

**TOTAL POLYPHENOL, FLAVONOID CONTENT AND ANTIRADICAL ACTIVITY
OF DRIED PARSLEY (*Petroselinum crispum*), CELERY (*Apium graveolens*)
AND DILL (*Anethum graveolens* L.).**

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Abstract

Parsley, celery and dill are good polyphenol source. It is necessary to define polyphenols in these products to evaluate the antiradical activity and to find the best extractant for this aim. The task of present research was to determine most appropriate solvent for polyphenol and flavonoid extraction. The following solvents- water; ethanol-water (1:1); pre-treatment with acetone and then ethanol-water (1:1); ethanol- acetone-water (7:7:6); methanol-water (1:1) was used for analyzing dried parsley, celery and dills, what were obtained using traditional convective and microwave-vacuum dryer. Total polyphenols, flavonoid content and antiradical activity was determined using JENWAY 6300 Spectrophotometer at 760 nm; 510 nm and 517 nm wavelength. Obtained results showed, that the best results was with ethanol-acetone-water mixture compared with other extractant. The higher polyphenol content was determined in traditionally dried celery and the higher flavonoid content was determined in traditionally dried dills.

Key words: parsley, celery, dill, extraction solvents, polyphenols, flavonoids, antiradical activity

INTRODUCTION

Plants are a good source of natural preparations containing effective bioactive compounds which can be used for different applications, particularly as food additives and health promoting ingredients in the formulations of functional foods and nutraceuticals (Ložiene et al., 2007). There is increasing interest in the industry and in scientific research for spices and aromatic herbs, because of their strong antioxidant and antimicrobial properties and are consumed as sources of fairly good amounts of several nutrients and it is widely accepted that they are significant nutritional sources of minerals. Other nutrients such as carotenoids and phenols are found in large amounts in these plants (Ozcan et al., 2005; Suhaj, 2006).

Products contain complex mixtures of polyphenols. Polyphenols are secondary plant compounds, which are divided into phenolic acids, flavonoids, stilbenes and lignans. They contribute to the taste, colour and nutritional value (Perla et al., 2012). Phenolic compounds have strong in vitro and in vivo antioxidant activities associated with their ability to scavenge free radicals, break radical chain reactions and chelate metals. Increased consumption of phenolic compounds has been correlated with reduced risk of cardiovascular diseases, certain cancers, has antioxidant and antimicrobial activities (Bonoli et al., 2004; Cetkovic et al., 2008)

In plants have been described more than 5000 different flavonoids. They are the most abundant polyphenols in human diets. There are six major flavonoid subgroups- flavones, flavonols, flavonones, flavanols, anthocyanidins and isoflavones. Their structural variation in each subgroup is partly due to degree and pattern of hydroxylation, methoxylation, prenylation or glycosylation. Most of the flavonoids

in plants are connected to sugars (glycosides), they can occasionally be found as aglycones (Dai & Mumper, 2010; Perla et al., 2012)

In addition to flavonoid free radical scavenging and antioxidant power, they have multiple biological activities, including vasodilatory, anticarcinogenic, anti-inflammatory, antibacterial, immune-stimulating, antiallergenic, antiviral and estrogenic effects, as well as being inhibitors of several enzymes such as phospholipase A2, cyclooxygenase, lipoxygenase, glutathione reductase and xanthine oxidase. However, the use of flavonoids in several domains is limited by their low stability and solubility in the fatty and aqueous phases (Chebil et al., 2006)

Free radical reactions occur in the human body and food systems. In the form of reactive oxygen and nitrogen species are an integral part of normal physiology. An over- production of these reactive species can occur due to oxidative stress brought about by the imbalance of the bodily antioxidant defence system and free radical formation. These reactive species can react with biomolecules, causing cellular injury and death. This may lead to the development of chronic diseases such as cancers and those that involve the cardio and cerebrovascular systems (Wong et al., 2006). Free radicals are known to take part in lipid peroxidation in food, which is responsible for rancid odours and flavours decreasing the nutritional quality. Therefore, synthetic and natural antioxidants are widely used in food industry as potential inhibitors of lipid peroxidation (Scherer & Godoy, 2009).

Minimally- processed vegetables and spices are a rapidly expanding segment of the fresh food industry due to convenience and increased demand by the consumers (Siddiq, 2013). The alternative of fresh product is their drying. Many of the published studies were conducted on the effect of dehydration on sensory qualities or some physical and chemical changes such as texture, color, flavour, aroma, taste of products (Ozcan et al., 2005).

Drying is the one of the storage methods, which has the capability of extending the consumption period maintaining products nutrition content. In drying process the moisture is removed by evaporation, this way the product can be stored for a long period, since the activities of the microorganisms, enzymes or ferments in the material are suppressed during drying (Alibas, 2007). High temperatures can destroy enzymes, vitamins, significantly reduce the polyphenols and determine the rancidity of fat- containing foods, but also can lead to the production of free radical scavenging substances, such as Maillard reaction products (Melanoids and Amadori rearrangement products). More frequently, in presence of suitable substrates, both reactions induced by the transformation can take place simultaneously and can influence each other (Vashisth et al., 2011; Dini et al., 2013).

More used method is convective dryer. The drying time of convective technique can be shortened by using higher temperatures which increase moisture diffusivity and by cutting products into small pieces. Increased drying temperatures entails higher costs and may cause biochemical changes that degrade the dried product quality; whereas subdividing the material in an additional process that results in mass losses and lowering of the product quality (Figiel, 2009).

Drying with the microwave method under vacuum is a modern, efficient method of food preservation. During microwave-vacuum drying the energy of microwave is absorbed by water located in the whole volume of the material being dried. This makes a large vapour pressure in the centre of the material, allowing rapid transfer of moisture to the surrounding vacuum and preventing structural collapse. As a result, the rate of drying is considerably higher than in traditional methods of dehydration. The decisive factor enhancing drying rate is the wattage of microwaves. Using this method, the rapid process of dehydration creates a porous texture food sample and stimulates obtaining a crispy and delicate texture, this way reducing the product's density as well as shrinkage (Rakcejeva et al., 2011). Microwave drying are gaining popularity of its inherent advantages over convective drying method such as reducing the

drying time of biological material without quality loss. In an industrial level, food processing using this technique has been reported to be both cost- effective and feasible (Arslan & Ozcan, 2010)

The extraction of bioactive compounds from plants is the first step in the utilization of phytochemicals in the preparation of dietary supplements or nutraceuticals, food ingredients, pharmaceutical and cosmetic products. Polyphenols can be extracted from fresh, frozen or dried plant samples. Usually before extraction samples are treated by milling, grinding and homogenization. The yield of chemical extraction depends on the type of solvents with varying polarities, extraction time and temperature, sample- solvent ratio as well as on the chemical composition and physical characteristics of the samples. The solubility of phenolics is defined by the chemical nature of the plant sample as well the polarity of used solvents (Dai & Mumper, 2010; Carrera, 2012).

For polyphenols and other antioxidants in plant materials three principal extraction techniques may be used: extraction using solvents, solid- phase extraction and supercritical extraction (Suhaj, 2006). Solvent extraction are used for antioxidant recovery from food material, but supercritical fluid extraction represents a viable alternative for solute extraction from natural matrixes because it offers solvent free products and prevails thermo degradation (Kitzberger et al., 2007)

Most used solvents can be petrol ether, toluene, acetone, ethanol, methanol, ethyl acetate and their combinations have been used for the extraction of phenolics, often with different proportions of water. Choosing the right solvent affects the amount and rate of polyphenols. Methanol is more efficient in extraction of lower molecular weight polyphenols (for catechin extraction) while the higher molecular weight flavanols are better extracted with aqueous acetone (e.g. better yield for procyanidins is obtained with 70% acetone). Ethanol is good solvent for polyphenol extraction and is safe for human consumption (Suhaj, 2006; Dai & Mumper, 2010).

The aim of this study is to study the changes of total polyphenol and flavonoid content, antiradical activity using different solvents on celery, dill and parsley dried using traditional convective and microwave-vacuum dryer.

METHODS AND MATERIALS

Plant materials and drying process

Vegetables were purchased from farm in Zemgale region, Latvia during vegetable seasoning. Then they were washed, removed no edible parts, peeled, cuted in equal small pieces. For each drying process was used about 1.00 kg of sample. One part of vegetables was dried in traditional convective dryer (Memmert, Modell 100- 800) at 45 ± 1 °C. Before microwave- vacuum drying all vegetables were steamed using a steam cooker (Tefal, Vitamin +, Model VC4003) and after that the samples were rapidly cooled in cool tap water. Celery, dill and parsley were steamed for 1.5 and 3.0 min. Steaming time was chosen based on spice colour and structure (Turkmen et al., 2005). The samples were dried using microwave-vacuum dryer (OOO Ingredient, Russia) at 35 ± 1 °C. The necessary amount of microwave energy was calculated using empirical formulas when the initial moisture of vegetables is known and the final is estimated ($9\pm 1\%$) (Rakcejeva et al., 2011).

Samples extraction

Sample extraction was made according to Chavan U.D. (2001) with modifications. Obtained dried sample was grounded using mechanical grinder. 3 g sample was extracted with 30 mL distilled water; 30 mL methanol- water (1:1) mixture; 30 mL ethanol- water (1:1) mixture; 30 mL acetone- ethanol- water (7:7:6) mixture for 30 min at 18 ± 1 °C. One extraction was made following-3 g sample was extracted with 30 mL pure acetone for 1 h, filtrated and residue was extracted with 30 mL ethanol- water

(1:1) solvent for 30 min at 18 ± 1 °C. All mixtures were filtrated and extracts were stored at 4 ± 1 °C till further analysis.

Determination of total polyphenol content

Total polyphenolic content was determined with Folin- Ciocalteu reagent according to Bozin B. (2008) with slight modifications. To 500 μ L of obtained extract was added 2.5 mL 0.2 M freshly prepared Folin- Ciocalteu reagent and 2.0 mL sodium carbonate solution (7.5 g $\cdot$ 100 mL distilled water) and mixture was kept in the dark at 18 ± 1 °C for 30 min to complete the reaction. The absorbance of the resulting solution was measured at 760 nm with a JENWAY 6300 Spectrophotometer. The concentration of total polyphenol content was expressed as mg gallic acid equivalents (GAE) per 100 g dried weight (DW), using a standard curve of gallic acid (120 mg $\cdot$ 1L). Analysis was triplicated.

Determination of total flavonoid content

Total flavonoid content was measured by a colorimetric assay according to Kim (2003) and Olivera (2008) with slight modifications. A 500 μ l aliquot spice extract or standard solutions of catechin was added in 5 ml flask containing 2.0 ml distilled water (H_2O), immediately added 150 μ l 5% $NaNO_2$. After 5 min 150 μ l 10% $AlCl_3$ was added. At 6 min 1.0 ml 1 M $NaOH$ was added to the mixture, then the reaction flask was diluted to volume with the addition of 1.2 ml distilled H_2O and mixed. Mixture was kept in the dark at 18 °C for 20 min and absorbance of the mixture was determined at 510 nm versus prepared blank solution (in extract place was used solvent) with JENWAY 6300 Spectrophotometer. A standard curve was constructed based on a range of catching concentrations (400 mg/l). Analysis was triplicated and results were expressed as mg catechin equivalents (CE)/ 100 g dry weight.

DPPH free radical scavenging assay

The antiradical activity of vegetable extracts was determined on the radical scavenging ability in reacting with stable 2, 2-diphenil-1-picrylhydrazyl (DPPH) free radical according to Afify (2012) with modifications. 4 mg of DPPH was diluted in 100 ml pure ethanol and 3.5 ml of this solution was added to 0.5 ml vegetable extract. This mixture was shaken and kept in dark at 18 ± 1 °C for 30 min, absorbance was measured at 517 nm using JENWAY 630 Spectrophotometer. The radical percentage was calculated using the following equation:

$$\% \text{ Antiradical activity} = ((A_{\text{blank}} - A_{\text{sample}}) / A_{\text{blank}}) \cdot 100$$

Statistical analysis

For the analytic data, mean values and standard deviation were reported. The data obtained were subjected to one- way analysis of variance (ANOVA) and least significant inference (LSD) was at $p < 0.05$.

RESULTS AND DISCUSSIONS

Total polyphenol content depends from chosen solvent and their mixtures with water. The amount vary widely for celery, parsley and dill samples from 671, 69 (celery dried in microwave-vacuum dryer, solvent acetone 1h +ethanol-water 1:1) to 2280, 75 (dill dried in conventive dryer, solvent water) mg GAE $\cdot$ 100 g DW (Table 1).

Table 1

Total polyphenol content on celery, parsley, dill dried in convective and microwave dryer using different solvents

Samples	Solvents				
	<i>water</i> (mg GAE ·100 g)	<i>acetone-ethanol-water (7:7:6)</i> (mg GAE·100 g)	<i>ethanol-water (1:1)</i> (mg GAE·100 g)	<i>methanol-water (1:1)</i> (mg GAE·100 g)	<i>acetone 1 h and ethanol-water (1:1)</i> (mg GAE·100 g)
Celery A	1006.53 ± 75.38	1944.44 ± 107.39	1680.44 ± 213.40	1226.10 ± 81.45	1708.33 ± 108.50
	879.36 ± 87.83	1059.62 ± 22.57	756.98 ± 74.66	1081.30 ± 183.00	671.69 ± 62.63
Parsley A	1346.49 ± 139.04	1754.93 ± 21.33	1488.70 ± 190.33	1341.00 ± 51.89	1429.65 ± 91.84
	671.61 ± 58.76	1237.08 ± 48.32	731.56 ± 80.31	841.12 ± 27.99	920.51 ± 68.21
Dill A	2280.75 ± 156.32	1124.68 ± 109.76	1451.96 ± 79.28	1595.35 ± 137.67	1484.76 ± 164.19
	1715.41 ± 44.09	731.28 ± 83.52	721.06 ± 188.86	1293.15 ± 39.05	1146.21 ± 64.39

(A- Samples dried in convective dryer; B- samples dried in microwave-vacuum dryer)

The highest polyphenol content was reached using acetone-ethanol-water and acetone (1 h)+ ethanol-water solvents. Higher polyphenol content was in samples dried in convective dryer comparing with samples dried in microwave-vacuum dryer. It can be explained, that in drying process samples in convective oven are dried for longer time period and polyphenols are forming as a result in Maillard reaction. According to Stankevičius M. et al. (2010), total polyphenol content in celery are 1637.1 ± 0.7 mg GAE·100 g DW, in parsley 1247.8 ± 0.9 mg GAE·100 g DW, in dill 1399.8 ± 2.4 mg GAE·100 g DW. Obtained our results compared data in literature (Stankevičius et al., 2010) are similar.

Total flavonoid content in analyzed spice extracts ranged from 110.00 (parsley dried in microwave-vacuum dryer, solvent methanol-water 1:1) to 1015.54 (dill dried in convective dryer, solvent water) mg CE ·100 g DW (Table 2)

From all used solvents, the best flavonoid content was reached using acetone-ethanol-water (7:7:6) solvent. The highest flavonoid content was in celery dried in microwave-vacuum dryer, but in other samples flavonoid content was lower. Using microwave-vacuum drying process, water was removed faster than in convective dryer. Pure water can not be considered as more suitable solvent, because they spoil in time faster than other analyzed solvents. According to Stankevičius M. et al. (2010), total flavonoid content in celery are 887.0 ± 4.1 mg CE·100 g DW, in parsley 119.0 ± 0.6 mg CE·100 g DW, in dill 650.1 ± 4.8 mg CE·100 g DW.

Radical scavenging activity was measured of analyzed samples on 2, 2- diphenyl-1- pycrylhydrazyl (DPPH) radical. In the DPPH assay, the ability of analyzed sample extracts are acting as donors of hydrogen atoms or electrons, transforming DPPH radical into its reduced form DPPH⁻ -H was analyzed. Results ranged from 8.57 (parsley dried in microwave-vacuum dryer, solvent methanol-water 1:1) to 91.93 (dill dried in microwave-vacuum dryer, solvent acetone-ethanol-water 7:7:6) % (Table 3)

Table 2

Total flavonoid content on celery, parsley, dill dried in convective and microwave dryer using different solvents

Samples		Solvents				
		<i>water</i> (mg CE ·100 g)	<i>acetone-ethanol-water (7:7:6)</i> (mg CE·100 g)	<i>ethanol-water (1:1)</i> (mg CE ·100 g)	<i>methanol-water (1:1)</i> (mg CE ·100 g)	<i>acetone 1 h and ethanol-water (1:1)</i> (mg CE ·100 g)
Celery	A	407,77 ± 21,71	430,20 ± 54,09	384,58 ± 8,19	344,94 ± 10,42	423,24 ± 38,67
	B	442,25 ± 12,59	495,69 ± 2,90	395,24 ± 13,61	384,71 ± 8,05	323,91 ± 12,13
Parsley	A	220,64 ± 22,48	202,16 ± 8,46	143,24 ± 22,79	204,94 ± 27,04	125,91 ± 15,13
	B	150,30 ± 41,84	181,76 ± 16,22	150,58 ± 22,11	110,00 ± 5,37	165,02 ± 31,73
Dill	A	1015,54 ± 38,89	438,04 ± 19,25	496,13 ± 22,12	370,92 ± 40,54	379,91 ± 9,51
	B	770,48 ± 103,70	273,14 ± 13,70	293,02 ± 12,71	428,62 ± 3,69	507,02 ± 14,11

(A- samples dried in convective dryer; B- samples dried in microwave-vacuum dryer)

Table 3

Sample antiradical activity on celery, parsley, dill dried in convective and microwave dryer using different solvents

Samples		Solvents				
		<i>Water (%)</i>	<i>acetone-ethanol-water (7:7:6) (%)</i>	<i>Ethanol-water (1:1) (%)</i>	<i>methanol-water (1:1) (%)</i>	<i>Acetone 1h and ethanol-water (1:1) (%)</i>
Celery	A	30,13 ± 21,27	80,14 ± 3,45	58,21 ± 11,35	48,48 ± 10,93	68,09 ± 4,74
	B	46,36 ± 16,92	86,55 ± 1,48	85,24 ± 2,31	76,80 ± 5,10	86,76 ± 0,51
Parsley	A	48,66 ± 6,26	74,57 ± 3,79	50,01 ± 17,44	55,75 ± 10,30	29,03 ± 2,24
	B	39,21 ± 17,45	65,52 ± 4,36	69,46 ± 6,49	8,57 ± 7,76	49,22 ± 2,51
Dill	A	19,45 ± 17,25	60,37 ± 3,57	50,12 ± 5,09	37,06 ± 17,74	52,63 ± 4,89
	B	68,18 ± 7,86	91,93 ± 2,75	62,75 ± 1,55	40,28 ± 16,77	87,21 ± 1,45

(A- samples dried in convective dryer; B- samples dried in microwave-vacuum dryer)

Comparing five different solvents, water extracts has not stable antiradical activity and samples spoiled faster than others. Highest antiradical activity can be reached using acetone-ethanol-water and acetone (1 h) + ethanol- water solvent. Using microwave-vacuum dryer, samples have higher antiradical activity and could be related with increased flavonoids content. From all samples, the best radical activity had celery dried in microwave-vacuum dryer, lowest radical activity had dill dried in convective dryer. According to Stankevičius M. et al. (2010), radical scavenging activity in celery are 71.6 ± 1.3 %, in

parsley 20.8 ± 0.9 %, in dill 51.7 ± 1.4 %. Obtained our results compared data in literature (Stankevičius et al., 2010) are similar.

CONCLUSIONS

The best solvent for reaching higher polyphenol amount from extraction process is mixture acetone-ethanol-water. Acetone, ethanol and water in different concentration may be considered as the best solvent for total polyphenol and flavonoid extraction for analyzed spices. Higher flavonoid content was reached using microwave - vacuum dryer on celery, but for parsley and dill higher flavonoid content was reached using convective dryer. Using microwave-vacuum drying, samples have higher antiradical activity.

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