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Effect of drying method and extraction solvent on the total phenolics and antioxidant activity of cauliflower (*Brassica oleracea* L.) extracts

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Abstract

Plants, being a rich source of medicinally important compounds such as antioxidants, have chemo-preventive role against the risk of oxidative stress-related diseases. There has been much interest in fruits and vegetable rich diets as a natural source of antioxidants and functional ingredients. As well as targeting plants high in antioxidant activity it is also important to optimize extraction parameters. Four extracting solvents, methanol, ethanol, aqueous methanol (80% v/v) and aqueous ethanol (80% v/v) were evaluated for their efficacy to extract antioxidants from cauliflower that had undergone different drying processes namely air-drying, sun-drying and oven-drying. There was a significant difference ($P < 0.05$) in the extracting ability of each of the solvents. The aqueous solvents were superior in their ability to extract the antioxidants and aqueous methanol was significantly more efficient than aqueous ethanol. This result was consistent across a number of parameters including extraction yield, total phenolic content and antioxidant activity. Furthermore, the samples drying process prior to extraction, also significantly influenced ($P < 0.05$) the extraction yield. Oven dried (40 °C) cauliflower had the highest yield of extractable antioxidants while air dried (ambient, approx 25 °C) had the lowest. Again, there was excellent correlation between extraction yield, antioxidant activity and total phenolic content.

Introduction

Plant-derived products contain a wide range of phytochemicals, including antioxidants, which are thought to have a protective role against risk of oxidative stress-related diseases such as cancer and cardiovascular diseases (Garcia-Alonso *et al*., 2004; Koksal and Gulcin, 2008). Therefore, a diet rich in vegetables and fruits and hence bioactive compounds, including natural antioxidants, has been associated with reduced risk of heart disease, cancer and diabetes (Dewanto *et al*., 2002a; Kaur and Kapoor, 2002). The *Brassica* vegetables (broccoli, cauliflower, cabbage, Brussels sprouts) have been identified as an excellent source of antioxidants, not only because of the high levels present but also because they are vegetables that are regularly included in the diet, consumed in relatively large amounts and are available worldwide. Much research has focused on the antioxidant activity of the Brassica vegetables, especially broccoli and cauliflower (Guo *et al*., 2001; Gulcin *et al*., 2004; Kaur *et al*., 2007; Koksal and Gulcin 2007). In addition to reports documenting antioxidant activity, the effect of food processing and storage on antioxidant activity has also been reported (Zhang and Hamauzu, 2004; Gliszcynska-Swiglo *et al*., 2006; Gawlik-Dziki 2008; Sultana *et al*., 2008; Roy *et al*., 2009). The key compounds that contribute to antioxidant activity have been identified and their concentrations measured by chromatographic methods (Koh *et al*., 2009; Lee *et al*., 2011).

Paralleling this increased awareness of the health benefits of a diet rich in antioxidants is an increasing concern about the use of synthetic additives, including antioxidants, in food. The commonly used synthetic antioxidants include butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butylhydroquinone (TBHQ) and the esters of gallic acid, and their sole purpose is to retard lipid oxidation. In response to these concerns, the potential of plant based extracts as a source of natural antioxidants for addition to food has been the subject of several

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research papers (Llorach *et al*., 2003; Gulcin *et al*., 2004; Liyana-Pathirana and Shahidi, 2006; Shahidi *et al*., 2007). The advantage of using naturally sourced antioxidants is their added role in protecting the body against cardiac diseases and cancer. Llorach *et al*. (2003) investigated the use of cauliflower by products (i.e. the leaves) as a source of bioactive extracts and found it to be rich in complex flavonoids. Shahidi's research group identified hazelnut by-products (Shahidi *et al*. 2007) and bran from the processing of cereal grain (Liyanal-Pathiriana, 2006) to be potential sources of natural antioxidants. Gulcin's research group have also investigated the potential of a number of plant products, herbs and spices as sources of natural antioxidants (Gulcin *et al*., 2004; Gulcin, 2006; Gulcin *et al*., 2007).

Extracting antioxidants from plant material most often involves the method of solvent extraction. The choice of solvent has been shown to have a significant influence on the concentration of antioxidants extracted (Sultana *et al*., 2009; Ahmad *et al*., 2011). On examining the literature relevant to the extraction of antioxidants from the *Brassica* vegetables, broccoli, cauliflower and curly kale, a full range of solvents, including water, ethanol, methanol, aqueous methanol and acidified methanol have been employed and in most cases without any experiments to determine optimal extraction conditions. However, investigations to determine the optimal solvent for extracting antioxidants are limited. In separate studies Koksal and Gulcin (2008) and Llorach *et al*. (2003) compared the solvents ethanol and water for the extraction of antioxidants in cauliflower. In each case ethanol was superior to water in extracting total phenolic contents (TPC). Also, Olsen *et al*., (2009) tested acetone and methanol as extracting solvents for the extraction of antioxidants from curly kale and found both had similar extraction efficiency.

Antioxidant activity and extraction yields of antioxidants have also been shown to be influenced by the drying procedure prior to extraction. Chan *et al*. (2008) reported that all thermal drying methods tested (microwave-, sun- and oven-drying) resulted in a decrease in the TPC of leaves and tea of ginger. Rhim *et al*. (2009) studied the effect of different drying techniques on the antioxidant activity of the herb, jiwhang, and also concluded that most forms of drying (including air-, hot air-, sun- and freeze-drying) had adverse effects on antioxidant activity. A study on the effect of drying conditions for broccoli suggested that, in fact, the critical factor was the drying time (Mrkic *et al*., 2006). The results suggested that shorter drying times (through use of higher temperature and increased air flow) maximised antioxidant activity.

Therefore, when attempting to identify potential sources of natural antioxidants, in addition to targeting plants high in antioxidant activity it is also wise to consider extraction and drying parameters. While much work has been conducted on the antioxidant content of cauliflower, there has been little work published on the effect of drying cauliflower prior to extraction or on the choice of extraction solvent. Therefore, this study evaluates how drying of cauliflower (air, sun and oven drying) prior to extraction and the subsequent extraction of antioxidants using four different solvent systems affect the TPC and antioxidant activity of cauliflower.

Materials and Methods

Reagents and standards

All the reagents used throughout the study were of analytical grade, and were procured either from Merck (Darmstadt, Germany) or Sigma Chemical Co. (St. Louis, MO, USA) unless stated otherwise. 1,1-diphenyl-2-picrylhydrazyl radical (DPPH•) (90.0%), linoleic acid, food grade synthetic antioxidant BHT (99.0%), Folin-Ciocalteu phenol reagent (2 N) were purchased from Sigma Chemicals Co (St, Louis, MO, USA).

Collection and processing of samples

Samples of cauliflower (*Brassica oleracea* L.), grown at Faisalabad, Pakistan without any pesticide application, at the same agricultural plots, were harvested randomly. Freshly collected samples were washed twice with tap water. Any remaining water was removed by air-blowing followed by chopping the samples into small pieces/slices (approx. 1 x 1 cm) using a steel chopper.

Drying of samples

The chopped vegetable material was divided into three portions (500 g each) to dry by different drying processes. One portion was air-dried (ambient conditions, 10 days), another portion was sun-dried (7 days) and a third portion was oven-dried at 40 ^{o}C (3 days). All samples were ground and the material that passed through 80-mesh was used for extraction purposes.

Extraction of antioxidant components

The antioxidants were extracted (in triplicate) from dried cauliflower material (20.0 g) using ethanol:water (80:20), 100% ethanol, methanol:water (80:20) and 100% methanol (200 mL). The extraction was performed by an orbital shaker for 24 hrs at room temperature and then the extracts filtered through filter paper (Whatman No. 1). The residue was re-

extracted with a further two aliquots of fresh solvent following the same procedure. The combined extracts were evaporated to dryness using a rotary evaporator (EYELA, SB-651, Rikakikai Co. Ltd. Tokyo, Japan). The crude concentrated extract was weighed and stored at 4°C.

Determination of total phenolic contents (TPC)

The TPC of each extract were determined spectrophotometrically using the Folin-Ciocalteu assay (Chaovanalikit and Wrolstad, 2004) and in triplicate. Briefly, 50.0 mg of the crude extract was mixed with Folin-Ciocalteu reagent (0.5 mL) and deionised water (7.5 mL). After 10 minutes at room temperature, 1.5 mL of 20 % sodium carbonate (w/v) was added. The mixture was heated in a water bath at 40 °C for 20 min and then cooled in an ice bath. The absorbance was measured at 755 nm using a UV/Visible spectrophotometer (U-2001, Hitachi Instruments Inc., Tokyo, Japan). Gallic acid standards in the range 10 – 100 ppm were treated in a similar manner to generate a calibration curve ($r^2 = 0.9986$). The TPC of the extracts were expressed as gallic acid equivalent (GAE) g/100 g dry weight.

DPPH• scavenging assay

The free radical scavenging activity of the extracts was measured using the method described by Iqbal *et al*. (2005). Briefly, to 1.0 mL of cauliflower extract (25µg crude extract in 1.0 mL methanol), 5.0 mL of freshly prepared methanolic solution of DPPH• (0.025 g/L) was added. The extract which reduced the initial DPPH• concentration by 50% was determined (25 µg crude extract in 1.0 mL methanol) and then used for the assay. The optimal time to record the fall in absorbance of the DPPH• solution on the addition of the antioxidant extract was determined by monitoring the absorbance (using a Hitachi U-2001 Spectrophotometer) at regular intervals (0, 0.5, 1, 2, 5 and 10 min). After 5 minutes the absorbance was stable and unchanging and this time was used to record the absorbance for all the extracts at a wavelength of 515 nm. The % DPPH• activity was calculated using the formula

% DPPH• activity= (A blank – A sample / A blank) × 100

A blank = Absorbance of DPPH• solution (containing all the reagents except test sample)
A sample = Absorbance of DPPH• solution, 5 minutes after adding the cauliflower extract.

Determination of antioxidant activity in a linoleic acid system

The antioxidant activity of the crude extracts was determined by measuring the inhibition of linoleic acid oxidation (Iqbal *et al*., 2005). Crude extract (5.0 mg) was added to a solution of linoleic acid (0.13 mL), 99.8% ethanol (10.0 mL) and 0.2 M sodium phosphate buffer (10.0 mL, pH 7). The mixture was made up to 25.0 mL with distilled water and incubated at 40°C for 360 hours. The extent of linoleic acid oxidation was measured using the thiocyanate method described by Yen *et al*. (2000). Briefly, ethanol (10.0 mL, 75% v/v), an aqueous solution of ammonium thiocyanate (0.2 mL, 30% w/v), incubated sample (0.2 mL) and ferrous chloride solution (0.2 mL, 20 mM in 3.5% HCl; v/v) were added sequentially. After 3 min of stirring, the absorbance values were measured at 500 nm using a spectrophotometer (U-2001, Hitachi Instruments Inc., Tokyo, Japan) and taken as peroxide contents. A control containing all reagents except the extracts was also prepared and the absorbance recorded. The synthetic antioxidant BHT was used as a positive control. Percentage inhibition of linoleic acid oxidation was calculated using the equation:

100 – [(increase in absorbance of sample at 360 h / increase in absorbance of control at 360 h) × 100].

Determination of reducing power

The reducing power of each extract at four different concentrations was determined according to the procedure described by Oyaizu (1978) with slight modification. Portions of each crude extract (2.5, 5.0, 7.5 and 10.0 mg) were mixed with sodium phosphate buffer (5.0 mL, 0.2 M, pH 6.6) and potassium ferricyanide (5.0 mL, 1.0%). The mixture was incubated at 50°C for 20 min. Then 5 mL of 10% trichloroacetic acid was added and the mixture centrifuged at 980 g for 10 min at 5°C (CHM-17; Kokusan Denki, Tokyo, Japan). The upper layer of the solution (5.0 mL) was decanted, mixed with 5.0 mL of distilled water and ferric chloride (1.0 mL, 0.1%) and the absorbance recorded at 700 nm using spectrophotometer (U-2001, Hitachi Instruments Inc., Tokyo, Japan).

Statistical Aanalysis

All experiments were analysed using analysis of variance (ANOVA) using SPSS (version 18). Equal variances between treatments were measured using Levene's test and where they were found to be unequal a log transformation was performed. Tukey's b multiple range test was performed to identify optimum treatments ($P < 0.05$).

Results and Discussion

The present study was conducted to evaluate the antioxidant activity of cauliflower (*Brassica oleracea* L.) and, in particular, it investigated the influence of different drying processes and extracting solvents had on antioxidant activity. Antioxidant evaluation was determined by several methods including measuring the yield of extraction, TPC by the Folin-Ciocalteau assay, percentage inhibition of linoleic peroxidation, DPPH radical scavenging activity and reducing power of the extracts.

Yield of extraction

The four extracting solvents, methanol, ethanol, aqueous methanol (80% v/v) and aqueous ethanol (80% v/v) were evaluated for their effectiveness to extract antioxidants from cauliflower that had been air-dried, sun-dried and oven-dried. There was a significant difference ($P < 0.05$) in the extracting ability of each of the solvents (Table 1). The aqueous solvents were superior in their ability to extract the antioxidants and aqueous methanol (30.0 g/100 g dry weight) was significantly more efficient than aqueous ethanol (27.5 g/100 g dry weight) towards extracting the antioxidants. Extraction with pure ethanol offered the least yield (7.5 g/100 g dry weight). These findings could be supported by other studies reported in the literature, where methanol and ethanol with some water content (typically 20 - 40%) have been found to be superior in extracting antioxidant compounds from a wide range of plants (Chatha *et al*., 2006; Chan *et al*. 2009; Katsube *et al*., 2009). Within the Brassica literature, the few studies that investigated optimal extraction solvents did not include aqueous organic solvents in their investigations (Koksal and Gulcin 2008; Olsen *et al*., 2009). One study compared ethanol and water (Koksal and Gulsin, 2008) and a second study, compared acetone and methanol.

Based on the trends of our present study, it could be suggested that aqueous based organic solvents are superior to recovering a higher extraction yield of antioxidant components from Cauliflower and thus might be considered in future extraction studies. However, it is important to point out that optimal extraction yield may not translate to higher antioxidant activity; the aqueous based solvents may just solubilise a larger range of compounds, some of which may have little or no antioxidant activity. The method of drying the cauliflower prior to extraction also significantly influenced ($P < 0.05$) the extraction yield (Table 1). Oven- dried (40°C) cauliflower had the highest extraction yield while air-dried (ambient, approx 25°C) had the lowest.

Table 1. Effect of extracting solvents and drying processes on the extraction yield (g/100g dry weight) from cauliflower (*Brassica oleracea*)

Extracting solvent	Extraction yield (g/100 g dry weight)		
	Air-dried	Sun-dried	Oven-dried
100% ethanol	$7.5 \pm 0.2^{a}_{a}$	$8.5 \pm 0.1^{a}_{b}$	$10.0 \pm 0.2^{a}_{cd}$
80% ethanol	$19.0 \pm 0.4^{b}_{a}$	$22.3 \pm 0.5^{b}_{b}$	$27.5 \pm 0.5^{b}_{d}$
100% methanol	$15.0 \pm 0.3^{c}_{ab}$	$16.5 \pm 0.4^{c}_{bc}$	$17.5 \pm 0.5^{c}_{cd}$
80% methanol	$22.5 \pm 0.4^{d}_{b}$	$25.5 \pm 0.5^{d}_{bc}$	$30.0 \pm 0.4^{d}_{cd}$

Values are expressed as means ± standard deviation (n = 3). Means with different superscript letters in columns and subscripts in rows indicate a significant difference using Tukey's B test ($P< 0.05$) for different extraction solvents and drying techniques, respectively.

Table 2. DPPH radical scavenging activity of cauliflower (*Brassica oleracea*) extracts obtained using different extracting solvents and drying processes

Extracting solvent	DPPH' scavenging activity (%)		
	Air-dried	Sun-dried	Oven-dried
100% ethanol	$62.6 \pm 0.2^{a}_{ab}$	$64.1 \pm 0.9^{a}_{bc}$	$65.2 \pm 0.5^{a}_{cd}$
80% ethanol	$66.0 \pm 0.6^{bc}_{a}$	$67.6 \pm 0.4^{b}_{b}$	$69.1 \pm 0.4^{b}_{c}$
100% methanol	$64.3 \pm 0.3^{ab}_{a}$	$66.4 \pm 0.3^{b}_{c}$	$67.3 \pm 0.2^{c}_{d}$
80% methanol	$67.4 \pm 0.2^{c}_{ab}$	$69.2 \pm 0.1^{c}_{bc}$	$70.0 \pm 0.9^{bc}_{cd}$

Values are expressed as means ± standard deviation (n = 3). Means with different superscript letters in columns and subscripts in rows indicate a significant difference using Tukey's B test ($P< 0.05$) for different extraction solvents and drying techniques, respectively.

DPPH radical scavenging assay

The DPPH radical is widely used as a reliable tool to measure free-radical scavenging and thus antioxidant activity of plant materials (Sanchez-Moreno *et al*., 1999). Cauliflower extracts obtained from air-dried, sun-dried, and oven-dried samples using different extraction solvents exhibited appreciable scavenging activity in the range 62.6-70.0% although less than that offered by BHT (80.0%) (Table 2). In support of these findings, Koksal and Gulcin (2008) reported DPPH radical scavenging activities of 51 and 64 % for water and ethanol cauliflower extracts, respectively. For this study, the DPPH radical scavenging activity varied considerably in relation to both the extracting solvents and drying processes. The oven-dried cauliflower extracted by aqueous methanol have the highest scavenging activity at 70.0%, however, ANOVA indicated that there was no significant difference between treatments.

Percentage inhibition of linoleic acid peroxidation

The measurement of inhibition of linoleic acid peroxidation was also used to measure antioxidant activity of cauliflower extracts. Linoleic acid (C18:2) is a polyunsaturated fatty acid, which forms peroxides by oxidation. The antioxidant activity of the crude extracts was determined by assessing their ability to protect linoleic acid from oxidation. The peroxides formed oxidize Fe^{+2} to Fe^{+3}, which can form a thiocyanate complex, the concentration of which can be determined spectrophotometrically at 500 nm. A high absorbance indicates a greater

Table 3. Percentage inhibition of linoleic acid peroxidation of cauliflower (*Brassica oleracea*) extracts obtained using different extracting solvents and drying processes

Extracting solvent	(%) Inhibition of linoleic acid peroxidation		
	Air-dried	Sun-dried	Oven-dried
100% ethanol	$58.9 \pm 0.7^{a}_{a}$	$66.8 \pm 0.4^{a}_{c}$	$70.0 \pm 1.0^{a}_{d}$
80% ethanol	$69.7 \pm 0.9^{b}_{a}$	$75.9 \pm 0.5^{b}_{c}$	$79.2 \pm 0.4^{b}_{d}$
100% methanol	64.0 ± 1.0^{c}	71.2 ± 0.3^{c}	75.3 ± 0.9^{c}
80% methanol	$74.5 \pm 1.0^{d}_{a}$	$80.3 \pm 0.7^{d}_{c}$	$85.0 \pm 0.4^{d}_{d}$

Values are expressed as means ± standard deviation (n = 3). Means with different superscript letters in columns and subscript in rows indicate a significant difference using Tukey's B test (P< 0.05) for different extraction solvents and drying techniques, respectively.

Table 4. Total phenolic content of cauliflower (*Brassica oleracea*) extracts (GAE g/100g dry weight basis) as affected by drying processes and extracting solvents

Extracting solvent	Total phenolic content (GAE g/100 g dry weight)		
	Air-dried	Sun-dried	Oven-dried
100% ethanol	$5.02 \pm 0.01^{a}_{a}$	$6.91 \pm 0.10^{a}_{c}$	$8.36 \pm 0.2^{a}_{d}$
80% ethanol	$6.24 \pm 0.60^{b}_{ab}$	$9.12 \pm 0.80^{b}_{bc}$	$10.12 \pm 0.5^{b}_{d}$
100% methanol	$5.99 \pm 0.8^{c}_{a}$	$8.00 \pm 0.4^{c}_{b}$	$9.02 \pm 0.5^{c}_{c}$
80% methanol	$7.68 \pm 0.2^{d}_{a}$	$10.36 \pm 0.3^{d}_{c}$	$11.36 \pm 0.4^{d}_{d}$

Values are expressed as means ± standard deviation (n = 3). Means with different superscript letters in columns and subscript in rows indicate a significant difference using Tukey's B test (P< 0.05) for different extraction solvents and drying techniques, respectively. GAE = gallic acid equivalent.

Table 5. Effect of different drying processes and extracting solvents on reducing power (as absorbance at 700 nm) of cauliflower (*Brassica oleracea*) extracts

Solvents	Concentration(mg/mL)	Air-dried	Sun-dried	Oven-dried
100% ethanol	2.5	$0.89 \pm 0.03^{a}_{a}$	$0.99 \pm 0.04^{a}_{ab}$	$1.20 \pm 0.07^{a}_{cd}$
	5.0	$1.00 \pm 0.07^{b}_{ab}$	$1.10 \pm 0.05^{b}_{b}$	$1.52 \pm 0.06^{b}_{d}$
	7.5	$1.13 \pm 0.07^{c}_{a}$	$1.24 \pm 0.06^{c}_{b}$	$1.78 \pm 0.07^{bc}_{c}$
	10.0	$1.20 \pm 0.06^{d}_{a}$	$1.84 \pm 0.07^{d}_{d}$	$1.99 \pm 0.05^{d}_{d}$
80% ethanol	2.5	$1.19 \pm 0.04^{a}_{a}$	$1.41 \pm 0.03^{a}_{b}$	$1.59 \pm 0.06^{a}_{c}$
	5.0	$1.47 \pm 0.04^{b}_{a}$	$1.67 \pm 0.03^{b}_{b}$	$1.96 \pm 0.02^{b}_{d}$
	7.5	$1.89 \pm 0.03^{c}_{a}$	$1.98 \pm 0.05^{c}_{ab}$	$2.30 \pm 0.05^{c}_{d}$
	10.0	$2.21 \pm 0.04^{d}_{ab}$	$2.40 \pm 0.06^{d}_{bc}$	$2.67 \pm 0.08^{d}_{d}$
100% methanol	2.5	$1.04 \pm 0.02^{ab}_{b}$	$1.18 \pm 0.07^{b}_{bc}$	$1.22 \pm 0.02^{a}_{bc}$
	5.0	$1.13 \pm 0.03^{b}_{a}$	$1.31 \pm 0.06^{b}_{b}$	$1.49 \pm 0.03^{b}_{c}$
	7.5	$1.20 \pm 0.04^{bc}_{a}$	$1.68 \pm 0.08^{c}_{c}$	$1.84 \pm 0.06^{c}_{d}$
	10.0	$1.84 \pm 0.07^{d}_{a}$	$2.10 \pm 0.04^{d}_{c}$	$2.50 \pm 0.06^{d}_{d}$
80% methanol	2.5	$1.38 \pm 0.05^{a}_{a}$	$1.47 \pm 0.04^{ab}_{b}$	$1.69 \pm 0.09^{ab}_{c}$
	5.0	$1.62 \pm 0.05^{b}_{a}$	$1.80 \pm 0.07^{b}_{c}$	$1.98 \pm 0.07^{b}_{d}$
	7.5	$1.90 \pm 0.06^{c}_{a}$	$2.21 \pm 0.04^{cd}_{c}$	$2.51 \pm 0.08^{cd}_{cd}$
	10.0	$2.25 \pm 0.03^{d}_{a}$	$2.53 \pm 0.06^{d}_{c}$	$2.80 \pm 0.08^{d}_{d}$

Values are expressed as means ± standard deviation (n = 3). Means with different superscript letters in columns and subscript in rows indicate a significant difference using Tukey's B test (P< 0.05) for different extraction solvents and drying technique, respectively.

magnitude of peroxides formed during the reaction; and consequently the lower the antioxidant activity (Sultana *et al*., 2007). Table 3 shows the peroxidation of linoleic acid after an incubation period of 360 h (15 days).

There was a significant difference in the percentage inhibition of linoleic acid peroxidation for each extract. The extracts which were obtained using aqueous based solvents exhibited the highest inhibition of oxidation and, as for extraction yield; the oven dried samples extracted with aqueous methanol exhibited the highest inhibition of oxidation (85.0%). The percentage inhibition of oxidation surpassed that of a BHT solution (80%) of the same concentration. Koksal and Gulcin (2008) also reported ethanol and water extracts of cauliflower having similar or superior percentage inhibition of oxidation when compared

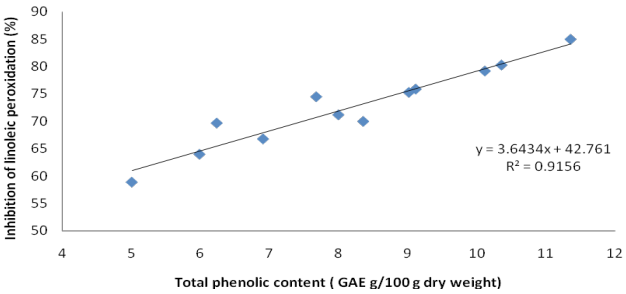

Figure 1. Plot of inhibition of linoleic acid peroxidation vs total phenolic contents

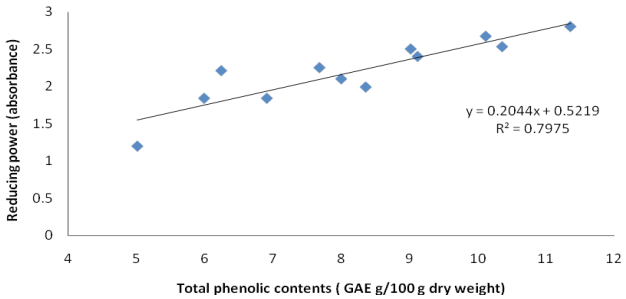

Figure 2. Plot of reducing power of cauliflower extracts (10 mg/mL) vs total phenolic contents

to the standards trolox and α-tocopherol. A plot of extraction yield versus percentage linoleic inhibition for all the extracts exhibited a strong correlation (r^2 = 0.805), indicating that the results of both extraction yield and percentage inhibition of linoleic acid assays strongly corresponded to each other. This supports the aspect that the aqueous organic solvent extracts of cauliflower with higher extraction yield have also higher percentage of inhibition of linoleic acid oxidation and thus superior antioxidant activity. The extract from oven- dried samples exhibited the highest inhibition of linoleic acid oxidation while that from air- dried the poorest (Table 3). This result is also consistent with the extraction yield data presented above.

Total phenolic contents (TPC)

The TPC of the different cauliflower extracts was determined by spectrophotometry using the Folin-Ciocalteau assay and expressed as gallic acid equivalent (GAE). Each solvent system did vary significantly ($P < 0.05$) in their ability to extract phenolic compounds (Table 4). Again, aqueous methanol was superior in extracting phenolics from cauliflower. A plot of TPC versus percentage of linoleic acid inhibition for all 12 extracts (varying in drying method and extracting solvent) exhibited a strong correlation (r^2 = 0.916) indicating that TPC is, in this case, also a useful predictor of antioxidant activity, see Figure 1. Llorach *et al*. (2003) reported similar findings for their study of ethanol and water based extracted of cauliflower. Interestingly, Koksal and Gulcin (2008) reported that TPC and antioxidant

activity (DPPH• assay) of ethanol extracts of cauliflower did not correlate strongly.

The TPC of air-dried, sun-dried and oven-dried cauliflower varied significantly ($P < 0.05$) with the oven-dried samples having the highest phenolic contents (Table 5). This effect of drying method has been consistently demonstrated with respect to antioxidant yield, antioxidant activity and TPC. What might be considered as the least aggressive drying method, sun- drying (approximately 25°C), has consistently produced extracts low in antioxidants whether it is measured as yield, activity or TPC and is independent of extraction solvent. In contrast, the more aggressive drying method, oven drying (40°C), consistently produced extracts high in antioxidants regardless of the extraction solvents used. The importance of a short drying time to maximise antioxidant activity of broccoli extracts reported by Mrkic *et al*. (2006) seems to be an important factor in this study also. The air-dried samples had been dried for 10 days, the sun- dried samples for 7 days while the oven-dried for 3 days indicating that as the length of drying time increased, antioxidant activity decreased. The use of higher temperatures for drying prior to extraction has been a focus of several studies (Dewanto *et al*., 2002a; Dewanto *et al*., 2002b; Katsube *et al*., 2009). Katsube *et al*. (2009) reported that a drying temperature of 60 °C (and lower) did not adversely affect TPC of mulberry leaf extracts, however, when temperatures of 70°C (and above) were employed TPC decreased significantly. Another study, this time involving tomatoes, thermal heating for 2, 15 and 30 minutes at 88°C had no significant effect on TPC (Dewanto *et al*., 2002a). While our study suggests shorter drying times (by employing heat, 40°C) is superior to an ambient- drying process, the point at which the temperature begins to significantly and adversely affect antioxidant activity is unknown and needs further investigation.

Reducing Power of extracts

The reducing power characterises the extract in the concentration range employed. For the extract concentrations, ranging between 2.5 to 10 mg/mL, the extracts from oven- dried cauliflower, consistently had superior reducing power. Likewise for the concentration range studied, the aqueous methanol extracts also had superior reducing power. Not surprisingly, the oven- dried cauliflower, extracted with aqueous methanol recorded the highest absorbance. There was a positive correlation between TPC and reducing power ($r^2 = 0.79$), see Figure 2.

Conclusions

Extraction solvent has a significant influence on the extraction of antioxidant compounds from cauliflower. Previous studies have mostly focused on single solvent system; however, this study clearly indicates that solvent systems involving both water and organic solvents are more effective towards recovering optimal amount of antioxidant components from cauliflower. This is supported by a number of experiments including: extraction yield, inhibition of linoleic acid oxidation, total phenolic contents and reducing power. Furthermore, a proper drying process, employed prior to extraction of the samples, can also significantly enhance the recovery of antioxidants from cauliflower.

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