCTION

Shea butter, the plant fat extracted from shea nuts, the seeds of shea trees (*Vitellaria paradoxa*) that belongs to the Sapotaceae family. Shea trees grow wild across a belt of savanna extending from Senegal, Mali, Côte d'Ivoire, Burkina Faso, Togo, Ghana, Benin, Nigeria, Niger, Cameroon to further east in Uganda, Sudan, and Ethiopia (Chalfin, 2004; Goreja, 2004). Shea butter, which has served as a versatile fat to many Africans, is used in African cuisine as nutritional edible oil and in African traditional medicine as a treatment for rheumatism, nostril inflammation, leprosy, for soothing and healing following circumcision and preventing stretch marks during pregnancy (Tella, 1979; Chalfin, 2004; Goreja, 2004; Olaniyan and Oje 2007). Shea butter has been used in international markets as cocoa butter equivalents (CBEs) for the manufacture of chocolate, especially Europe due to shea butter's similar physical and chemical properties (Lipp and Anklam, 1998; Alander, 2004). More recently, shea butter market is expanding in cosmetics and personal care industries due to excellent emollient activity and medicinal properties, such as anti-inflammatory and antioxidant activities (Alander and Andersson, 2002; Alander, 2004; Maranz and Wiesman, 2004; Masters *et al*., 2004). Shea butter generally consists of more than 90% triglycerides and a minor, though significant, unsaponifiable fraction. The triglyceride fraction is

comprised of more than 50% of unsaturated fatty acids, constituents that are prone to oxidation (Alander, 2004; Di Vincenzo *et al*., 2005). Shea butter is also known to contain relatively large amounts of unsaponifiables (4-11 %) and tocopherols that are known to provide stability to oils and fats (Itoh *et al*, 1974; Itoh *et al*., 1980; Hamilton and Rossell, 1986; Lipp and Anklam, 1998; Alander, 2004). Due to the considerable amounts of unsaturated fatty acids in shea butter, however, traditional extraction procedures that involve boiling or improper processing or storage can cause oxidative degradation of shea butter, leading to inconsistent quality and limited shelf-life (Masters *et al*., 2004; Lovett, 2004; Moharram *et al*., 2006). Such lipid oxidation degrades edible oils, producing sensory and chemical changes along with lowered nutritional values (Velasco and Dobarganes, 2002). To prevent the oxidation of plant-derived oils, antioxidants are added to the plant oils and fats.

During the past few decades, the use of natural antioxidants and plant extracts have received increased interest due to the concerns about possible ill health effects generated by the use of synthetic antioxidants (Abramovic and Abram, 2006; Kowalski, 2007; Azizkhani and Zandi, 2009). The protective effects of antioxidants on products are generally determined by measuring several variables, including the induction period under normal storage conditions and oxidation accelerating conditions.

Research reports on the protective effects of antioxidants on shea butter against oxidation are scarce in the literature. The effectiveness of various antioxidants are generally determined under several conditions that include the induction period, normal storage conditions, and oxidation-accelerating conditions, such as high temperatures and airflow (Judde *et al*., 2003; Kowalski, 2007; Jennings and Akoh, 2009). The usual parameters measured in determining the effectiveness of antioxidants include sensory characteristics, volatile headspace oxidation products, peroxide values, conjugated dienes and trienes, p-anisidine values, thiobarbituric acid reactive substances (TBARS), and fatty acid composition (Pokorny *et al*.,2001; Judde *et al*., 2003; Abramovic and Abram, 2006; Azizkhani and Zandim, 2009; Kowalski,2007; Bouaziz et al. 2008; Jennings, et al., 2009).

The objective of this study was to assess the effectiveness of synthetic butylated hydroxyltoluene (BHT), and natural antioxidants (rosmarinic acid and gallic acid) on the oxidative stability of shea butter. Such information would provide new approaches to maintain quality and extend shelf-life of shea butter.

MATERIALS AND METHODS

Plant material. A shea butter sample produced from the nuts of *Vitellaria paradoxa*, subspecies *paradoxa* in August 2009, was graciously provided by The Pure Company (Buipe, Northern Ghana). Before being used in experimental studies, the shea butter was filtered through P8 coarse filter paper (Fisher Scientific, Pittsburgh, PA) at 45-50°C in an oven to remove foreign materials. The filtered shea butter was stored at 4°C in a refrigerator until used in experimental trials.

Chemicals and reagents. The chemicals, 1-butanol, 95 % heptadecanoic acid, potassium dichromate, potassium hydroxide, potassium iodide, sodium sulfate, starch, and ACS grade sodium carbonate, were purchased from Acros Organics (Morris Plains, NJ). Sulfuric acid, HPLC grade chloroform, hexane, isooctane, methanol, and ACS grade glacial acetic acid and hydrochloric acid were purchased from Fisher Scientific (Fairlawn, NJ). Iodine was purchased from Mallinckrodt Chemical, Inc., (Hazelwood, MO), 2-thiobarbituric acid, ammonium chloride, sodium thiosulfate, butylated hydroxyltoluene (BHT), gallic acid, rosmarinic acid were purchased from Sigma Chemical (St. Louis, MO).

Experimental. For the oxidative stability studies, the antioxidants BHT, rosmarinic acid, and gallic acid (0.02% each) were added to separate samples of shea butter, based on the concentration of antioxidant used by Kowalski (2007) and represent the maximum added antioxidant levels used in foods (0.02%) (Pokorny *et al*., 2001). Antioxidants (0.02 g) were dissolved in 10 mL of ethanol and then added to 100 g of shea butter following the procedure of Kowalski (2007). The ethanol was then evaporated at 45^{o}C for 15 min to avoid over-oxidation and to enable the antioxidants to thoroughly mix with the

sample. Control samples contained ethanol without the addition of antioxidants.

The oxidation process was accelerated using the procedure of Kowalski (2007) in which all the experimental shea butter samples were placed in an oven (internal volume 15 L) at 90°C with a stream of air (flow 1 Lh^{-1}). Subsamples of the shea butter were removed from the oven at 0, 72, and 144 h and stored at 4°C in a refrigerator until tested for oxidation. Peroxide values, widely used to measure the primary oxidative deterioration of oils and fats (Pokorny et al., 2001), were determined on 3 g samples according to the IUPAC 2.501 procedures (Paquot and Hautfenne, 1987).

The formation of conjugated dienes was measured according to the procedure of Abuzaytoun and Shahidi (2006). Subsamples of the shea butter (20-40 mg) were placed in 25 mL of isooctane and thoroughly mixed for 5 min, and then the absorbance of the solution was determined at 234 nm using 10 mm Hellma quartz cell in a Hewlett-Packard 8453 diode array spectrophotometer (Agilent, Wilmington, DE) against the blank (pure isooctane). Conjugated diene (CD) content was calculated as: CD = A/(C x d), where A = absorbance, C = concentration of the solution (g/100 mL, and d is the length (cm) of the cell.

TBARS were determined according to procedures outlined by Abuzaytoun and Shahidi (2006). Subsamples (50-200 mg) of shea butter were mixed with 25 mL of 1-butanol, warmed in an oven at 45 °C for 3 min to avoid the solidification of the shea butter, and then sonicated for 5 min. A 5 mL aliquot of the mixture was transferred to a dry test tube, 5 mL of 2-TBA reagent (0.2 g of 2-TBA/100 mL of 1-butanol) was added to the same tube, and the solution was well mixed and then heated at 100 °C for 2 h in a water bath. The intensity of the colored complex was measured at 532 nm with a spectrophotometer (HP8453) against 1-butanol as the blank. The TBARS value was calculated as: TBARS (μmol/g) = 0.415B, where B is the absorbance reading at 532 nm and 0.415 is a constant determined from a standard using 1,1,3,3-tetramethoxypropane as a precursor of malonaldehyde (Abuzaytoun *et al*., 2006).

Changes in the fatty acid content were determined by gas chromatography (GC), using heptadecanoic acid as internal standard. The fatty acids (5 g) were transesterified using methanol in an acid-catalyzed reaction. A total of 1 mL of a 1% hexane solution (w/v) was added to the sample and the mixture was refluxed with 5 mL of a 0.5 N potassium hydroxide methanolic solution for 5 min. The mixture was then refluxed with 15 mL of ammonium chloride and sulfuric acid in methanol solution for 3 min. After the final reflux, the mixture was cooled, 10 mL of hexane was added, and a 1.5 mL solvent fraction containing fatty acid methyl esters (FAMEs) was recovered using a separatory funnel. The solvent fraction was dried over sodium sulfate and centrifuged at 9,500 g for 5 min and the dried supernatant containing the FAMEs was subjected to GC analysis using an Econo-Cap^{TM}EC^{TM}-WAX capillary column (length 30 m, internal diameter 0.25 mm, phase polyethyleneglycol, film 0.25 μm, Alltech, Deerfield, IL) in a gas chromatograph (Agilent 6890 series, Wilmington, DE) equipped with a flame ionization detector (FID) and an automated injector. Samples were injected at an initial oven temperature of 60°C, held for 1 min, and the column temperature was then increased at a rate of 10°C min^{-1} to 200°C. The injector and the FID temperature were set to 220°C. Helium was used as the carrier gas. The levels of the major fatty acids, palmitic (16:0), stearic (18:0), oleic (18:1), and linoleic (18:2) acids, were calculated by using heptadecanoic acid (17:0) as the internal standard. Pure FAMEs standards (palmitate, stearate, oleate and linoleate) were used for peak identification.

Statistical analysis. Statistical analysis was done using GraphPad Prism 5 (GraphPad Software Inc. La Jolla, CA). A one-way analysis of variance (ANOVA) was used to determine whether the measured oxidative parameters significantly increased or decreased over time within each sample. For samples in which a significant increase in oxidation was observed at $P<0.05$, post-tests for multiple comparisons were conducted using Tukey's multiple comparison test at $P<0.05$. In addition, a two-way ANOVA with two variables, treatment and time was used to determine the protective effects of the anti-

oxidants on shea butter against oxidation and to determine which antioxidant was the most effective. When significant differences were observed at $P<0.05$, further multiple comparisons were done on all sample pairs with the Bonferroni post test at $P<0.05$. All results were presented as means ± standard error (SE).

RESULTS

The peroxide values in control shea butter sample subjected to accelerated oxidation significantly increased from 10.89 to 25.22 mEq kg^{-1} ($P<0.01$) during the first 72 h and to 354.30 mEq kg^{-1} by 144 h (Table 1). In contrast, the samples of shea butter samples containing BHT, rosmarinic acid, and gallic acid had no significant increases in peroxide values over the 144 h test period. In the gallic acid treated sample, the peroxide values decreased.

Table 1. Effects of antioxidants on oxidation peroxide values of shea butter over time.

Antioxidant[1]	Oxidation treatment time (h)		
	0	72	144
	(peroxide values in mEq/kg)[2]		
Control	10.9 ± 0.6	25.2 ± 0.7	354.3 ± 3.2
BHT	10.2 ± 0.6	17.3 ± 0.7	18.5 ± 0.5
Rosmarinic acid	10.6 ± 0.6	14.1 ± 0.2	16.0 ± 0.8
Gallic Acid	8.8 ± 0.2	5.0 ± 0.3	4.4 ± 0.3

[1]Indicated antioxidant was mixed with shea butter at 90°C; control had no antioxidant added.
[2]Means ± standard error, higher numbers indicate more oxidation.

Conjugated diene levels increased rapidly in shea butter between 72 and 144 h in the control samples with no antioxidants, but remained almost without variations in the samples treated with antioxidants (Table 2). In the control sample, the conjugated dienes were significantly increased from 2.72 to 4.52 (0-72 h) and from 4.52 to 13.08 (72-144 h). No significant changes in conjugated dienes were observed in samples containing BHT, rosmarinic acid, and gallic acid. Only a slight decrease in conjugated dienes was observed in the gallic acid samples from 3.16 to 2.95 for 0 and 72 hours, respectively ($P<0.01$). At 72 h, control CD (4.52) was significantly higher than those in the samples with BHT (3.01), rosmarinic acid (3.06), and gallic acid (2.95) at 72 ($P<0.05$). The levels of conjugated dienes observed at 144 h in samples treated with antioxidants were similar to those in samples at 72 h. No significant differences among the antioxidants were observed.

The change in TBAR levels in shea butter treated with antioxidants was insignificant compared with the control sample not treated with an antioxidant (Table 3). The TBAR level in the control increased from 0.05 to 0.13 μmol/g between 72 h and 144 h ($P<0.001$). The lack of TBAR formation in the shea butter over the 144 h was similar in all samples treated with an antioxidant. All the tested antioxidants were equally effective in reducing the formation of secondary oxidation products.

Table 2. Effects of antioxidants on the formation of conjugate dienes in lipids of shea butter over time.

Antioxidant[1]	Oxidation treatment time (h)		
	0	72	144
	(Abs 234 nm)[1]		
Control	2.7 ± 0.1[2]	4.5 ± 0.0	13.1 ± 0.0
BHT	2.6 ± 0.4	3.0 ± 0.5	3.5 ± 0.5
Rosmarinic acid	2.7 ± 0.2	3.1 ± 0.2	3.1 ± 0.5
Gallic acid	3.2 ± 0.0	3.0 ± 0.0	3.0 ± 0.1

[1]Indicated antioxidant was mixed with shea butter at 90°C; control had no antioxidant added.
[2]Means ± standard error, the higher the absorbance the higher the level of conjugated dienes formed.

Table 3. Effects of antioxidants on oxidation forming TBAR substances in shea butter over time.

Antioxidant[1]	Oxidation treatment time (h)		
	0	72	144
	(TBARS in μmol/g)[2]		
Control	0.05 ± 0.00	0.05 ± 0.00	0.13 ± 0.00
BHT	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00
Rosmarinic acid	0.06 ± 0.00	0.05 ± 0.00	0.05 ± 0.00
Gallic acid	0.05 ± 0.00	0.05 ± 0.00	0.04 ± 0.00

[1]Indicated antioxidant was mixed with shea butter at 90°C; control had no antioxidant added.
[2]Means ± standard error, higher numbers indicate formation of more TBARS.

The major saturated fatty acids in shea butter were only slightly affected by oxidation (Table 4). Except for the addition of gallic acid, the response to the addition of antioxidant compounds to the shea butter was similar to that of the control at 144 h. In the shea butter samples containing gallic acid, the levels of palmitic and stearic acids were reduced as compared with the control and the other antioxidants tested.

Unsaturated fatty acids in shea butter were

significantly reduced in the control sample and in the gallic acid sample at 144 h after initiation of accelerated oxidation (Table 5). Oleic acid was reduced by 21.4% in the control and by 14.8% in the gallic acid treatment at 144 h of accelerated oxidation. In shea samples treated with BHT and rosmarinic acid, oleic acid levels were similar throughout the oxidation. The effects of the antioxidant treatments were on linoleic acid, a polyunsaturated fatty acid, essentially similar to those observed for oleic acid.

Table 4. Effects of antioxidants on palmitic and stearic acids levels in shea butter over time.

Antioxidant[1]	Oxidation treatment time (h)		
	0	72	144
	(% Palmitic acid oxidized)[2]		
Control	2.84 ± 0.04	2.71 ± 0.21	2.72 ± 0.05
BHT	2.56 ± 0.11	3.05 ± 0.11	2.83 ± 0.07
Rosmarinic acid	2.85 ± 0.08	2.97 ± 0.10	2.77 ± 0.14
Gallic acid	2.91 ± 0.08	2.55 ± 0.08	2.42 ± 0.01
Antioxidant[1]	(% Stearic acid oxidized)[2]		
Control	34.76 ± 0.59	33.04 ± 2.37	33.34 ± 0.59
BHT	30.80 ± 1.07	37.20 ± 1.54	34.26 ± 0.96
Rosmarinic acid	33.87 ± 0.75	35.48 ± 1.50	33.03 ± 1.97
Gallic acid	35.33 ± 1.55	31.58 ± 1.01	30.45 ± 0.20

[1]Indicated antioxidant was mixed with shea butter at 90°C; control had no antioxidant added.
[2]Means ± standard error; higher percentages indicate more oxidation.

Table 5. Effects of antioxidants on oxidation of oleic and linoleic acids over time.

Antioxidant[1]	Oxidation treatment time (h)		
	0	72	144
	(% Oleic acid oxidized)[2]		
Control	34.33 ± 0.47	32.34 ± 2.39	26.98 ± 0.45
BHT	30.99 ± 1.47	37.44 ± 1.34	34.35 ± 0.93
Rosmarinic acid	34.29 ± 0.88	35.53 ± 1.54	33.50 ± 2.01
Gallic acid	35.12 ± 1.55	31.27 ± 1.00	29.92 ± 0.01
Antioxidant[1]	(% Linoleic acid oxidized)[2]		
Control	4.79 ± 0.05	4.09 ± 0.29	0.78 ± 0.02
BHT	4.47 ± 0.22	5.29 ± 0.16	4.83 ± 0.15
Rosmarinic acid	4.96 ± 0.13	4.96 ± 1.50	4.75 ± 0.26
Gallic acid	4.97 ± 0.22	4.40 ± 0.15	4.13 ± 0.02

[1]Indicated antioxidant was mixed with shea butter at 90°C; control had no antioxidant added.
[2]Means ± standard error; lower percentages indicate more oxidation.

DISCUSSION

The accelerated oxidation experiments demonstrated that the addition of BHT and natural antioxidants can significantly limit oxidation of shea butter. Signs of oxidation in shea butter samples containing the added antioxidants were low as compared with the control samples not containing an antioxidant. The current study also demonstrated that the selected natural antioxidants (rosmarinic and gallic acids) could be as effective as BHT, a well-known synthetic antioxidant, against oxidation in some tests. The results support the use of natural antioxidants, a subject of interest to consumers seeking natural options.

In some tests for antioxidant activity, gallic acid proved more effective (lower amounts of peroxides) than BHT and rosmarinic acid in inhibiting oxidation. Gallic acid was less effective in protecting linoleic acid against oxidation. This may suggest that an effective natural antioxidant strategy would consist of using a mixture of two or more natural antioxidants.

The saturated fatty acids, palmitic and stearic, were quite stable during the accelerated oxidation tests as no significant decrease in the acid level, as compared with the control, was observed. As the number of double bonds increased, the contents of fatty acids decreased under the accelerated oxidation. These results are in agreement with a previous study showing the degree of unsaturation was proportionally related to the oxidation rate (Shahidi and Zhong., 2010) with the ratio of oxidation of stearic (18:0), oleic (18:1), and linoleic (18:2) acids being 1:100:1200, respectively (deMan, 1999). A similar trend was noted in this study in which the oxidation of stearic, oleic, and linoleic acids during 144 h oxidation process was 4.08%, 21.4%, and 83.7%, respectively, in comparison with the control.

The current study also demonstrates that the formation of primary oxidation measures (peroxide value) increased at higher rates as compared with the formation of secondary oxidation products (TBARS). This observation suggests that in this study the antioxidants were effective in inhibiting the initial stages of lipid peroxidation (Shahidi and Zhong, 2009). Further studies, however, are needed to evaluate the long term effects of antioxidants on the oxidative stability of shea butter over extended periods of time to resemble real shelf life processes.

The result of this study may be used in the development of shea butter products with better stability and extended shelf life using natural plant products.

ACKNOWLEDGEMENTS

We wish to acknowledge Victor Attafua and John Addaquay, the Pure Company (Buipe, Northern Ghana) for providing many of the samples for use in this study; to Dan Acquaye and Julie Asante-Dartey and ASNAPP-Ghana for their assistance. Funds from the New Use Agriculture and Natural Plant Products Program and the New Jersey Agricultural Experiment Station are also acknowledged.

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