Optimization of drying temperature for mango peel with maximum antioxidant activity

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Abstract
The present study was carried out to study the effect of oven drying temperature on antioxidant activity of mango peel with reference to freeze drying. Freeze drying allowed the peels to retain maximum antioxidant activity. Using three sets of drying temperature along with freeze drying it was observed that oven drying at 60 °C had a negative effect on the antioxidant potential mainly due to an effect on the phenol, flavonoid and beta carotene content of peels.

Keywords: Mango peel, antioxidant activity, drying, phenol, flavonoid, beta carotene

Introduction
Mango (*Mangifera indica*) belonging to the family of *Anacardiaceae*, is one of the most important tropical fruit and India ranks first among the world’s mango producing countries accounting to about 52 per cent of world’s mango production. In India, about 1500 varieties are grown with 1000 commercial varieties. It is rich source of antioxidants, including ascorbic acid, beta carotenoid and phenolic compound. Flavonol and xanthone glycosides as well as gallotannins and benzophenone derivatives have been demonstrated to be present mainly in the peels, though pronounced inter-varietal differences have been observed in terms of quantitative composition of these compounds (Ribeiro *et al.*, 2008).

As mango is a seasonal fruit, about 20 per cent of fruits are processed for products such as puree, nectar, leather, pickles and canned slices, among others, which have worldwide popularity. During processing of ripe mango, peel is a major by-product and several tons of mango wastes are produced annually from factories. Peels contribute to 15 – 20 per cent of the fruit (Ashoush and Gadallah, 2011). Negro *et al.* (2003) reported that the mango peel is not currently utilized for any commercial purposes and is discarded as a waste, becoming a source of pollution. Huge amount of bio waste is produced by the food industries. In mango bio-waste processing, drying may be an essential step to inactivate enzymes responsible for degrading many active compounds and to decrease the rate of microbial growth. The researcher also proposed that this waste should be treated as a specialized residue due to high levels of phenolics, flavonoid and beta carotene content. Ajila *et al.* (2007) also reported that mango peel contains a number of valuable compounds such as polyphenols, carotenoids, enzymes and dietary fibre. U.S. Patent application US 2002/0187239 A1 have proposed the use of mango by-products as a source of nutritional constituents (Miljkovic and Bignami, 2002).
According to Laxmi and Radhapriya, (2005) among the various types of processing techniques, drying is considered to be the best as it is inexpensive, and imparts properties that are unmatched by other preservation technologies. According to Genin and Rene, (1995) vacuum freeze drying is the best method of water removal with the final products of highest quality compared to the other methods of food drying, as it is based on the on the principle of dehydration by sublimation of a frozen product. Due to the absence of the liquid water and the low temperature required for the process, most of the deterioration and microbiological reactions are stopped which gives the final product of an excellent quality.

Ratti (2001) points out that despite several advantages freeze drying has been recognized as the most expensive process for manufacture of dehydrated products. While, Ratti and Mujimdar (1997) observed that hot air drying providing uniformity and hygiene are inevitable for industrial food drying processes even today. Thus, in the present study freeze drying was taken as the reference to optimise oven drying temperature for stabilizing antioxidant activity of mango peels. However, drying temperature and time affect the activity and stability of bioactive compounds due to chemical and enzymatic degradation, losses by volatilisation and/or thermal decomposition as at higher drying temperature dehydration may lead to phenolic depletion, while carotenoids are mainly degraded due to large amount of oxygen and high temperature.

Radical scavenging activity and levels of polyphenolic compounds in mulberry leaves air-dried at 60 °C or below were not different from those of freeze-dried leaves, whereas both values in mulberry leaves air-dried at 70 °C and over decreased significantly (Katsube et.al., 2009). Wolfe and Liu (2003) highlighted that the air-dried and freeze-dried apple peel retained much better their phenols, flavonoids and anthocyanins (with similar contents to those of the fresh apple peel) than the oven-dried samples at 40, 60 or 80 °C. Therefore, drying conditions play an important role in determining the quality of the final product, especially in terms of its antioxidant activity. No information about the bioactive compounds’ stability and antioxidant activity of mango peel was found. The aim of this work was to study the effect of drying methods (freeze-drying and oven-drying) on the polyphenol and beta carotene content and antioxidant activity of mango peels. The three temperatures of oven drying viz., 40°, 50° and 60 °C were employed. Moreover, statistical methods were used to identify the oven drying temperature best suitable for preserving antioxidant activity of mango peel.

Materials and method

Mangoes of desi variety grown in Udaipur city were purchased from the local market, washed thoroughly with water and then peeled off the fruit. The peels were removed using a sharp knife and the underlying pulp removed by gently scraping with its blunt edge; peeling was done in a linear fashion of about 2- 3 inches size, so that uniform drying occurs. The peels were then subjected to thorough washing and then dried (Figure 1). Freeze dried samples were taken as reference. The dried mango peels were then grounded and sieved through a sieve to obtain a powder with a particle size of 841 microns.
Determination of antioxidant components

The total phenol content was determined according to Folin- Ciocalteu’s reagent method (McDonald et al., 2001). One gram of sample was ground with ten times the volume of 80 per cent ethanol which was then centrifuged for 20 minutes at 10,000 rpm to prepare the extract and the supernatant was collected. The residue was re-extracted and then evaporated to dryness. Then dissolved in known volume of distilled water and an aliquot of 1ml was pipetted out and made up to a volume of 3 ml, to this 0.5 ml of Follin-Ciocalteau reagent and two ml of sodium carbonate (20%) was added and placed in boiling water for just one min. The aliquot was then cooled and read at 650nm. The concentration of phenol was expressed as mg of catechol equivalent per g of sample against the standard curve of catechol.

Determination of beta carotene content

β carotene analysis was done using HPLC technique as suggested by Chiosa et.al., 2005. Five grams of samples was extracted with acetone: hexane (4:6). After the extraction, the solvent was evaporated to dryness under a stream of nitrogen and the residue was reconstituted with 1 ml of eluent solution (acetonitrile - tetrahydrofuran - methanol – ammonium-acetate (68.4 % (v:v) : THF 22.0 % (v:v) : 6.8 % (v:v) : 2.8 % (v:v) (1% (w:v)). The reconstituted sample was collected in a screw-cap for HPLC analysis. The flow rate of the mobile phase was 1.5 ml/minute and detection was done at 450nm by diode array method for 15 minutes. The analysis was carried out on chromeleleon client 6.80SR 11d build 3302 (196279) software.

Extraction method

The dried powder of mango peel was extracted individually by cold percolation method (Parekh and Chanda, 2007) using methanol to determine the flavonoid and antioxidant activity. Ten grams of dried powder was taken with 100 ml of methanol in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 120 rpm for 24 hours. After 24 hours, the extract was filtered with eight layers of muslin cloth; centrifuged at 5000 rpm for 10 mins. Supernatant was collected and the solvent was evaporated. Then 100 ml of methanol was added to the residue in the conical flask and plugged in with cotton and rotated on a rotatory shaker at 120 rpm for 24 hours. Again after 24 hours, the extract was filtered with eight layers of muslin cloth; centrifuged at 5000 rpm for 10 mins, the supernatant was collected and the solvent was evaporated and the dry extract was stored at 4ºC in air tight bottles. The residue so obtained was used for the determination of the flavonoid and antioxidant activity.

Determination of flavonoid content

The flavonoid content was determined according to aluminium chloride colorimetric method (Chang et. al., 2002). The reaction mixture consisted of a final volume of 3 ml, of which 1.0 ml was of sample (1 mg/ml) 1.0 ml of methanol and 0.5 ml of aluminium chloride (1.2 per cent) and 0.5 ml potassium acetate (120 mM) was incubated at room temperature for 30 mins. The absorbance of all the samples was measured at 415 nm. Quercetin was used as positive control. Flavonoid content was expressed in terms of quercetin equivalent (mg g⁻¹ of extracted compound).
Determination of antioxidant activity

The free radical scavenging activity was measured by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) by the modified method of McCune and Johns (2002). The reaction mixture consisting of DPPH in methanol (0.3 mM, 1 ml) 1 ml methanol and different concentrations of the solvent extracts (1 ml) was incubated for 10 min in dark, after which the absorbance was measured at 517 nm. Ascorbic acid was used as positive control (Blois, 1958).

Statistical analysis

Data were recorded as mean ± standard deviation. All the data were subjected to oneway analysis of variance (ANOVA) test to determine significant difference between the means. All the experiments were carried out in triplicate.

Results

Drying of the mango peel

Mango peel was dried via hot air oven at different temperatures viz., 40°, 50° and 60 °C and in freeze drier. The moisture content of freeze dried mango peel was found to be 3.82 ± 0.24 per cent while for those dried in oven ranged from 2.90 ± 0.96 to 3.94 ± 0.50 per cent (Table 1). Statistically no significant difference was found in the moisture content of dried mango peel. The results were in line with the findings of Hassan et al. (2011), who reported a moisture content of 3.90 per cent of the powder prepared by drying the peels of Bambangan variety of mango at similar temperature.

Findings of Sudhakar and Maini (2000) were observed to be higher, as the investigators reported the moisture content of dried mango peel in a range of 5.80 to 8.60 per cent. Ashoush and Gadallah (2011) documented a moisture content of 4.92 per cent in MPP which was slightly higher than the results of the present investigation. Similar results were indicated by Sheraji et al. (2011). The major reason for the difference in the moisture content of the dried mango peel might be owing to the difference in the drying method. Open air drying was used for drying of the samples which resulted in higher moisture content in comparison to the present investigation.

The drying time required for drying of the samples varied according to the temperature used while drying in oven. The time taken for drying of mango peel in oven ranged between 7 and 13 hours (Table 1). Significant difference was observed in the time taken for drying of mango peel at different temperatures. Doymaz (2004) indicated that the drying time of 9.5 to 13.5 hours was required for drying of carrot peel at 50° and 60 °C which are in agreement with the current findings. Chantaro et al. (2008) reported that the time needed for drying of carrot peel in oven at different temperatures (i.e., 60° – 80 °C) was in a range of 5 to 15 hours. While, Kulkarni and Vijayanand (2010) observed that drying of passion fruit peel at 60 °C was accomplished in 6 hours, which was slightly less than the time taken in the present study.
Table 1. Moisture content and drying time of mango peel

<table>
<thead>
<tr>
<th>Drying condition</th>
<th>Moisture content (g/100g)</th>
<th>Drying Time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T_{FD}</td>
<td>3.82 ± 0.24</td>
<td>23.00 ± 0.00</td>
</tr>
<tr>
<td>T_{OD1}</td>
<td>3.94 ± 0.50</td>
<td>13.00 ± 0.50</td>
</tr>
<tr>
<td>T_{OD2}</td>
<td>2.90 ± 0.96</td>
<td>9.00 ± 0.50</td>
</tr>
<tr>
<td>T_{OD3}</td>
<td>3.59 ± 0.24</td>
<td>7.00 ± 0.00</td>
</tr>
</tbody>
</table>

CD (P ≤ 0.05) 0.33 NS 1.45*

T_{FD} – Freeze dried, T_{OD1} – Oven dried at 40°C, T_{OD2} – Oven dried at 50°C, T_{OD3} – Oven dried at 60°C

NS – Non Significant, * - Significant

It is evident from the data presented in Table 1 that drying of mango peel in freeze drier took 23 hours, which was the maximum time needed for drying of mango peel. The reason for this might be that drying time differs depending on the sample surface area, volume, thickness, the vacuum obtained, the temperature of the collector, eutectic point and solute concentration of the sample. Carapella et al. (2001) documented that longer time may be required for drying of the samples as freeze drying involves freezing, primary and secondary drying processes involving formation of ice, sublimation of the ice core and complete vanishing of the ice core and the time required for sublimation of one gram of the ice core could be infinite. Asami et al. (2003) reported that freeze drying of marionberries and strawberries took 22 hours whereas corn required approximately 20-24 hours.

Antioxidant components

Phenol content

Phenols, are commonly found in fruits and have been reported to exhibit antioxidant activity and are termed as free radical terminators. Shahidi and Wanasundara, (1992) agree that phenols have the ability to scavenge free radicals, via hydrogen donation or electron donation. According to Lagouri and Boskou, (1996) and Kähkönen et al. (1999) among the plant phenolics responsible for antioxidant capacity, phenolic acids and flavonoids play the major role. Pourcel et al. (2007) documented that phenolic compounds have been found to protect against abiotic stress like excessive UV light and free radicals. A causative relationship between total phenolic content and antioxidant activity has been reported by Jayaprakasha and Patil, (2007) which is mainly due to the reactivity of the phenol moiety (Tabart et al., 2009).

Perusal of data in Table 2 indicates that the phenol content of MPP ranged between 81.08 ± 0.23 and 156.50 ± 0.50 catechol equivalents mg per gram. Significant difference was noted between freeze dried mango peel and those dried in oven at different temperatures. The results of the present findings are in conformity with the observations of Ajila et al. (2007) and Dorta et al. (2012). Berardini et al. (2005) observed that total polyphenols of Tommy Atkins variety of mango to be 129 mg /g in freeze dried peels, whereas in the present study it was found to be 103.73 ± 0.66 CE mg per g. In several studies lower phenol content have been reported, which might have been due to difference in the variety selected and the method of drying (Larrauri et al., 1996; Vergara-Valencia et al., 2007; Kim et al., 2010; Ma et al., 2011 and Palafox-Carlos et al., 2012).
The drying temperature was observed to exert an effect on the bioactive compounds. The phenolic content was then observed to increase with the drying temperature. This might have been owing to the fact that at high temperature bound phenolic compounds are released and also partial degradation of lignin occurs which could again have led to the release of phenolic acid derivatives (Larrauri, 1999). Similarly, Kim et al., (2010) concluded that the total phenolic content in whole grape seed extract and powdered grape seed extract were significantly increased by heat treatment. Que et al. (2008) highlighted that the formation of phenolic compounds at high temperatures might be because of the availability of precursors of phenolic molecules by non-enzymatic inter-conversion between phenolic molecules. VegaGálvez-Vega et al. (2009) also noticed an increase in the total phenolic content of red pepper while hot air oven drying. The difference in content of present investigation to the other researches can be attributed to the difference in the extraction solvents (Kalpana, 2011, Ma et al., 2011) as several investigators have shown a huge variation in phenolic content of same mango peel variety extracted via different solvents.

### Beta carotene content

Beta carotene is a very effective antioxidant present in foods. Its potentiality and role as an antioxidant has been established via several studies. Beta carotene content of MPP ranged between 112.51 ± 3.65 and 197.29 ± 7.02 µg per g (Table 2). Statistically at 5 per cent level of significance, significant difference was observed between MPP dried in oven at different temperatures. Ajila et al. (2007) who observed the total carotenoid content in mango peel in a range of 1400 – 3945 µg per g. The researchers estimated total carotenoid while in the present study only the beta carotene content was estimated which could be reason for difference in the content reported by Ajila et al. (2007). Decrease was observed in the beta carotene content with the increase in the drying temperature. Mohamed and Hussein (1994) also found that during dehydration of carrots, the loss of carotenoids were higher at 60°C than at 40°C as they were more sensitive to high temperatures. This might have been due to the fact that carotenoids are very sensitive to light, heat, air and other variables (Chiossa et al., 2005, Kalpana, 2011, Ma et al., 2011).

Similar results have been reported by Suman and Kumari (2002) and Kamel et al., (2013).
Flavonoid content

Flavonoids are very effective antioxidants. They are large group of naturally-occurring including flavones, flavonols, isoflavones, flavonones and chalcones. They contain a characteristic structure, with free hydroxyl groups attached to aromatic rings, and inhibit lipid oxidation by scavenging radicals. Pietta (2000) pointed that flavonoids are potent antioxidants with beneficial health effects. Yanishlieva Maslarova, (2001) documented that singlet oxygen quenching, metal chelation, and lipoxygenase inhibition are the possible mechanisms of flavonoids for preventing lipid oxidation.

The flavonoid content of the MPP ranged between 7.32 ± 0.32 and 13.00 ± 0.13 quercetin equivalents mg per g (Table 2). Highest content was found in freeze dried mango peel samples and lowest in mango peel dried at 60 °C. No significant difference was noted between samples of mango peel dried in oven at 40° and 50 °C whereas significant difference was found for the remaining samples (P ≤ 0.05). The results of freeze dried samples in the present study are in close proximity with Maisuthisakul et al. (2007) who documented total flavonoid content of mango peel as 14.60 ± 0.10 mg RE per g. Abu Bakar, et al. (2009) found total flavonoid content in Mangifera pajang to be 7.50 mg GAE / g in the mango peel. Kalpana (2011) also reported a flavonoid content of 10.24 mg per g in ripe mango peel which is in accordance with present findings.

Higher flavonoid content was observed by Kim et al. (2010), who studied the flavonoid content of ripe mango peel and reported a content of 21.16 mg RE/g. While lower content has been documented by González-Aguilar et al. (2007), Ma et al. (2011), Palafox-Carlos et al. (2012) and Ajila and Prasada, (2013) which portrays that the flavonoid content varies from variety to variety of mango.

The flavonoid content of MPP was observed to decrease with the increase in drying temperature. This decrease might have been due to thermal degradation (Maillard and Berret, 1995 and Larrauri, 1999). Im et al. (2003) reported that rutin content i.e., flavonoid content in buckwheat grit cakes decreased as the heating temperature or heating time increased. 
Harbourne, et al. (2009) also noted a decrease in the flavonoid content with the increase in temperature.

DPPH Antioxidant activity

Antioxidants are compounds in foods that neutralise chemicals called free radicals (unstable molecules) produced by oxidation. Antioxidant activity of a compound depends several factors such as antioxidant structure, composition of lipid fraction, availability of oxidants, presence of various other inhibitors or promoters of oxidation, presence of non-lipidic components, moisture, microstructure, temperature etc. DPPH assay measures the amount of free radical that is scavenged by the antioxidants present in the food.

DPPH radical scavenging activity of the MPP ranged between 64.40 ± 0.86 and 89.49 ± 0.50 per cent (Table 3). Maximum antioxidant activity was observed in freeze dried samples whereas minimum activity was noticed for samples dried in oven at 60 °C. No significant difference was found for MPP dried in freeze drier and in samples dried in the oven at 40° and
50 °C. But MPP samples dried in oven at 60 °C was found to differ significantly from the rest (P ≤ 0.05).

Vasco et al. (2008) reported the DPPH radical scavenging activity as 84.00 ± 3.00 per cent in mango peel (dry weight basis). Kim et al. (2010) also found that the DPPH radical scavenging activity of MPP was 81.86 per cent. While, Ribeiro et al. (2008) and Ayala-Zavala et al. (2010) reported lower antioxidant capacity of dried mango peel evaluated by DPPH assay.

Table 3  DPPH antioxidant activity of MPP

<table>
<thead>
<tr>
<th>Drying condition</th>
<th>DPPH antioxidant activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T_{FD}</td>
<td>89.49 ±0.50</td>
</tr>
<tr>
<td>T_{OD1}</td>
<td>85.52 ± 0.21</td>
</tr>
<tr>
<td>T_{OD2}</td>
<td>88.33 ± 0.61</td>
</tr>
<tr>
<td>T_{OD3}</td>
<td>64.40 ±0.86</td>
</tr>
<tr>
<td>CD (P ≤ 0.05)</td>
<td>4.63*</td>
</tr>
</tbody>
</table>

T_{FD} – Freeze dried, T_{OD1} – Oven dried at 40°C, T_{OD2} – Oven dried at 50°C, T_{OD3} – Oven dried at 60°C * - Significant,
Values are expressed on dry weight basis

Selection of the drying temperature

Based on the results demonstrated in Table 3 it is evident that the antioxidant activity of MPP in the present study decreased with increase in temperature beyond 50 °C, which might have been due to change in the content of phenol, flavonoid and beta carotene. Since the antioxidant activity was found to have no significant difference statistically when dried in oven at 40 and 50 °C and in freeze drier, 40 °C was selected for further study and is recommended as a suitable temperature for drying based on the fact that at higher temperature valuable components such as beta carotene, certain vitamins and minerals are lost due to their susceptibility to heat.

Several researchers have documented that antioxidant activity decrease with increase in temperature suggesting that appropriate drying temperature is essential for maintaining a high antioxidant activity. Studies conducted by Kahkonen et al. (1999), revealed that the flavonoids, isoflavones, flavones, anthocyanin, catechin, phenolics, and beta carotene were the majority of compounds showing the antioxidant activity of plant material. The results are in conformity with Reyes and Cisneros-Zevallos (2007) and Ruenroengklin et al. (2008).

Statistical analysis showed that no significant difference existed between freeze dried and oven dried samples at 40° and 50 °C, for antioxidant activity. Several studies have shown that drying temperature has an effect on the nutritional value of the foods with increasing temperature reduction in several nutrients like beta carotene etc., takes place (Di Scala and Crapistie, 2008; Galvez-Vega et al., 2009; Mirinda et al. 2010; Idah et al., 2010). A gradual decrease was observed in the phenol and flavonoid compounds with rise in temperature after 50 °C with an exception of beta carotene, which continuously decreased.
Larrauri, et al. (1997) studied the effect of drying temperature on polyphenol content and antioxidant activity of red grape pomace peel and reported that a decrease was observed a decrease of 18.60 and 28.00 per cent at 60 °C and 100 °C respectively. High-temperature minimised the antioxidant activity, total polyphenols and free polyphenols of broccoli owing to the negative effect of drying temperature on antioxidant activity (Mrkic, et al., 2006).

Garrau, et al. (2007) also studied the antioxidant activity of orange pulp and peels and observed drying at higher temperatures (i.e. 80° and 90 °C) or at temperatures which implied longer drying times (i.e., 30 °C) promoted a decrease of the antioxidant capacity. Drying at 80 °C reduced the antioxidant activity of winery waste by 21 per cent and at 100 °C by 33 per cent, with respect to drying at 60 °C (Lafka, et al., 2009). DPPH radical scavenging activity in mulberry leaves air-dried at 40° and 60 °C did not significantly differ from that of freeze-dried leaves, such scavenging activity in leaves air-dried at 70 °C and over decreased significantly (Katsube et al., 2009). Harbourne, et al. (2009) studied the effect of different drying conditions on herbs and found that freeze-drying, air-drying and oven or tray drying of herbs at 30 °C yielded extracts high in phenols, active ingredients and had a desirable colour for incorporation into a beverage.

Lopez et al. (2010) reported that with the rise in temperature the antioxidant activity increased up to a given temperature but later decreased. The antioxidant activity of winery waste was significantly affected by the temperature of drying. Kong et al. (2010) found that oven drying at 43.80 °C was the most efficient conditions for the drying of decanted pink guava by product with high lycopene content and antioxidant capacity. Similar results have been documented by other researchers. Hossain, et al. (2010) reported that vacuum oven-dried herbs did not show significant difference (P ≤ 0.05) in total phenol content and antioxidant capacity from the freeze-dried samples of herbs. Anwar et al. (2013) also reported that 40 °C was the most suitable temperature for drying of cauliflower in reference to the antioxidant activity and at the same time it also helped in preserving vital nutrients.

Conclusions

Mango peels can be stabilised through drying and also favour its capacity to inhibit lipid peroxidation and scavenge free radicals. This behaviour may be due to the fact that a large percentage of phenol, flavonoid and beta carotene are bound to the cellular structures. Results reveal that suitable drying temperature may help in preserving maximum antioxidant activity of mango peel. Similarly Lopez et al. (2010) reported that with the rise in temperature the antioxidant activity increased up to a given temperature but later decreased.
References


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Procurement of ripe mangoes of *desi* local variety

Washing

Separating the peel from the fruit manually

Washing of the peel

Oven drying at 40 °C, 50 °C and 60 °C

Freeze drying

Powdering of the dried sample and sieving

*Figure 1. Drying of mango peel*