Effects of sulphur preservative on phytochemical and antioxidant capacity of peels of mango cultivars (*Mangifera indica* L.) produced in South Africa

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Abstract:

Fruits have abundant phytochemicals that contribute as bioactive molecules with ability to lower incidence of diseases. Mangoes are rich in polyphenols and antioxidants. In this study, peels of six selected mango cultivars (Tommy Atkins, Peach, Saber, Sunshine, Keitt and Vhavenda) were treated with sulphur dioxide solutions (0, 10, 20, 50, 100, 150, 200, 250 and 300 ppm) as preservative of phytochemicals and antioxidants capacity. Regardless of cultivar, sulphur concentration has effect on composition of polyphenols and antioxidant capacity of mango peels, reaching a plateau at 50ppm. Vhavenda cultivar has significantly highest polyphenols and antioxidant capacity than the other cultivars evaluated. This study reveals that mango peels are a prospective source of natural antioxidants as they constitute significantly higher total antioxidant capacity and phenolic content.

Key words: antioxidants, mango cultivars, peels, phytochemicals, sulphur.

INTRODUCTION

Phytochemicals are compounds that act as free radical scavengers to help eliminate the highly charged oxygen molecules that are byproducts of metabolized oxygen (Khalid, 2007), and are believed to offer various health benefits (Van Duyn and Pivonka, 2000; Min et al., 2013). Antioxidants are substances that can prevent or delay the oxidative damage of lipids, proteins and nucleic acids by reactive oxygen species (ROS), which include free radicals such as hydroxyl, peroxyl and non-radicals such as hydrogen peroxides (Saikat et al., 2010). According to Pietta (2000), phenolic antioxidants are thought to neutralize ROS before they can cause damage.

The most of the abundant antioxidants in fruits are polyphenols, vitamin C, A and E and carotenoids to a lesser extent in some fruits (Lim et al., 2007). According to Fleuriet and Macheix (2003), most of these polyphenols are flavonoids and are present in the form of ester and glycoside in fruits. Flavonoids commonly found in fruits and vegetables have been linked to reduced risk of mortality from the coronary heart diseases (Wang et al., 2011).

Phenolic compounds are plant secondary metabolites that are biosynthesized through the shikimic acid pathway (Tomas-Baraeran and Espin, 2001). Phenolic compounds are associated with the health benefits deriving from consuming high levels of fruits (Parr and Bolwell, 2000; Aberoumand, 2012). Mango is regarded as a valuable source of phytochemical compounds (Kim et al., 2007; Ashoush and Gadallah, 2011; Mudau et al., 2013); among these compounds, polyphenolics are widely distributed secondary metabolites and the

predominant antioxidants present. Several studies have reported phenolic compounds in mango flesh and peels, including various flavonoids, xathones, phenolic acids and gallotannins (Schieber et al., 2000; Berardini et al., 2005) and variability of these compounds have been observed in different mango cultivars (Souza et al., 2011; Taing et al., 2012).

Mangoes are seasonal fruits with limited shelf-life with fruit quality dropping off rapidly once fully ripe. Mango fruits are processed for various products (Loelillet, 1994) and by-products such as peels and kernels are generated (Ashoush and Gadallah, 2011). These by-products are rich sources of natural bioactive compounds which play an important role in prevention of diseases (Ashoush and Gadallah, 2011). Studies have been conducted on the utilization of mango kernels as a source of fat (Arogba, 2002), natural antioxidants (Kaur, 2004), starch (Moharram and Moustafa, 1982), flour (Puravankara et al., 2000) and feed (Ravindran and Sivakanesan, 1996) but studies on peels are scarce (Berardini et al., 2005). Most of the studies on the exploitation of mango peels dealt with their use as a source of pectin, which is considered a high quality dietary fiber (Beerh et al., 1976; Srirangarajan and Shrikhande, 1976; Tandon et al., 1991; Pedroza-Islas and Aguilar-Esperanza, 1994; Tandon and Garg, 1999).

The mango peels have been reported to a good source of dietary fiber containing high amount of extractable polyphenolics (Larrauri et al., 1996). According to Ojokoh (2007), mango peel fibre is a good source of dietary fiber and its chemical composition may be compared to citrus fibre. Mango peels also

demonstrated higher values of anticancer properties because of polyphenolic extracts (Noratto, 2010) and glucose retardation (Reyers and Vega, 1988). Chemical in peels of certain mango cultivars have also been shown to prevent the formation of fat cells through disrupting adipogenesis (Taing et al., 2012) which is the key in development of obesity (Min et al., 2013).

The use of sulphur solution as a preservative in dried mango has become a commercial drying standard for the industry in South Africa with limited studies evaluating the phytochemical and antioxidant capacity of peels of mango cultivars. As a result, data that describe the use of preservatives in mango byproducts is lacking. Therefore, the objective of this study was to determine the effect of sulphur concentration on polyphenol content and total antioxidant capacity of mango peels for potential usefulness as a preservative.

MATERIALS AND METHODS

Location. The experiment was conducted at Agro-food Laboratory, University of Limpopo. The ripe fruits of mango cultivars *inter alia*; Tommy Atkins, Peach, Saber, Sunshine and Keitt were randomly collected from a commercial orchard in Hoedspruit. Vhavenda cultivar was collected from another commercial orchard in Vhembe District (23 °N 50’ E, 30 °S 17’E); alt 610 m; subtropical-type climate (i.e. summer rainfall and cold, dry winter) of Limpopo Province, South Africa.

Plant materials. Fresh, healthy and disease free fruits from six selected cultivars were washed and manually peeled. The peels were soaked in a sodium metabisulfite solution (BASF chemical company, Germany). Nine different

concentrations of SO_2 (in 3 L of water) were used. Treatment concentrations were 0, 10, 20, 50, 100, 150, 200, 250 and 300 ppm. Mango peels from the six cultivars were soaked for 5 minutes in the solution, and then immediately dried in a hot air oven at 58 ^{o}C for 28 h. Samples of dried peels were stored at -30^{o}C until their analysis.

Sample preparations (Extraction)

For the analysis, 10 g dried fruit peel samples were weighed and transferred to a waring commercial blender (Instrulab, Johannesburg, South Africa) containing 100 ml of methanol, and then blended at a high speed for 2 min (stopping occasionally to avoid accumulation of fumes). The mixture was removed and let stand in the beaker to achieve separation. After 6-8 minutes, the supernatant was collected, centrifuged at 12 000 *x g* for ten minutes and stored. The residues were blended again with 50 ml methanol, supernatant decanted as above, combined with the first one, filtered with MN-615 (240 mm) filter papers (Bethlehem, USA) and stored at –4 ^{o}C until analysis.

Determination of Total phenolics content

Total polyphenol content (TPC) for fruit peel samples, was determined using Waterman and Mole method (1994). In this method, 50 ml volumetric flasks were used, each containing 10 ml of water. Ten (10) ml of water, 0.5 ml of the sample extracts were added; 2.5 ml of the Folin-Ciocalteau's reagent was added. Within 2-8 min, 7.5 ml of sodium carbonate was added and the flasks were filled with water to 50 ml of volumetric flask mark. The flasks were swirled and allowed to stand for 2 h in the dark. The absorbance was measured at 760 nm using

Genesis 20 Spectrophotometer (Thermo Electron Corporation, Madison, USA). Data were calculated using a pre-prepared Gallic acid calibration curve. A stock solution was prepared by dissolving 0.1 g Gallic acid in 100 ml methanol. Results were expressed as milligrams of Gallic acid per 100 ml of sample extracts.

Determination of Total antioxidant activity

The ABTS assay was used to measure the total antioxidant activity (TAC) of the mango peel extracts. 8 mM ABTS and 3 mM potassium persulfate ($K_2S_2O_8$) were dissolved in 25 ml distilled water each, and then the equal volumes of the two were mixed. The reaction mixture was left to stand at room temperature overnight (12-16 h) in the dark before usage. The resultant intensely coloured ABTS (mother solution) was diluted with phosphate buffered (pH 7.4) solution to make a working solution. The assay was first carried out on Trolox, the water-soluble α-tocopherol (vitamin E) analogue, which served as standard. Working solution (2900 µl) was added to 100 µl serial Trolox dilutions, swirled and left to react for 15 minutes.

For sample analysis, dilutions were made by adding 1 ml of the sample extract to 4 ml of the solvent (methanol), and then 2900 µl of the working solution was added to 100 µl sample extracts, swirled and left to react for 30 minutes. Absorbance was measured at 734 nm using Genesis 20 Spectrophotometer (Thermo Electron Corporation, Madison, USA). The assay was performed by triplicates and data were calculated using a Trolox calibration/standard curve. Fresh ABTS solution was prepared everyday due to self-degradation of the radical. The results were expressed as µmol Trolox equivalents (TEAC).

Statistical analysis

All data were reported as mean ± standard error of three replicates. Analyses of variance (ANOVA) were performed on data using the GLM (General linear model) procedure of SAS version 8.0 (SAS Institute Inc., 1999). Differences at $P<0.05$ were considered significant. Treatment means found to were separated using Duncan's Multiple Range Test (DMRT).

RESULTS

Total phenolics composition of mango peels

The secondary metabolites composition of dried mango peels treated with sulphur at different concentrations showed that the total polyphenolic content at low concentrations (0, 10 and 20 ppm) was similar (Table 1). However, at 50 ppm of concentrations the composition of polyphenols was significantly different to the low and high concentrations tested ($P<0.01$) (Table 1). The trend was shown in all six cultivars evaluated.

There were differences in the polyphenols on the different mango cultivars ranging from 2.3 mg/100 mg of Keitt mango peels to 2.5 mg of Gallic acid/100 mg of Vhavenda mango peels (Table 2). The composition of polyphenols in Vhavenda mango peels was greater than that of the other five cultivars ($P<0.01$) (Table 2) whilst polyphenolic content of Peach, Saber, Tommy Atkins and Sunshine were significantly the same ($P<0.01$).

Total antioxidant capacity of mango peels

The total antioxidant capacity of dried mango peels treated with sulphur at different concentrations showed that the antioxidant activity at low concentrations (0, 10 and 20 ppm) was similar (Table 1), with the same trend shown in all mango cultivars. However, at 50 ppm of concentrations the antioxidants activity was significantly different to the low and high concentrations tested ($P<0.01$) (Table 1). At high concentrations (100, 150, 200, 250 and 300 ppm), total antioxidants activity values were similar to the low concentrations (Table 1).

There were no significant differences in the total antioxidant activity observed on the different mango cultivars ranging, with values ranging from 413.9 µmol/ g of Saber mango peels to 422.9 µmol/ g of Vhavenda mango peels (Table 2).

DISCUSSION

Our results demonstrated that dipping mango peels in 50ppm of SO_2 solution was effective in maintaining their bioactive compounds. In this way, previously it was demonstrated that at the same concentration can effectively preserve dried mango flesh with maximum proximate composition attained at this level, and the pretreatment of most fruits prior to drying as it enhances inactivation of pathogenic bacteria during dehydration (Mudau et al., 2013; DiPersio et al., 2006). Besides reducing the pathogenic bacteria, treatment in preservatives also maintains the colour of dried fruit products (Davidek et al., 1990) increasing their marketability. Simple pretreatment methods prior to drying of mango slices also lead to significant retention of β-carotene of which it has high amount of Vitamin A and antioxidative capacity (Muoki et al., 2009; Mercandate and Rodriguez-Amaya, 1998).

We found that mango peels possess high contents of the secondary metabolites than the flesh in this work. Similar results were reported by other authors, who found that mango peels containing high levels of polyphenols and dietary fiber (Larrauri et al., 1996; Kim et al., 2010). Also, it was reported that apple peels had higher antioxidant activities than the edible portion of the fruit

(Wolfe et al., 2003). In a recent study reported, it was suggested that mango peel extracts can inhibit adipogenesis likely to be due to higher concentrations and types of polyphenols in the peel extracts when they are compared with flesh extracts (Taing et al., 2012). For the same cultivars evaluated in this study, there are significantly more bioactive compounds in peels than the flesh (Mudau et al., 2013). Other studies have also shown the content of total polyphenol and antioxidant activities being higher in the peel than the pulp at any stage of mango fruit development (Lakshminarayana et al., 1979; Reyers and Vega, 1988; Ueda et al., 2000; Ajila et al., 2007) and several extraction techniques have been investigated for utilisation of these bioactive compounds (Ashoush and Gadallah, 2011; Palmeira et al., 2012, Meneses et al., 2013).

The variation in polyphenol content of peels of these cultivars as seen in this study may be associated to genetic factors (Mercandate and Rodriguez-Amaya, 1998) and also factors such as soil conditions (Rodríguez Pleguezuelo et al., 2012) and phytosanitary status (Tahir et al., 2002). This variability in polyphenol and antioxidant content has also been observed on mango flesh with causes extended to cultivar (Mercandate and Rodriguez-Amaya, 1998; Othman and Mbogo, 2009), cultivation practice (Hofman et al., 1995), climatic conditions (Léchaudel and Joas, 2006), ripeness at harvest (Jacobi et al., 1995; Lalel et al., 2003), and even postharvest storage conditions of the fruit (Hofman et al., 1997; Nunes et al., 2007).

Genetic variability in mango cultivars can ultimately lead to differences in carotenoids (Mitra, 1997) and anthocyanin (Lizada, 1991) content. Souza et al.

(2011) reports that certain Brazilian mango cultivars have genomic similarities of as little as 7% showing a significant genetic diversity that could contribute to phytochemical differences between mango fruit cultivars. These phytochemical different cultivars offer potential in diverse uses, including in obese individuals. In a study done by Taing et al. (2012), where mango peel extracts were reported to be more effective at inhibiting adipogenesis in 3T3-L1 pre-adipocytes, it was further found that the degree of inhibition was cultivar-dependent. This difference in activity could be due to genetic variability between mango cultivars resulting in different types or relative amounts of phytochemicals (Taing et al., 2013).

CONCLUSION

From this work, we conclude that the natural bioactive compounds can effectively accumulate if mango peels are preserved at 50ppm of SO_2 solutions in all cultivars evaluated. Also, from all cultivars studied in this work; Vhavenda have highest values of total polyphenols (2.5 mg/100 mg) and antioxidants (422.9 µmol/ g); and their peels could be used as a source of natural antioxidants. In general, mango peels with high total antioxidants activity showed the highest polyphenolic contents.

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Table 1. Total antioxidant activity value and polyphenols content of dried mango peels treated with sulphur (different concentrations)

Sulphur concentration (ppm)	Saber				Peach				Keitt				Tommy Atkins				Vhavenda				Sunshine			
	AA	Std err	TP	Std err	AA	Std err	TP	Std err	AA	Std err	TP	Std err	AA	Std err	TP	Std err	AA	Std err	TP	Std err	AA	Std err	TP	Std err
0	393.9^{b}	±0.2	2.0^{b}	±0.1	392.7^{b}	±0.2	1.9^{b}	±0.1	382.7^{b}	±0.2	1.9^{b}	±0.1	372.8^{b}	±0.2	1.7^{b}	±0.1	393.7^{b}	±0.2	1.9^{b}	±0.1	382.7^{b}	±0.2	1.9^{b}	±0.1
10	409.9^{b}	±0.3	2.1^{b}	±0.1	409.6^{b}	±0.2	2.2^{b}	±0.1	409.6^{b}	±0.2	2.3^{b}	±0.1	419.3^{b}	±0.2	2.3^{b}	±0.1	429.9^{b}	±0.3	2.3^{b}	±0.1	411.6^{b}	±0.3	2.3^{b}	±0.1
20	436.6^{b}	±0.2	2.3^{b}	±0.1	435.6^{b}	±0.2	2.3^{b}	±0.1	435.6^{b}	±0.2	2.4^{b}	±0.1	435.5^{b}	±0.2	2.5^{b}	±0.1	446.6^{b}	±0.2	2.5^{b}	±0.1	425.6^{b}	±0.2	2.4^{b}	±0.1
50	465.9^{a}	±0.2	3.2^{a}	±0.1	467.3^{a}	±0.2	3.3^{a}	±0.1	457.3^{a}	±0.2	3.4^{a}	±0.1	467.1^{a}	±0.2	3.7^{a}	±0.1	475.9^{a}	±0.2	3.8^{a}	±0.1	487.3^{a}	±0.2	3.4^{a}	±0.1
100	416.5^{b}	±0.2	2.3^{b}	±0.1	416.3^{b}	±0.2	2.4^{b}	±0.1	426.3^{b}	±0.2	2.2^{b}	±0.1	446.3^{b}	±0.2	2.4^{b}	±0.1	426.5^{b}	±0.2	2.5^{b}	±0.1	426.3^{b}	±0.2	2.2^{b}	±0.1
150	408.9^{b}	±0.2	2.5^{b}	±0.1	410.5^{b}	±0.2	2.5^{b}	±0.1	420.5^{b}	±0.2	2.3^{b}	±0.1	430.6^{b}	±0.2	2.5	±0.1	418.9^{b}	±0.2	2.5^{b}	±0.1	420.5^{b}	±0.2	2.3^{b}	±0.1
200	409.4^{b}	±0.2	2.2^{b}	±0.1	410.2^{b}	±0.2	2.3^{b}	±0.1	420.2^{b}	±0.2	2.2^{b}	±0.1	420.1^{b}	±0.2	2.2^{b}	±0.1	419.4^{b}	±0.2	2.4^{b}	±0.1	410.2^{b}	±0.2	2.3^{b}	±0.1
250	398.2^{b}	±0.3	2.4^{b}	±0.1	405.3^{b}	±0.3	2.3^{b}	±0.1	425.3^{b}	±0.3	2.2^{b}	±0.1	415.3^{b}	±0.3	2.3^{b}	±0.1	400.2^{b}	±0.3	2.4^{b}	±0.1	425.3^{b}	±0.3	2.3^{b}	±0.1
300	384.9^{b}	±0.2	2.7^{b}	±0.1	405.1^{b}	±0.2	2.6^{b}	±0.1	395.1^{b}	±0.2	2.1^{b}	±0.1	395.3^{b}	±0.2	2.1^{b}	±0.1	394.9^{b}	±0.2	2.2^{b}	±0.1	415.1^{b}	±0.2	2.2^{b}	±0.1

AA- total antioxidant activity (µmol/ g), TP- total polyphenol content (mg of Gallic acid/100 mg)
Means with different superscripts along the same column are significantly different (P < 0.01).

Table 2. Total antioxidant activity value and polyphenols content of dried mango peels (different cultivars)

Mango cultivars	Total antioxidants activity (µmol/ g)	Std err	Total polyphenols content (mg of Gallic acid/100 mg)	Std err
Saber	413.9^{a}	0.3	2.4^{b}	0.2
Peach	417.0^{a}	0.3	2.4^{b}	0.2
Keitt	419.2^{a}	0.3	2.3^{c}	0.2
Tommy Atkins	422.5^{a}	0.3	2.4^{b}	0.3
Vhavenda	422.9^{a}	0.3	2.5^{a}	0.1
Sunshine	422.7^{a}	0.3	2.4^{bc}	0.2

Means with different superscripts along the same column are significantly different (P < 0.01).