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RESEARCH ARTICLE

Enhanced polyphenol content and antioxidant capacity in the edible portion of avocado dried with superheated-steam

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Abstract

In order toevaluatethe effects of drying methods on assay, this present study investigated the effect of superheated-steam drying at three different temperatures (130°C, 150°C and 170°C), in comparison with freeze drying on phenolic and flavonoid contents as well as antioxidant capacities in avocado (*Persea americana*Mill) pulp. The results showed that superheated-steam dried (SHSD) avocado pulp gave significantly higher total phenolic and total flavonoid contents compared to its freeze dried (FD) counterpart. The IC_{50} values from the DPPH assay were significantly lower (higher antioxidant activity)in SHSD pulp at all the temperatures used (6.69 – 12.16 mg/ml) compared to FD (35.02 mg/ml). The ORAC values also showed significantly higher radical absorbing capacitywhen SHSD at 170°C (26.58 µmol TE/1g)compared to FD (15.18 µmol TE/1g).The results showed that the drying methods significantly increased antioxidant capacity of SHSD avocado pulp in comparison with the FD.Superheated-steam drying at 170°C is proposed as an appropriate drying technique and condition to preserve pulp in avocado.

Introduction

The avocado (*Persea Americana* Mill) is sometimes known as the Alligator Pear, reflecting its shape and the leather-like skin. It is classified in the Lauraceae family and is native to Mexico and Central America (Chen et al., 2008).About 25% of the edible portion of avocado is comprised of lipid, which the principle components are monounsaturated fatty acids (Hierro et al., 1992; Swhisher, 1988). This type of fatty acids when added in a diet is well known to offer cardiovascular benefits. Many studies have also reported on the bioactive phytochemicals of this fruit. They include carotenoids (Lu et al., 2005), many phenolic acids and flavonoids (Kosińska et al., 2012; Rodríguez-Carpena et al., 2011). In medicinal usage, avocado has been applied in stimulating hair growth, wound healing, treating dysentery and diarrhoea as well as an emmenagogue and aphrodisiac(DerMarderosian and Beutler, 2002). Avocado fruit extracts have been reported to possess anticancer, antifungal and antioxidant activities (Domergue et al., 2000; Lu et al., 2005; Wang et al., 2010). Recent study has shown that addition of avocado oil in burger patties gave the highest oxidative stability compared to olive and sunflower oils, thus leading to possibility of manufacturing food products with enhanced nutritional properties (Rodríguez-Carpena et al., 2012).

In food industry, drying is an important process. Drying is involved in food preparation as well as to extent its shelf life. The nutrients and quality of dried food products, however, can significantly be affected by the drying process. One of the most common techniques applied in drying process of food materials is freeze drying. This drying method enables food to maintain its colour and nutrient composition, thus producing good quality product. However, this method of drying also comes with disadvantages.The initial investment cost for freeze drying is expensive andthe process also involves high energy consumption (Jiang et al., 2013; Zotarelli et al., 2012). Superheated-steam drying is a more recent and emergingmethod of drying, where steam with a temperature above the saturation or boiling point is applied as the drying medium. It offers advantages by saving energy consumption, improves production efficiency, involves no product oxidation and retains vitamin C content (Mujumdar and Law, 2010; Tang and Cenkowski, 2000). Previous investigations of superheated steam drying on assay for foods have mainly tended to focus on meat, fish, vegetable, and grain(Sa-adchom et al., 2011;Blasco et al., 1999;Choi et al., 2009;Niamnuy et al., 2011). Their studies have concentrated on food cooking and also activation of nutrition components by superheated-steam method. A study investigating on mangosteen fruit describes that antioxidant activity of xanthones in mangosteen rind was enhanced through the superheated steam treatment (P. Suvarnakuta et al., 2011). This study is one of a few that look at the effect of superheated steam drying on assay for fruit in nutrition field.Polyphenols and antioxidant activity in avocado have been reported in many studies involving fresh or freeze dried fruit, but no information is available on the effects of heat treatment on these content and activity in this fruit.

This study was therefore carried out to evaluate the phenolics and flavonoids contents as well as antioxidant activity in avocado undergoing heat treatment, in particularly superheated-steam. In our approach, effect of drying process was evaluated by application of superheated-steam drying and the commonly used freeze drying method was used as a comparison. These data will provide useful information on how different drying process can affect antioxidant capacity of fruit.

2. Materials andmethods

2.1 Chemicals

Folin-Ciocalteu's phenol reagent, gallic acid ($C_7H_6O_5$), sodium nitrite ($NaNO_2$), aluminum chloride ($AlCl_3$), rutin ($C_{27}H_{30}O_{16}$) were purchased from Sigma-Aldrich, Germany. Sodium carbonate (Na_2CO_3), sodium hydroxide (NaOH) were purchased from Merck, Germany. DPPH (2,2-diphenyl-1-picrylhydrazil) ($C_{18}H_{12}N_5O_6$), Fluorescein sodium salt ($C_{20}H_{10}Na_2O_5$), AAPH: 2,2'-Azobis(2-methyl propionamidine)dihydrochloride ($C_8H_{18}N_6$.2HCl), Trolox (6-Hydroxy -2,5,7,8-tetramethyl-chroman-2-carboxylic acid) were purchased from Sigma-Aldrich Inc., USA. Phosphate buffer saline (PBS) was purchased from Invitrogen Corporation, CA, USA. All chemicals were of analytical grade.

2.2 Sample preparation

Fresh avocado fruits (Hass avocado) were purchased from the local grocery store in Kitakyushu, Japan and left at room temperature to ripen naturally. Once ripened, the fruits were cleaned and the pulp was manually separated from the seed and skin. The pulp was then dried and the moisture losswas measured using Moisture Balance MOC-120H (Shimadzu Corporation, Japan).

2.3 Drying methods

Pulp taken from three kilogramof avocadofruit were divided into two portions, where each was subjected to superheated-steam drying or freeze drying in order to produce superheated-steam dried (SHSD) or freeze dried (FD) samples respectively. The SHSD process was conducted in a superheated steam oven (DC Quto QF-5200C, Naomoto, Japan) at different steam temperature of 130°C, 150°C and 170°C. The FD treatment was operated at -50°C in a freeze dryer (EYELA FDU-1200, Tokyo Rikakikai Co. Ltd., Japan). The pulp was dried until they reached the final moisture content of 10% maximum, measured using Moisture Balance MOC-120H (Shimadzu Corporation, Japan). Dried pulpwas ground to fine powder using Waring commercial blender 8011S (Connecticut, USA) and kept at -40°C until further use.

2.4 Sample extraction

One gram of dried sample wasextracted in 80% ethanol with ratio of solid:liquid was at1:30 (wt/vol). The mixture was ultrasonicated for 15 min using a 37kHz ultrasonic generator (UT-106, SHARP, Japan). Then the mixture was

centrifuged at 400 rpmat the temperature of 40°C for 30 min using Heidolph Instrument Unimax 1010DT orbital shaker, Germany. The extracts were then filtered using a Whatman No. 4 filter paper and the resulting ethanolic extract was used for further analysis.

2.5 Total phenolic content

Total phenolics were determined using a modified Folin-Ciocalteau colorimetric method (Waterhouse, 2002; Wolfe et al., 2008). A volume of 0.25ml of ethanolic extract was mixed with 1ml distilled water in a test tube. 0.25ml Folin-Ciocalteau reagent was added to the solution and allowed to react for 6 min. Then, 2.5ml of 7% sodium carbonate solution was added into the test tubes, and the mixture was diluted to 6ml with deionized water. Each sample was allowed to stand for 90 min, and the absorbance was measured at 760 nm usingUV-Vis spectrophotometer UV1601 (Shimadzu Corporation,Australia). The measurement was compared to a standard curve of gallic acid concentrations and expressed as milligrams of gallic acid equivalents (GAE) per 100g dried sample.

2.6 Total flavonoid content

The measurement of total flavonoid content was determined using a colorimetric method by (Wolfe et al., 2003)with slight modification. A volume of 0.5 ml of ethanolic extract was mixed with 2.5ml of distilled water in a test tube. This solution was then mixed with 0.15ml of 5% sodium nitrite solution. After 5 minutes, 0.3ml of 10% aluminum chloride solution was added. After 6 minutes, 1ml of 1M sodium hydroxide was added and mixed. The total volume of mixture was made up to 5ml with distilled water. The sample absorbance was read immediately at 510nm using UV-Vis spectrophotometer UV1601(Shimadzu Corporation, Australia). All measurements were compared to a standard curve of rutin solutions. The flavonoids content was expressed as milligrams of rutin equivalents (RE) per 100g dried plant sample.

2.7 DPPH radical scavenging activity

The DPPH (2,2-diphenyl-1-picrylhydrazil) radical scavenging activity of avocado pulp was carried out using colorimetric method (Othman et al., 2007)with modification. Briefly, 1 ml of ethanolic extract was mixed with 2 ml 0.15mM DPPH in ethanol. The mixture was left in the dark for 30 min before measuring the absorbance at 517nm using a UV-Vis spectrophotometer UV1601 (Shimadzu Corporation, Australia). The ethanol solution of DPPH served as a control. The percentage inhibition value was calculated according to the following equation:

Scavenging activity (%) = $[(A_0 – A_s) / A_0] \times 100\%$,

Where A_s is the absorbance of the sample and A_0 is the absorbance of the blank control. The inhibition percentage was plotted against the appropriate known concentrations and the sample concentration providing 50% inhibition (IC_{50}) of the DPPH radical was determined from this graph. All tests were carried out in triplicates.

2.8 Oxygen radical absorbing capacity (ORAC)

The peroxyl radical scavenging efficacy of the avocado pulp extracts were measured using the ORAC assay (Ahmad Aufa et al., 2014). Briefly, a volume of 150 μL of 10nM fluorescein in 10mM sodium phosphate buffer, pH 7.4 (working buffer) was added to each well in a black, clear-bottom, 96-well microplate. Then 25 μL of blank (working buffer), Trolox standard or sample was added to triplicate wells. No outside wells were used in order to avoid results with greater variation. The mixture was then incubated at 37°C for at least 10 min. 25 μL of freshly prepared 240 mM 2,2'-Azobis(2-methylpropionamidine)-dihydrochloride (AAPH) in working buffer were added using a 12-channel pipetter. The microplate was immediately inserted into a Fluostar Omega microplate reader (BMG LabTech GmbH, Ortenburg, Germany) at 37°C. The decay of fluorescence at emission wavelength 520nm was measured with excitation at 485 nm every 1.5 min for 3 hrs. The areas under the fluorescence versus time curve for the samples minus the area under the curve for the blank were calculated and compared to a standard curve of the areas under the curve for 25, 50, 100, 200 and 400 μM Trolox standards minus the area under the curve for blank.

ORAC value = $(AUC_{sample} – AUC_{blank}) / (AUC_{Trolox} – AUC_{blank})$, where AUC is area under the curve.ORAC values were expressed as mean micromoles of Trolox equivalents (TE) per 1g of dried fruit sample.

2.9 Statistical analysis

All experiments were performed in triplicates. The results were expressed as mean ± standard deviation (S.D). The experimental data were analyzed using analysis of variance (ANOVA) (Microsoft Excel 2010). The mean values

were considered at the 95% confidence level (p = 0.05). Correlation between the phenolic contents and the antioxidant activity was determined using IBM SPSS Statistics (version 19).

3. Results and discussion

In order to evaluate the effect of drying process in fruit, two types of different samples, i.e. SHSD and FD avocado pulp, produced by superheated-steam drying and freeze drying respectively were applied in the evaluation of total phenolic, total flavonoid and antioxidant activity of avocado fruit pulp in this research. It has been reported that the moisture content of avocado averages 74% (David Klein, 1998). The finding from this study also showed the same value in the pulp of avocado, which was at 74.43 ± 2.18% (n=3).

Table 1 shows percentage yield of avocado pulp dried with freeze dryer and superheated-steam dryer (at three different steam temperatures of 130°C, 150°C and 170°C). In SHSD samples, duration of the drying process taken for the pulp to reach their final moisture of ≤10% was 3 hr, which was much shorter than the FD samples (data not shown). The yield of the dried pulp averaged at 28.59% when SHSD and was at 26.97% when FD.SHSD technique made it possible to produce dried avocado pulp in a shorter time than FD technique.

Table 1. Percentage yield and final moisture of dried avocado pulp.

	Yield (%)	Final moisture (%)		
FD	26.97	4.03	±	0.83
[a,b]SHSD, 130°C	31.67	2.29	±	0.25
[a]SHSD, 150°C	27.18	2.89	±	0.53
[a]SHSD, 170°C	26.92	1.49	±	0.15

Results expressed as mean ±S.D. (n=3). [a] 3 hr SHS drying. [b] additional 30 min of drying time.

3.1 Total phenolic content

Ripened avocado contained the highest levels of phenolic compounds (Villa-Rodríguez et al., 2011). The total phenolic contents of the ripe avocado pulp were measured using the Folin-Ciocalteu method, which relies on electron transfer from phenolic compounds to the Folin-Ciocalteu reagent in alkaline medium (Singleton and Rossi, 1965).Figure 1(a) shows TPC of avocado pulp dried using freeze dryer and superheated-steam dryer (steam temperature of 130°C, 150°C and 170°C). In this study, the pulp showed the lowest TPC value when FD (520.55 mg GAE/100g dried sample). When SHSD, the high temperature ofthe superheated-steam applied in the drying process seemed to give a positive effect to the TPC in the pulp of avocado, where the TPC valueswere significantly higher at all three different temperatures (735.06 - 934.61 mg GAE/100g dried sample) compared to its FD counterpart. The TPC value in SHSD pulp increased with the increase in temperature, where the highest TPC was observed at the steam temperature of 170°C. The TPC values from this finding still shows higher TPC values in both SHSD and FD pulp compared to a study conducted on fresh pulp of avocado (Wolfe et al., 2008), indicating that dried avocado pulp may contain higher total polyphenols compared to the fresh ones.

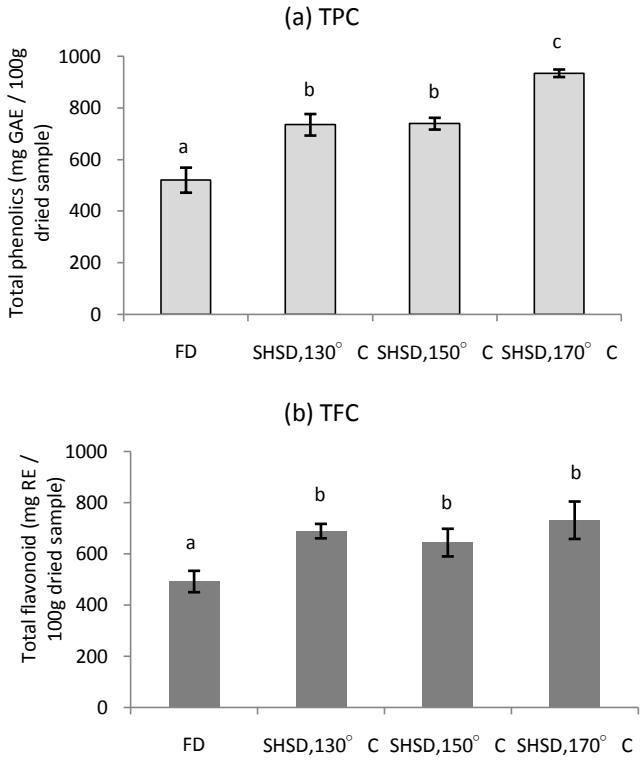

Figure 1. (a) Total phenolic content and (b) Total flavonoid content of avocado pulp dried under different conditions (mean ± S.D., n=3). Different letters indicate significant differences ($p<0.05$).

3.2 Total flavonoid content

Figure 1(b) shows TFC of avocado pulp dried using freeze drier and superheated-steam dryer at the temperature of 130°C, 150°C and 170°C. Just like in TPC, the same pattern was also observed in TFC, where the FD pulp gave the lowest TFC value (492.08 mg RE/100g dried sample), but this value rise significantly higher when the pulp was SHSD (643.92 - 731.31mg RE/100g dried sample). The highest TFC value was also observed in the pulp dried at the highest superheated-steam temperature used in this study. This is possibly due to the liberation of phenolic compounds from the matrix during the process.One plausible explanation for these differences in TFC is the thermal degradation of the ripe avocado pulpthrough higher temperature of SHSD(Nordin et al., 2013).

3.3 DPPH radical scavenging activity

In this study, inhibition concentration (IC_{50}), which is the amount of substance required to inhibit the initial concentration of DPPH radical by half was calculated from a series of dose-response data. The lower the concentration to deplete the DPPH, the better is the antioxidant activity displayed by the substance. The scavenging activity of the pulp showed a similar trend as portrayed by the TPC and TFC as discussed above.In this case, the high temperature of the superheated-steam seemed to increase the antioxidant capacity of the pulp by increasing its radical scavenging activity as indicated by the significantly lower IC_{50} (6.69 – 12.16 mg/ml) compared to its FD counterpart (35.02 mg/ml) (Table 2).The IC_{50} was cut down to almost half (45%) when the superheated-steam temperature was increased from150°Cto 170°C.In this study, superheated-steam drying seemed to be the more favourable method of drying for the pulp. A study conducted on tomatoes also shows that the sample dried under heat gave a higher DPPH radical scavenging activity compared to its FD and fresh sample (Chang et al., 2006).It is assumed thathigh temperature SHSD processing as similar as hot-air-drying process would deactivate oxidative and/or hydrolytic enzymes and avoid the loss of phenolic acids and, therefore, lead to the increase of TPC.

Table 2. Antioxidant capacities of avocado pulp.

	DPPH	ORAC
	IC_{50}(mg/ml)	µmol TE/1g

FD	35.02	±	3.41	15.18	±	1.85a
SHSD, 130°C	*11.79	±	0.42	5.37	±	0.76b
SHSD, 150°C	*12.16	±	0.47	15.13	±	1.24ac
SHSD, 170°C	*6.69	±	0.22	26.58	±	1.07d

Results expressed as mean ±S.D. (n=3).DPPH, 2,2-diphenyl-1-picrylhydrazil; ORAC, Oxygen radical absorbing capacity. *p<0.05 compared to FD sample.Different letters in the same group indicate significant differences (p<0.05).

3.4 Oxygen radical absorbing capacity (ORAC)

The ORAC assay measures the degree of inhibition of peroxy-radical-induced oxidation by the compounds of interest. It measures the performance of the compounds against a standard, Trolox (a water soluble derivative of vitamin E), and the results are reported in Trolox Equivalents (TE).The results show that antioxidant activity in FD pulp was comparable to the result given by SHSD pulp dried at 150°C (Table 2). However, when the superheated-steam temperature was increased to 170°C, this value was significantly increased by 43% (from 15.13 to 26.58 µmol TE/1g) in the SHSD pulp. The increasing ORAC value with the increase in temperature in this study indicated that just like in the DPPH radical scavenging activity, the high superheated-steam temperature applied during the drying process also enhanced the oxygen radical absorbing capacity of avocado pulp.

3.5 Correlation among phenolic content, flavonoid content and antioxidant capacities

The TPC and TFC when correlated with DPPH IC_{50}, Pearson correlation gave very strong correlation (r = -0.886 and -0.89 respectively). This suggests that the radical scavenging activity might be contributed by the phenolic and flavonoid contents in the avocado pulp. On the other hand, the TPC and ORAC values were moderately correlated (r = 0.492), while the TFC and ORAC values were modestly correlated with each other (r = 0.152).

In avocado, the pulp is the part of the fruit which is usually consumed for its nutritional benefit. Since the significantly higher TPC, TFC and antioxidant activities in avocado pulp, superheated steam drying might cause the damage of cell structures of avocado pulp and resulted in more increase of antioxidant components from the pulp itself (Huang et al., 2006).Studies have shown that thermally processed foods, especially fruits and vegetables, have higher biological activities due to their various chemical changes during heat treatment(Dewanto et al., 2002).Though the seed and skin contain the very high amount of polyphenols and antioxidant activities in comparison with the pulp(Wang et al., 2012), this study thus provides some advantages by highlighting that certain heat treatment such as superheated-steam can actually enhance phenolic and flavonoid contents as well as antioxidant activities in fruit pulps.Superheated-steam drying may be offered especially in food industry as an alternative drying method. The large amount of energy released from superheated-steam can be used in both heating and drying of food (Iyota et al., 2001). Yoshida & Hyodo (1966)demonstrated that drying food using superheated-steam yielded better colour, lower percentage of oxidisation and nutrient loss. Fraile & Burg(1997)also reported that superheated steam had great potential for high-starch foods. The lack of oxidative reaction during dehydration with superheated-steam could improve the quality of some food products (Wang et al., 2012).

4. Conclusion

The effects of drying methods and conditions on avocado pulp undergoing freeze drying and superheated-steam drying were compared in this study, where TPC, TFC and antioxidant activities in avocado pulp were evaluated. Even though freeze drying is a method well known to produce high quality food product, the results showed that all the values of TPC, TFC, as well antioxidant activities were enhanced when avocado pulp was dried with superheated-steam. The results showed that the polyphenols contents as well as antioxidant activities in avocado significantly increased during SHSD in comparison with FD. The presented results in this work strongly suggest that superheated-steam drying is a preferred drying method for avocado pulp as it enhanced its polyphenol contents and antioxidant activities. Unfortunately, very little is known about how antioxidant capacity correlates with the number of phenoliccompounds or the type of antioxidant found before and after SHSD.The increase inthe range of applications for superheated steam technology would encourage the growth of this area of research. Thepolyphenol contents in SHSD avocado pulp shallbe elucidated in upcomingstudies.In near future, we hope to identify the key antioxidant determinants by analysis of thermally degradablecomponents in avocado on these work characteristics.

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