

# Antioxidant effect of a crude phenolic extract from sorghum bran in sunflower oil in the presence of ferric ions

F.E. Sikwese, K.G. Duodu \*

*Department of Food Science, University of Pretoria, Pretoria 0002, South Africa*

Received 7 September 2006; received in revised form 10 October 2006; accepted 16 November 2006

## Abstract

Whole grain condensed tannin sorghum, its bran and a crude phenolic extract (CPE) prepared from the bran were evaluated for total phenols (TP), condensed tannins (CT) and antioxidant activity (AA). Antioxidant effect of the CPE from the sorghum bran was evaluated in sunflower oil in the presence of ferric ions by measuring peroxide values (PVs) and anisidine values (AVs) during storage at 65 °C, in comparison with tertiary butyl hydroquinone (TBHQ). Sorghum bran contained three times more TP and AA, and seven times more CT than the whole grain. The CPE had highest levels of TP, CT and AA. Sunflower oil with CPE had lower PVs and AVs compared to control samples. Oil samples with TBHQ had PVs lower than, but AVs similar to samples containing CPE. In the presence of ferric ions, the CPE was less effective in reducing PVs, but was more effective than TBHQ in reducing AVs.

© 2006 Elsevier Ltd. All rights reserved.

*Keywords:* Sorghum bran; Phenolic extract; Antioxidant activity; Lipid oxidation; Ferric ions; Peroxide value; Anisidine value

## 1. Introduction

Oxidation is one of the major causes of deterioration of fats and oils leading to the development of rancid odours and taste (Moure et al., 2001), and causing a reduction in the shelf life of the fat or oil. Oxidation can also decrease the nutritional quality and safety of lipids through the formation of toxic products in foods after cooking and processing (Frankel, 1996; Madhavi, Deshpande, & Salunke, 1996; Moure et al., 2001). Lipid oxidation occurs through a chain reaction that involves three stages: initiation, propagation and termination (Coulter, 2002; Gordon, 1990; Hamilton, Kalu, Prisk, Padley, & Pierce, 1997; Madhavi et al., 1996). Highly reactive free radicals are formed that react with oxygen or other fatty acids to form more free radicals and hydroperoxides in a chain reaction. During the termination stage, stable deterioration products, mainly carbonyl compounds are formed, which

are responsible for the perception of rancidity in oxidized oils.

The presence of high amounts of polyunsaturated fats such as linoleic and linolenic acids in oils make them more susceptible to oxidation (Chu & Kung, 1998; Nawar, 1996; Tan, Che Man, Selemat, & Yusoff, 2002). The problem is further exacerbated if the oil is exposed to factors such as oxygen (Nawar, 1996; Tan et al., 2002), light (Abdalla, Tirzite, Tirzitis, & Roozen, 1999; Eskin & Przybylski, 2001; Nawar, 1996), high temperatures (Nawar, 1996; Suzuki, Yasui, Matsukura, & Terao, 1996), or trace metals (mainly transition metals such as Fe and Cu) (Fomuso, Corredig, & Akoh, 2002; Gordon & Weng, 1992; Luzia et al., 1998; Satue-Gracia, Frankel, Rangavajhyala, & German, 2000). The transition metals enhance the rate of oxidation of edible oils by increasing the rate of generation of free radicals from fatty acids or hydroperoxides (Nawar, 1996; Paiva-Martins & Gordon, 2005), and presence of metals at concentrations as low as 0.1 ppm is known to increase the rate of oxidation (Nawar, 1996).

Synthetic phenolic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA)

\* Corresponding author. Tel.: +27 12 420 4299; fax: +27 12 420 2839.  
E-mail address: [gyebi.duodu@up.ac.za](mailto:gyebi.duodu@up.ac.za) (K.G. Duodu).

and tertiary butyl hydroquinone (TBHQ) are commonly added to lipid foods to stabilize the lipids against oxidation (Chu & Hsu, 1999; Moure et al., 2001; Stauffer, 1996). Chelating agents such as citric acid may be used to remove metals and thereby prevent their catalytic effect on oxidation (O'Brien, 2004). However, there is concern about the use of these synthetic antioxidants due to their reported negative effects such as carcinogenicity (Whysner, Wang, Zang, Iatropoulos, & Williams, 1994) and toxicity (Moure et al., 2001; Wanasundara & Shahidi, 1998). This has led to an increasing interest in the search for naturally occurring antioxidants (Duve & White, 1991; Malecka, 2002). Phenolic compounds from plant sources may act as antioxidants by scavenging lipid radicals, and in the presence of transition metal ions, phenols may act as both radical scavengers and metal chelators (Paiva-Martins & Gordon, 2005).

Sorghum (*Sorghum bicolor* (L.) Moench) contains phenolic compounds in the form of phenolic acids, flavonoids and condensed tannins (Waniska, 2000), which have been shown to have antioxidant activity (Awika, Rooney, Wu, Prior, & Cisneros-Zevallos, 2003; Awika, Rooney, & Waniska, 2004; Hagerman et al., 1998). These phenolic compounds, which are concentrated in the outer layers of the sorghum grain (pericarp and testa) (Beta, Rooney, Marovatsanga, & Taylor, 2000; Hahn, Rooney, & Earp, 1984; Rooney, Blakely, Miller, & Rosenow, 1980; Yousef, 1998), possess structural features favourable for radical scavenging and/or metal chelation, which would enable them to be effective antioxidants. The pericarp and testa are often removed and disposed of as bran during utilization of sorghum. A potential therefore exists to use sorghum bran as a cheap source of natural antioxidants to prevent the development of oxidative rancidity in edible oils and other lipid food systems. The antioxidant effect of extracts from navy bean hulls (Onyeneho & Hettiarachchy, 1991) and durum wheat bran (Onyeneho & Hettiarachchy, 1992) in soy and sunflower oils have been investigated.

The aim of this study was to evaluate the antioxidant effect of a crude phenolic extract from sorghum bran in sunflower oil in the presence of ferric ions in comparison with tertiary butyl hydroquinone (TBHQ), a commonly-used synthetic antioxidant.

## 2. Materials and methods

### 2.1. Samples and reagents

A brown-coloured condensed tannin sorghum variety (*Phatafuli*) grown during the 2002/2003 season on a local farm in Chikwawa district of Malawi was obtained through Kasinthula Research Station, Ministry of Agriculture and Irrigation, Malawi. Refined, bleached and deodorized (RBD) sunflower oil without added antioxidants was supplied by Continental Oil Mills (Johannesburg, South Africa). TBHQ was obtained from Merck (Germany). All other chemicals used were of analytical reagent grade.

### 2.2. Preparation of milled sorghum whole grain and bran

The sorghum grain was hand-cleaned and sorted to remove broken, diseased or insect-infested grain, glumes and other foreign material. The grain was then decorticated (10% bran yield) using a sorghum dehuller (Rural Industries Innovation Centre, Kanye, Botswana). The whole grain and bran were separately ground to pass a 0.5 mm sieve using a Laboratory Mill 3100 (Falling Number, Huddinge, Sweden).

### 2.3. Preparation of freeze-dried crude phenolic extract (CPE) from sorghum bran

Sorghum bran (40 g) was extracted with a total of 450 ml 75% aqueous acetone (75% acetone and 25% water) as follows: 150 ml solvent was added to 40 g bran in a centrifuge tube and vortex mixed every 10 min for 2 h to improve extraction efficiency. The mixture was centrifuged at 3500 rpm for 10 min (25 °C) using a Medifriger centrifuge (J.P. Selecta, s.a.) and decanted. The sample residue was re-extracted twice each with 150 ml solvent, centrifuged and decanted. The three supernatants were pooled and concentrated in a Buchi Rotavapor RE 120 rotary evaporator (Laboratoriums Technik AG, Switzerland) at 35 °C. The resulting mixture was freeze-dried and thoroughly mixed to obtain a fine powder. The freeze-dried crude phenolic extract (CPE) was stored at –20 °C.

### 2.4. Preparation of ferric palmitate

Ferric palmitate was prepared as described by Gordon and Weng (1992). Sodium hydroxide pellets (4.2 g) were dissolved in 100 ml distilled water and palmitic acid (25.6 g) was added. The mixture was stirred for 5 min, diluted to 400 ml with distilled water followed by heating with stirring to 90 °C. The resulting sodium palmitate solution was poured into a beaker containing ferric chloride hexahydrate (20 g), and the mixture was heated to 80 °C with stirring for 20 min. The brown precipitate formed was removed by filtration and washed three times with hot distilled water at 80 °C. The solid was dried in a vacuum oven for 8 h. When analyzed by atomic absorption spectrophotometry, the ferric palmitate prepared contained approximately 6.8% iron, the theoretical value of the iron content for ferric palmitate.

### 2.5. Determination of total phenols

The Folin-Ciocalteu method (Singleton & Rossi, 1965) as described by Waterman and Mole (1994) was used to determine total phenols in the milled sorghum whole grain, bran and CPE from sorghum bran. This method is based on the reducing power of the phenolic hydroxyl groups (Hahn et al., 1984), which react with the Folin-Ciocalteu phenol reagent to form chromogens that can be detected spectrophotometrically at 760 nm. Sorghum whole grain

and bran samples (0.25 g) were extracted using 15 ml 75% aqueous acetone (Kaluza, McGrath, Roberts, & Schroeder, 1980) as follows: 5 ml of solvent was added to 0.25 g sample in glass centrifuge tubes and the samples were vortex mixed every 10 min for 2 h to improve extraction efficiency. The samples were then centrifuged at 3500 rpm for 10 min (25 °C) using a Medifriger centrifuge (J.P. Selecta, s.a.) and decanted. Each sample residue was rinsed twice with 5 ml of solvent, vortex mixed for 5 min, centrifuged as above and decanted. The three supernatants were then combined and used for analysis. CPE (0.02 g) was reconstituted in 15 ml 75% aqueous acetone, and 0.5 ml of the reconstituted sample was used to determine total phenols. Tannic acid was used as a standard and results were expressed as mg tannic acid equivalents/100 mg sample, dry weight basis.

### 2.6. Determination of condensed tannins

The vanillin–HCl method (Price, Van Scoyoc, & Butler, 1978) was used for the determination of condensed tannins in the milled sorghum whole grain, bran and CPE from sorghum bran. This method is based on the ability of the condensed tannin units to react with the vanillin reagent in the presence of mineral acids to produce a red colour that is measured spectrophotometrically at 500 nm. Whole grain and bran samples (0.2 g) were extracted with 10 ml 100% methanol (Price et al., 1978) step-wise as follows: 5 ml solvent was added to the sample in a centrifuge tube and vortex mixed every 5 min for 20 min, followed by centrifuging at 3500 rpm for 10 min (25 °C) using a Medifriger centrifuge (J.P. Selecta, s.a.), and decanting. The sample residue was rinsed with 5 ml of solvent, vortex mixed and centrifuged as above and decanted. The two supernatants were combined and used for analysis. CPE (0.02 g) was reconstituted in 15 ml methanol and used for the condensed tannin assays. The extracts and reagents were maintained at 30 °C in a thermostat-controlled water bath before mixing the reactants. The methanolic extract (1 ml) was added to 5 ml vanillin reagent (4% HCl in methanol and 0.5% vanillin in methanol) and mixed. Sample blanks were done with 4% HCl in methanol replacing the vanillin reagent. The reactants were maintained at 30 °C and absorbance was read at 500 nm after 20 min. Catechin was used as a standard and the results were expressed as mg catechin equivalents/100 mg sample, dry weight basis.

### 2.7. Determination of antioxidant activity

Antioxidant activity (by free radical scavenging) of the milled whole grain, bran and CPE was determined using the Trolox Equivalent Antioxidant Capacity (TEAC) assay as described by Awika et al. (2003). TEAC is a spectrophotometric technique that measures the relative ability of hydrogen-donating antioxidants to scavenge the ABTS<sup>•+</sup> radical cation chromogen in relation to that of Trolox, the water-soluble Vitamin E analogue, which is used as an

antioxidant standard. The ABTS radical cations (ABTS<sup>•+</sup>) were produced by mixing equal volumes of 8 mM ABTS with 3 mM potassium persulfate prepared in distilled water and allowed to react in the dark for at least 12 h at room temperature before use. The ABTS<sup>•+</sup> solution was diluted with a phosphate buffer solution (pH 7.4) prepared by mixing 0.2 M of NaH<sub>2</sub>PO<sub>4</sub>, 0.2 M of Na<sub>2</sub>HPO<sub>4</sub> and 150 mM NaCl in 1 l of distilled water, with pH adjustment using NaOH where necessary. This solution was made fresh for each analysis. The ABTS<sup>•+</sup> solution (2900 µl) was added to the 75% aqueous acetone sorghum bran and grain extracts (100 µl), CPE solution (100 µl) (0.02 g CPE redissolved in 15 ml 75% aqueous acetone and further diluted 1:20 with the same solvent) or Trolox (100 µl) in a test tube and mixed. Absorbance readings (at 734 nm) were taken 30 min (for the samples) and 15 min (for the standard) after mixing of the samples and standard, respectively, using a Lambda EZ150 spectrophotometer (Perkin–Elmer, USA). The results were expressed as mM Trolox equivalents/g of sample, dry weight basis.

### 2.8. Evaluation of the antioxidant effect of CPE in sunflower oil

CPE from the condensed tannin sorghum bran was added to 80 g sunflower oil sample aliquots at concentrations of 1000, 1500 and 2000 ppm in screw-capped 100 ml Schott glass bottles and covered with aluminium foil to exclude light. The CPE was introduced into the oil with the aid of absolute methanol to facilitate dispersion. The synthetic antioxidant, TBHQ, was also added to an 80 g sunflower oil sample at the legal limit of 200 ppm (Shahidi & Wanasundara, 1992; Stauffer, 1996).

To assess the effect of the CPE on the oxidative stability of the oil in the presence of ferric ions, a similar set of the above samples were prepared with the addition of 2.2 ppm ferric ions (32.35 mg ferric palmitate/kg oil) (Gordon & Weng, 1992; Luzia et al., 1998) or 4.4 ppm ferric ions (64.70 mg ferric palmitate/kg oil) to the oil. All samples were thoroughly mixed for 5 min and stored in the dark in a Labcon forced circulation oven Type FSOE 16 (Labcon Pty Limited, Roodeport, South Africa) at 65 °C. Oxidative stability of the oil was evaluated by determining the peroxide values (PVs) using the AOAC Official Method 965.33 (AOAC, 2000), and anisidine values (AVs) using the International Union of Pure and Applied Chemistry (IUPAC) standard method 2.504 (IUPAC, 1979), at 2-day intervals during 14 days of storage.

### 2.9. Statistical analysis

The experiments were replicated three times, and all measurements were done in duplicate. Data for total phenols, condensed tannins and antioxidant activity of the extracts was subjected to analysis of variance (ANOVA), and the Fisher's LSD test was used to identify differences among the means at  $p < 0.05$ . Data for the evaluation of

the oxidative stability of sunflower oil was subjected to ANOVA computed using the SAS Generalized Linear Model (GLM) (SAS Institute, 2000). *F*-tests were conducted to determine differences among means at  $p < 0.05$ .

### 3. Results and discussion

#### 3.1. Phenolic content and antioxidant activity of sorghum whole grain, bran and CPE from sorghum bran

Table 1 shows the total phenol content, condensed tannin content and antioxidant activity of the sorghum whole grain, bran and CPE from sorghum bran. Levels of total phenols of sorghum reported in the literature vary widely due to differences in extraction solvents, test methods and standards used. This makes it difficult to do direct comparisons. Though tannic acid has been used as a standard for sorghum in the past (Beta, Rooney, Marovatsanga, & Taylor, 1999), it must be stated that sorghum does not contain any tannic acid. Recent research indicates that for cereals such as sorghum, catechin (Towo, Svanberg, & Ndossi, 2003) or gallic acid (Awika et al., 2003; Dicko, Gruppen, Traore, van Berkel, & Voragen, 2005) are normally used as standards during total phenol assays. However, some results obtained in our laboratory recently on a different sorghum variety indicate that though the absolute total phenol values may be different, the trends obtained between different samples are similar when either tannic acid or catechin is used as a standard. The level of condensed tannins obtained in the whole grain in this study (6.12% catechin equivalents) (Table 1), is comparable to levels reported for condensed tannin sorghums (type III) using the vanillin–HCl method. Beta et al. (1999) reported condensed tannin level of 5.48 mg catechin equivalents/100 mg sample for DC-75, a condensed tannin sorghum variety, which they classified to be possibly, a type III sorghum. The antioxidant activities for bran (0.60 mM TE/g) and whole grain (0.19 mM TE/g) in Table 1, fall within the same range as reported by Awika et al. (2003) for bran (0.512–0.768 mM TE/g) and whole grain (0.108–0.226 mM TE/g) of the condensed tannin-containing sorghums, Hi-tannin and sumac respectively.

The bran contained approximately three times more total phenols and antioxidant activity, and seven times more condensed tannins than the whole grain, which shows

that phenolic compounds in sorghum are highly concentrated in outer layers of the grain (Beta et al., 2000; Hahn et al., 1984; Hahn & Rooney, 1986; Rooney et al., 1980). Sorghum bran, a waste product during sorghum milling, could be regarded as a potential source of antioxidants for use in lipid foods. The CPE had significantly higher levels of total phenols, condensed tannins and antioxidant activity than the bran. The freeze-drying process concentrates the phenolics further by removal of excess moisture. It may be more appropriate to incorporate phenolic extracts from sorghum into lipid foods such as sunflower oil in their freeze-dried form for optimum antioxidative effects.

#### 3.2. Effect of CPE from condensed tannin sorghum bran on the oxidative stability of sunflower oil

Fig. 1 shows the effect of the sorghum CPE and TBHQ on the peroxide value, PV (Fig. 1a) and anisidine value, AV (Fig. 1b) of sunflower oil over a 14-day storage period. The initial PV and AV of the freshly refined oil were 1.29 meq/kg oil and 2.59, respectively. According to Rossell (1986), freshly refined oil should have a PV of less than 1 meq/kg, and oils that have been stored for some time after refining could have a PV of up to 10 meq/kg, and that an AV of less than 10 indicates good oil. The PV and AV levels of the sunflower oil used in this study were therefore within acceptable limits for good quality oil.

At the three concentrations (1000, 1500 and 2000 ppm), the CPE reduced formation of primary oxidation products (hydroperoxides) as shown by lower PVs (Fig. 1a) compared to the control oil sample without any additive. In all the samples, PVs generally increased from the beginning of the storage period to the last day, showing the progression of oxidation. The lowest rate of increase of PVs was in the samples containing TBHQ. For the control oil sample, the progressive increase in PV was significantly ( $p < 0.05$ ) higher throughout the storage period compared to the samples containing the CPE or TBHQ. For most of the storage period, PVs of oil samples containing 1000 ppm CPE did not seem to be significantly ( $p < 0.05$ ) different from the PVs of oil samples containing 1500 and 2000 ppm CPE.

The antioxidant effect of the sorghum CPE may be attributed to the presence of phenolic compounds, such as condensed tannins in the extract. Phenolic compounds

Table 1

Total phenols, condensed tannins and antioxidant activity of whole grain sorghum, bran and freeze-dried crude phenolic extract from sorghum bran

Sample	Total phenols (mg TAE/100 mg)	Condensed tannins (mg CE/100 mg)	Antioxidant activity (mM TE/g)
Whole grain	3.09 <sup>a</sup> a <sup>b</sup> (0.40) <sup>c</sup>	6.12 a (0.52)	0.19 a (0.01)
Bran	10.12 b (0.59)	45.16 b (4.39)	0.60 a (0.05)
CPE	58.35 c (3.24)	491.80 c (25.54)	4.60 b (0.50)

TAE, tannic acid equivalents; CE, catechin equivalents; TE, trolox equivalents; CPE, freeze-dried crude phenolic extract from sorghum bran.

<sup>a</sup> Results are means of six determinations expressed on a dry basis.

<sup>b</sup> For each sample, means within each column followed by a different letter are significantly different ( $p < 0.05$ ).

<sup>c</sup> Standard deviations are given in parentheses.

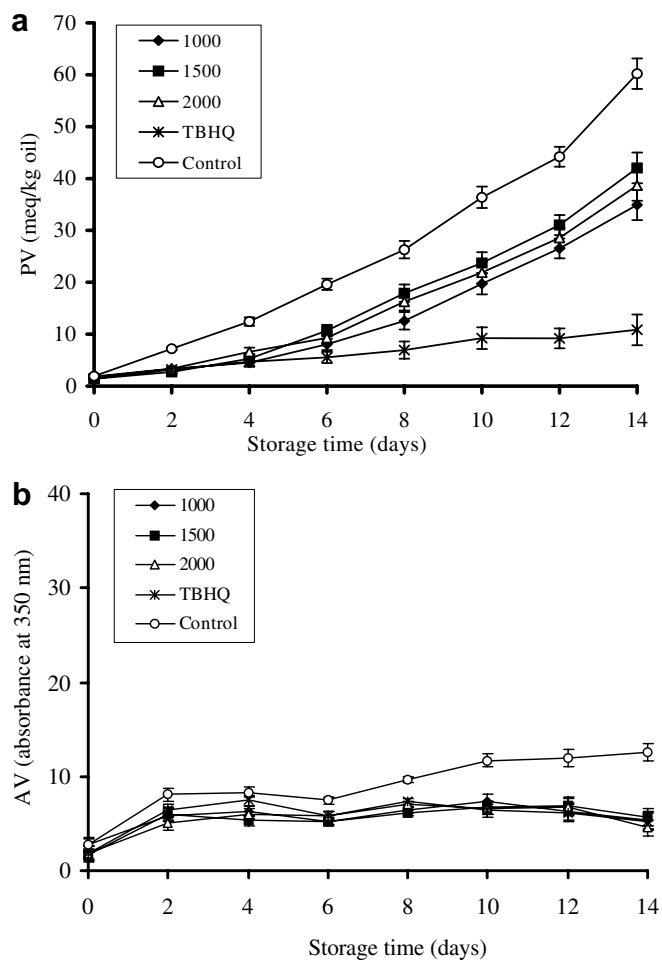


Fig. 1. Effect of sorghum crude phenolic extract (CPE) and TBHQ on peroxide value, PV (a) and anisidine value, AV (b) of sunflower oil over a 14-day storage period. Each point on the graphs is the average value of six determinations obtained in three separate experiments. Bars represent standard error of the mean.

are able to scavenge and stabilize lipid radicals by donating hydrogen atoms (Eskin & Przybylski, 2001; Frankel, 1996), hence reducing the rate of chain propagation of the oxidation process. The ability of plant extracts to inhibit lipid oxidation due to their phenolic content has been previously shown (Gordon & Weng, 1992; Onyeneho & Hettiarachchy, 1991; Onyeneho & Hettiarachchy, 1992; Wanasundara & Shahidi, 1998).

From day 4 to the end of the storage period, AVs for the control oil sample were significantly ( $p < 0.05$ ) higher than the oil samples containing CPE or TBHQ, which had similar AVs throughout the storage period (Fig. 1b). This indicates that the sorghum CPE had a similar ability to inhibit formation of secondary oxidation products in the oil as TBHQ.

While the PVs for all samples showed a steady increase during the storage period (Fig. 1a), AVs for oil samples containing CPE and TBHQ were almost constant with only the control oil sample showing a gradual increase (Fig. 1b). This indicates that a high rate of hydroperoxide formation did not necessarily lead to a high rate of generation of secondary oxidation products. This may be due to

the ability of the CPE or TBHQ to scavenge free radicals (such as alkoxyl radicals) arising from the decomposition of the hydroperoxides, thus preventing the radicals from further reaction to form compounds such as aldehydes and ketones (Eskin & Przybylski, 2001). Similar results were obtained by Abdalla and Roozen (1999) who reported better inhibition of hexanal (secondary oxidation product) generation than formation of conjugated dienes (primary oxidation products) by thyme and lemon balm extracts in sunflower oil.

### 3.3. Effect of ferric ions on the oxidative stability of sunflower oil

Promotion of lipid oxidation by transition metals is a well-known phenomenon (Fomuso et al., 2002; Gordon & Weng, 1992; Satue-Gracia et al., 2000). Previous studies have reported higher PVs in oil containing ferric ions (Gordon & Weng, 1992; Luzia et al., 1998). Results of this study however show significantly lower PVs for oil with added ferric ions compared to the control without added ferric ions (Fig. 2a). The lower PVs for oil with added ferric ions may however not be due to lower lipid oxidation rates. Iron has been shown to have the ability to decompose lipid peroxides and prevent their accumulation, which leads to lower PVs (Mancuso, McClements, & Decker, 1999). It may therefore be suggested that in this study, ferric ions accelerated the breakdown of hydroperoxides to secondary oxidation products (Satue-Gracia et al., 2000), and prevented their accumulation. Oil samples containing ferric ions would therefore be expected to have higher AVs compared to control samples due to an increase in secondary oxidation products, and this can be observed in Fig. 2b. Similar observations have been reported (Fomuso et al., 2002; Halliwell & Gutteridge, 1989; Van der Merwe, 2003).

The higher PVs and AVs of oil containing 4.4 ppm ferric ions compared to that containing 2.2 ppm illustrates the dependence of the extent of oxidation on the concentration of ferric ions as shown by previous studies (Fomuso et al., 2002; Satue-Gracia et al., 2000).

### 3.4. Effect of CPE from condensed tannin sorghum bran on the oxidative stability of sunflower oil in the presence of ferric ions

The PVs of sunflower oil samples containing CPE and ferric ions at various concentrations over the storage period are presented in Table 2. On the average, PVs of oil samples containing CPE at 1000 ppm and 2.2 ppm ferric ions, were similar to control oil samples, while oil samples containing CPE at 1000 ppm and 4.4 ppm ferric ions and CPE at 1500 and 2000 ppm generally had higher PVs compared to the control samples. This may be because the peroxides formed in the control samples did not accumulate but were decomposed by the ferric ions to secondary oxidation products (Fomuso et al., 2002; Mancuso et al., 1999), as demonstrated by the corresponding higher AVs for the

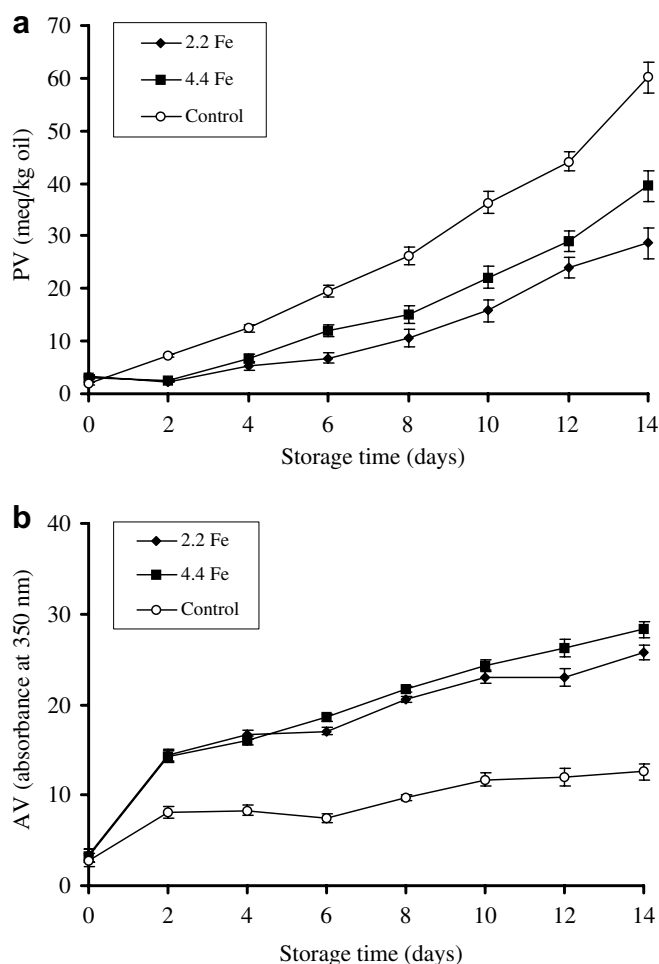


Fig. 2. Effect of ferric ions on peroxide value, PV (a) and anisidine value, AV (b) of sunflower oil. Each point on the graphs is an average of six determinations obtained from three separate experiments. Bars represent standard error of the mean.

control oil compared to the samples containing CPE and ferric ions (Table 3). For oil samples containing CPE, possible chelation of the ferric ions by phenolic compounds in the CPE (such as condensed tannins) may render the ions unavailable and catalytically inactive to decompose hydroperoxides to secondary oxidation products (Fernandez, Mira, Florencio, & Jennings, 2002; Shahidi & Wanasundara, 1992; Sugihara, Arakawa, Ohnishi, & Furuno, 1999). Condensed tannins have been shown to form metal complexes with ferric ions (Porter, 1992; Slabbert, 1992). Since secondary oxidation products (aldehydes in particular), are the source of the characteristic odour of rancid fats (Coulate, 2002), the CPE may be considered as potential agents in preventing development of rancidity in sunflower oil.

From day 2 to the end of the storage period, oil samples containing TBHQ and ferric ions had lower PVs (Table 2) but higher AVs (Table 3) than oil containing CPE (at all the three concentrations) and ferric ions. TBHQ does not chelate metal ions (Shahidi & Wanasundara, 1992). Therefore for samples containing TBHQ, ferric ions will be available to break down hydroperoxides to secondary oxidation products leading to lower PVs but higher AVs.

The dependence of the extent of oxidation on ferric ion concentration as shown in Fig. 2 is again shown by higher PVs (Table 2) and AVs (Table 3) in the oil samples with increasing ferric ion concentration.

Though PVs are often used as an indicator of initial stages of oxidation (O'Brien, 2004), hydroperoxides decompose rapidly during storage, upon heating or in the presence of ferric ions; hence PV may not necessarily indicate the full extent of lipid oxidation. This study demonstrates that in the presence of ferric ions, PV measurements alone may not give a true representation of the rate of lipid oxidation. Decomposition of hydroperoxides into secondary oxidation products could be accelerated

Table 2

Effect of storage time on peroxide values of sunflower oil samples containing either freeze-dried crude phenolic extract from sorghum bran (at different concentrations) or TBHQ, both in the presence of ferric ions

Treatment	Storage time (days)							
	0	2	4	6	8	10	12	14
Control (+2.2 ppm Fe)	1.79 <sup>a</sup> a <sup>b</sup>	2.37 a	5.37 b	6.82 bc	10.69 bc	15.81 ab	23.94 bc	28.68 a
Control (+4.4 ppm Fe)	1.95 a	2.51 a	6.79 bc	12.08 de	15.03 cd	22.07 cd	29.07 cd	39.47 b
TBHQ + 2.2 ppm Fe	1.82 a	1.09 a	1.60 a	2.44 a	5.32 a	10.92 a	14.08 a	21.90 a
TBHQ + 4.4 ppm Fe	1.90 a	1.14 a	3.59 ab	4.77 ab	9.62 ab	13.17 a	19.57 ab	26.25 a
1000E <sup>c</sup> + 2.2 ppm Fe	1.59 a	3.79 ab	5.66 b	7.57 bc	11.51 bc	15.98 abc	21.61 b	28.84 a
1000E + 4.4 ppm Fe	1.74 a	3.44 a	7.09 bc	10.68 cd	17.19 cd	24.60 de	28.37 cd	38.32 b
1500E + 2.2 ppm Fe	2.09 ab	3.06 a	6.85 bc	11.75 de	17.93 de	25.16 de	30.84 de	41.47 b
1500E + 4.4 ppm Fe	1.89 a	3.78 ab	9.54 d	14.82 ef	23.09 f	31.44 f	39.41 f	52.92 d
2000E + 2.2 ppm Fe	1.80 a	3.63 ab	6.06 bc	9.49 cd	15.18 cd	21.46 bcd	28.06 cd	39.38 b
2000E + 4.4 ppm Fe	1.96 a	4.31 b	9.89 d	15.15 f	22.24 ef	29.62 ef	36.52 ef	51.04 c
SEM <sup>d</sup>	0.22	0.24	0.8	1.09	1.68	2.07	1.92	1.92

<sup>a</sup> Results are means of six determinations obtained in three independent experiments.

<sup>b</sup> Means within each column followed by a different letter are significantly different ( $p < 0.05$ ).

<sup>c</sup> 1000E = 1000 ppm crude phenolic extract from sorghum bran in sunflower oil (similar interpretation for 1500E and 2000E).

<sup>d</sup> Standard error of the mean.

Table 3  
Effect of storage time on anisidine values of sunflower oil samples containing either freeze-dried crude phenolic extract from sorghum bran (at different concentrations) or TBHQ, both in the presence of ferric ions

Treatment	Storage time (days)							
	0	2	4	6	8	10	12	14
Control (+2.2 ppm Fe)	3.39 <sup>a</sup> a <sup>b</sup>	14.46 c	16.67 c	17.03 c	20.61 c	23.06 b	22.99 b	25.75 cd
Control (+4.4 ppm Fe)	3.27 a	14.22 c	15.98 bc	18.59 d	21.63 d	24.31 bc	26.22 c	28.26 d
TBHQ + 2.2 ppm Fe	2.62 a	11.46 b	14.73 b	18.26 cd	20.58 c	22.99 b	23.14 b	25.25 c
TBHQ + 4.4 ppm Fe	2.96 a	11.76 b	15.44 bc	18.70 d	22.00 d	26.38 c	26.44 c	27.80 cd
1000E <sup>c</sup> + 2.2ppm Fe	2.64 a	5.77 a	5.23 a	5.74 a	7.56 ab	7.75 a	7.40 a	6.29 ab
1000E + 4.4 ppm Fe	2.37 a	6.53 a	6.64 a	7.66 b	8.01 b	8.67 a	8.54 a	6.50 ab
1500E + 2.2ppm Fe	2.20 a	5.63 a	6.09 a	5.46 a	6.89 a	6.79 a	6.57 a	6.34 ab
1500E + 4.4 ppm Fe	1.92 a	5.05 a	5.95 a	5.74 a	7.05 a	6.68 a	7.08 a	8.43 b
2000E + 2.2 ppm Fe	1.85 a	5.45 a	5.56 a	6.53 ab	7.14 ab	6.70 a	6.45 a	5.69 a
2000E + 4.4ppm Fe	2.05 a	6.70 a	6.10 a	7.13 b	6.84 a	7.70 a	7.77 a	7.33 ab
SEM <sup>d</sup>	0.73	0.67	0.51	0.43	0.32	0.71	0.91	0.87

<sup>a</sup> Results are means of six determinations obtained in three independent experiments.

<sup>b</sup> Means within each column followed by a different letter are significantly different ( $p < 0.05$ ).

<sup>c</sup> 1000E = 1000 ppm crude phenolic extract from sorghum bran in sunflower oil (similar interpretation for 1500E and 2000E).

<sup>d</sup> Standard error of the mean.

by the ferric ions, hence preventing their accumulation in the oil samples, resulting in low PVs, which could be wrongly interpreted as indicative of good quality oil.

#### 4. Conclusions

Freeze-dried crude phenolic extract (CPE) from condensed tannin sorghum bran inhibits the formation of both primary and secondary oxidation products in sunflower oil due to the presence of phenolic compounds that possess antioxidant properties. Ferric ions have an influence on the effectiveness of the sorghum CPE as well as that of TBHQ as antioxidants. The CPE exhibits a lower ability to suppress formation of primary oxidation products compared to TBHQ both in the absence and presence of ferric ions. In the absence of ferric ions, the CPE and TBHQ show a similar ability to inhibit formation of secondary oxidation products but the CPE is more effective in the presence of ferric ions, probably due to the ability of phenolic compounds in the CPE to chelate ferric ions, which prevents the ferric ions from decomposing hydroperoxides to secondary oxidation products. The CPE appears to exert an antioxidant effect by acting as both radical scavengers and metal chelators. Condensed tannin sorghum bran may be considered a cheap potential source of phenolic compounds for application as antioxidants in lipid foods.

#### Acknowledgement

The authors greatly acknowledge financial support from the German Academic Exchange Service (DAAD).

#### References

Abdalla, A. E., & Roozen, J. P. (1999). Effect of plant extracts on the oxidative stability of sunflower oil and emulsion. *Food Chemistry*, 64, 323–329.

- Abdalla, A. E., Tirzite, D., Tirzitis, G., & Roozen, J. P. (1999). Antioxidant activity of 1,4-dihydropyridine derivatives in beta-carotene-methyl linoleate, sunflower oil and emulsions. *Food Chemistry*, 66, 189–195.
- Association of Official Analytical Chemists (2000). In W. Horwitz (Ed.), *Official methods of analysis of the AOAC international* (17th ed.). Gaithersburg, Maryland: AOAC International. AOAC Official Method 965.33.
- Awika, J. M., Rooney, L. W., Wu, X., Prior, R. L., & Cisneros-Zevallos, L. (2003). Screening methods to measure antioxidant activity of sorghum (*Sorghum bicolor*) and sorghum products. *Journal of Agricultural and Food Chemistry*, 51, 6657–6662.
- Awika, J. M., Rooney, L. W., & Waniska, R. D. (2004). Properties of 3-deoxyanthocyanins from sorghum. *Journal of Agricultural and Food Chemistry*, 52, 4388–4394.
- Beta, T., Rooney, L. W., Marovatsanga, L. T., & Taylor, J. R. N. (1999). Phenolic compounds and kernel characteristics of Zimbabwean sorghums. *Journal of the Science of Food and Agriculture*, 79, 1003–1010.
- Beta, T., Rooney, L. W., Marovatsanga, L. T., & Taylor, J. R. N. (2000). Effect of chemical treatments on polyphenols and malt quality in sorghum. *Journal of Cereal Science*, 31, 295–302.
- Chu, Y., & Hsu, H. (1999). Effects of antioxidants on peanut oil stability. *Food Chemistry*, 66, 29–34.
- Chu, Y., & Kung, Y. (1998). A study on vegetable oil blends. *Food Chemistry*, 62, 191–195.
- Coulter, T. P. (2002). *Food: the chemistry of its components* (4th ed.). Cambridge: The Royal Society of Chemistry (pp. 73–125).
- Dicko, M. H., Gruppen, H., Traore, A. S., van Berkel, W. J. H., & Voragen, A. G. J. (2005). Evaluation of the effect of germination on phenolic compounds and antioxidant activities in sorghum varieties. *Journal of Agricultural and Food Chemistry*, 53, 2581–2588.
- Duve, K. J., & White, P. J. (1991). Extraction and identification of antioxidants in oats. *Journal of the American Oil Chemists' Society*, 68, 365–370.
- Eskin, N. A. M., & Przybylski, R. (2001). Antioxidant and shelf life of foods. In N. A. M. Eskin & D. S. Robinson (Eds.), *Food shelf life stability: chemical, biochemical and microbiological changes* (pp. 176–202). Boca Raton, Fla: CRC Press.
- Fernandez, M. T., Mira, M. L., Florencio, M. H., & Jennings, K. R. (2002). Iron and copper chelation by flavonoids: an electrospray mass spectrometry study. *Journal of Inorganic Biochemistry*, 92, 105–111.

- Fomuso, L. B., Corredig, M., & Akoh, C. C. (2002). Metal catalyzed oxidation of a structured lipid model emulsion. *Journal of Agricultural and Food Chemistry*, 50, 7114–7119.
- Frankel, E. N. (1996). Antioxidants in food and their impact on food quality. *Food Chemistry*, 57, 51–55.
- Gordon, M. H. (1990). The mechanism of antioxidant action in vitro. In B. J. F. Hudson (Ed.), *Food antioxidants* (pp. 1–18). England: Elsevier Science Publishers Ltd.
- Gordon, M. H., & Weng, X. C. (1992). Antioxidant properties of extracts from tanshen (*Salvia miltiorrhiza Bunge*). *Food Chemistry*, 44, 119–122.
- Hagerman, A. E., Riedl, K. M., Alexander Jones, G., Sovik, K. N., Ritchard, N. T., Hartzfeld, P. W., et al. (1998). High molecular weight plant polyphenolics (tannins) as biological antioxidants. *Journal of Agricultural and Food Chemistry*, 46, 1887–1892.
- Hahn, D. H., & Rooney, L. W. (1986). Effect of genotype on tannins and phenols of sorghum. *Cereal Chemistry*, 63, 4–8.
- Hahn, D. H., Rooney, L. W., & Earp, C. F. (1984). Tannins and phenols of sorghum. *Cereal Foods World*, 29, 776–779.
- Halliwell, B., & Gutteridge, J. M. C. (1989). *Free radicals in biology and medicine* (p. 236). Oxford: Clarendon Press.
- Hamilton, R. J., Kalu, C., Prisk, E., Padley, F. B., & Pierce, H. (1997). Chemistry of free radicals in lipids. *Food Chemistry*, 60, 193–199.
- International Union of Pure and Applied Chemistry (1979). *Standard methods for the analysis of oils and fats and derivatives* (6th ed.). Method 2.504.
- Kaluza, W. Z., McGrath, R. M., Roberts, T. C., & Schroeder, H. H. (1980). Separation of phenolics of *Sorghum bicolor* (L) Moench grain. *Journal of Agricultural and Food Chemistry*, 28, 1191–1196.
- Luzia, M. R., Trugo, L. C., Da Paixao, K. C. C., Marcilio, R., De Maria, C. A. B., & Quinteiro, L. M. C. (1998). Effect of 5-caffeoylquinic acid in the presence of metal chelators on soybean oil oxidative stability. *Lebensm.-Wiss. U.-Technol*, 31, 64–68.
- Madhavi, D. L., Deshpande, S. S., & Salunkhe, D. K. (1996). *Food antioxidants: technological, toxicological and health perspectives* (pp. 2–7). New York: Marcel Dekker Inc..
- Malecka, M. (2002). Antioxidant properties of the unsaponifiable matter isolated from tomato seeds, oat grains and wheat germ oil. *Food Chemistry*, 79, 327–330.
- Mancuso, J. R., McClements, D. J., & Decker, E. A. (1999). The effects of surfactant type, pH, and chelators on the oxidation of salmon oil-in-water emulsions. *Journal of Agricultural and Food Chemistry*, 47, 4112–4116.
- Moure, A., Cruz, J. M., Franco, D., Dominguez, J. M., Sineiro, J., Dominguez, H., et al. (2001). Natural antioxidants from residual sources. *Food Chemistry*, 72, 145–171.
- Nawar, W. W. (1996). Lipids. In O. R. Fennema (Ed.), *Food chemistry* (3rd ed., pp. 225–319). New York: Marcel Dekker Inc.
- O'Brien, R. D. (2004). *Fats and oils: formulating and processing for applications* (2nd ed.). London: CRC Press (pp. 192–199).
- Onyeneho, S. N., & Hettiarachchy, N. S. (1991). Effect of navy bean hull extract on the oxidative stability of soy and sunflower oils. *Journal of Agricultural and Food Chemistry*, 39, 1701–1704.
- Onyeneho, S. N., & Hettiarachchy, N. S. (1992). Antioxidant activity of durum wheat bran. *Journal of Agricultural and Food Chemistry*, 40, 1496–1500.
- Paiva-Martins, F., & Gordon, M. H. (2005). Interactions of ferric ions with olive oil phenolic compounds. *Journal of Agricultural and Food Chemistry*, 53, 2704–2709.
- Porter, L. J. (1992). Structure and chemical properties of the condensed tannins. In R. W. Hemingway & P. E. Laks (Eds.), *Plant polyphenols: synthesis, properties significance* (pp. 245–258). New York: Plenum Press.
- Price, M. L., Van Scoyoc, S., & Butler, L. G. (1978). A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. *Journal of Agricultural and Food Chemistry*, 26, 1214–1218.
- Rooney, L. W., Blakely, M. E., Miller, F. R., & Rosenow, D. T. (1980). Factors affecting the polyphenols of sorghum and their development and location in the sorghum kernel. In J. Hulse (Ed.), *Polyphenols in cereals and legumes: proceedings of a symposium held during the 36th annual meeting of the Institute of Food Technologists, St. Louis, Missouri, 10–13 June 1979* (pp. 25–38). Ottawa: International Development Research Centre.
- Rossell, J. B. (1986). Classical analysis of oils and fats. In R. J. Hamilton & J. B. Rossell (Eds.), *Analysis of oils and fats* (pp. 1–32). England: Elsevier Applied Science.
- SAS Institute (2000). *SAS/STAT user's guide*. Cary, NC: SAS Institute, 27513.
- Satue-Gracia, M. T., Frankel, E. N., Rangavajhyala, N., & German, J. B. (2000). Lactoferrin in infant formulas: effect on oxidation. *Journal of Agricultural and Food Chemistry*, 48, 4984–4990.
- Shahidi, F., & Wanasundara, P. K. J. P. D. (1992). Phenolic antioxidants. *Critical Reviews in Food Science and Nutrition*, 32, 67–103.
- Singleton, U. L., & Rossi, J. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagent. *American Journal of Enology and Viticulture*, 16, 144.
- Slabbert, N. (1992). Complexation of condensed tannins with metal ions. In R. W. Hemingway & P. E. Laks (Eds.), *Plant polyphenols: synthesis, properties, significance* (pp. 421–436). New York: Plenum Press.
- Stauffer, C. E. (1996). *Fats and oils*. Minnesota, USA: Eagan Press (pp. 1–14).
- Sugihara, N., Arakawa, T., Ohnishi, M., & Furuno, K. (1999). Anti- and pro-oxidative effects of flavonoids on metal-induced lipid hydroperoxide-dependent lipid peroxidation in cultured hepatocytes loaded with  $\alpha$ -linolenic acid. *Free Radical Biology & Medicine*, 27, 1313–1323.
- Suzuki, Y., Yasui, T., Matsukura, U., & Terao, J. (1996). Oxidative stability of bran lipids from rice variety [*Oryza sativa* (L.)] lacking lipoxygenase-3 in seeds. *Journal of Agricultural and Food Chemistry*, 44, 3479–3483.
- Tan, C. P., Che Man, Y. B., Selemat, J., & Yusoff, M. S. A. (2002). Comparative studies of oxidative stability of edible oils by differential scanning calorimetry and oxidative stability index methods. *Food Chemistry*, 76, 385–389.
- Towo, E. E., Svanberg, U., & Ndossi, G. D. (2003). Effect of grain pretreatment on different extractable phenolic groups in cereals and legumes commonly consumed in Tanzania. *Journal of the Science of Food and Agriculture*, 83, 980–986.
- Van der Merwe, G. H. (2003). Quality parameters for the prediction of mono- and polyunsaturated oil shelf-life. PhD. Food Science thesis. University of Pretoria.
- Wanasundara, U. N., & Shahidi, F. (1998). Antioxidant and pro-oxidant activity of green tea extracts in marine oils. *Food Chemistry*, 63, 335–342.
- Waniska, R. D. (2000). Structure, phenolic compounds and antifungal proteins of sorghum caryopses. In A. Chandrashekar, R. Bandyopadhyay & A.J. Hall (Eds.), *Technical and institutional options for sorghum grain mold management: proceedings of an international consultation* (pp. 72–106). ICRISAT.
- Waterman, P. G., & Mole, S. (1994). *Analysis of plant metabolites* (pp. 1–103). Oxford: Alden Press Limited.
- Whysner, J., Wang, C. X., Zang, E., Iatropoulos, M. J., & Williams, G. M. (1994). Dose response of promotion by butylated hydroxyanisole in chemically initiated tumours of the rat fore stomach. *Food and Chemical Toxicology*, 32, 215–222.
- Yousef, A. M. (1998). Extractability, fractionation and nutritional value of low and high tannin sorghum proteins. *Food Chemistry*, 63, 325–329.