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Isolation and optimization of polyphenols from the peels of orange fruit

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ABSTRACT

80% of cultivated oranges are processed in food industries generating huge amounts of industrial orange peel wastes abundant in polyphenols. In this study, we aimed to explore the potential to develop a commercially feasible extraction method to recover these polyphenols. Different drying techniques, extraction temperatures, solvents and duration were attempted. All the extracts were analyzed for total polyphenol content by FolinCiocalteu method. Though extraction of sundried samples with acidified aqueous methanol at 90ºC for 5 hours yielded the highest polyphenol content, for commercial purpose we recommend extraction of oven dried samples with aqueous methanol at 90ºC for 3 hoursas the process would be faster and more efficient with better control of the process parameters.

KEY WORDS: Orange peel, Polyphenol, FolinCiocalteu, Gallic acid.

1. INTRODUCTION

Natural antioxidants are preferred over synthetic antioxidants because of presumed safety, potential nutritional benefits and therapeutic effects (M.Lopez-Velez, 2003; Lydia A, 2002). Among all the natural antioxidants, polyphenols gain significance owing to their high redox potential which allows them to act as reducing agents, hydrogen donors and singlet oxygen quenchers (Marina Kajdzanoska, 2011). Moreover, polyphenols are abundantly present in our diet (Augustin Scalbert, 2000). The benefits of dietary polyphenols have been studied extensively over the last decade. They have been established to play a significant role in the prevention of degenerative diseases like cancer, cardiovascular, neuro diseases, inflammation, high blood pressure, cholesterol increase (Aleksandra Duda-Chodak, 2007) etc. Fruits, vegetables, leguminous plants and some cereals are rich sources of polyphenols. Polyphenols are often present in higher concentration in the outer non edible part of the fruits like peel compared to inner edible part (Gianmaria F, 2011; Joe A.Vinson, 1995; Kelly Wolfe, 2003; Joe A Vinson, 1998; 2001). Annually more than 55 million tons of oranges are grown globally out of which 80 percentage of the oranges produced are processed in industry for juice production. Citrus fruit extracts are used as functional ingredients in several industrial products (E.Belajova, 2004). All these industrial activities generate humongous quantities of wastes rich in polyphenol that are often disposed into the environment apart from using only as animal feed. Main phenolic constituents of Citrus fruits are flavonone and flavone glycosides, hydroxycinnamates, coumarines, psoralens and polymethoxyalted flavones (Maria de Lourdes, 2007; John A.Manthey, 2001). The objective of this study was to explore the potential of developing a commercially feasible extraction method to recover the maximum amounts of polyphenols from Orange peel. Different drying techniques, extraction temperatures, solvents and duration were attempted. All the extracts were analyzed for total polyphenol content by FolinCiocalteu method.

2. MATERIALS AND METHODS

2.1. Chemicals: FolinCiocalteu reagent and Gallic acid were procured from Hi media and Sigma Aldrich respectively. Other laboratory reagents including methanol, hydrochloric acid and sodium carbonate were obtained from Merck.

2.2. Samples: Outer Peels of fresh oranges purchased from nearby market were cut into small pieces of ~2mm X 2mm dimension using Stainless steel scissors. 50 g of the fresh peel was dried by each of the following different techniques, viz., freeze dried (FD) in lyophilizer at -50ºC for 36 hours (B B LI, 2006), oven dried (OD) at 50ºC for 36 hours and sundried (SD) for 2 days in bright sunlight. Also 50 g of the fresh peel was retained un dried (UD). Dried samples were stored in air tight containers at -18ºC till extraction (B B LI, 2006).

2.3. Extraction: Freeze dried (FD), oven dried (OD), sundried (SD) and un dried (UD) samples were removed from freezer and allowed to attain room temperature (RT). 250 mg of FD, OD & SD samples and 1000mg of UD sample were weighed and transferred into separate screw capped sample tubes. Modified methanol extraction (Joe A Vinson, 2001) was adopted to extract all the samples as methanol is a common industrial solvent. To each of the sample tubes, 25 mL of aqueous methanol (1:1) was added. Sample tubes were placed in $\pm$ 1ºC sensitive digital water baths set at 30ºC, 50ºC, 70ºC and 90ºC for the required duration. Samples were vortexed for 30 seconds at 30 minute intervals and replaced into the water bath. Finally samples were cooled to RT and made up to 50 mL with methanol and filtered using Whatman filter paper (No1). Filtrates were stored in air tight tubes at -18ºC till

the analysis was performed (BB Li, 2006). Extractions were done in triplicates. The above extraction procedure was repeated using 25 mL of acidified aqueous methanol (5 mL of 1.2 M Hydrochloric acid in water and 20 ml of 1:1 aqueous methanol) instead of aqueous methanol.

2.4. Estimation of polyphenol content: Polyphenol content was estimated by modified FolinCiocalteu method. In this test method, two strong inorganic oxidants namely phosphotungstic and phosphomolybdic acids (Roberto Stevanato, 2004)reduce the molecules by chemical oxidation. Gallic acid is used as standard owing to its high molar absorption of around 23,000. An economical A standard curve of Gallic acid with a coefficient of correlation of 0.99 [Figure1] was obtained using 10 ppm to 100ppm concentration solutions. 1mL of known dilution of the sample that were previously made up with methanol were taken in 10 mL volumetric flasks. 0.5 mL of FolinCiocalteu reagent was added and allowed to stand for 3 minutes. 1.5 mL of 10% Sodium carbonate was added and volume made up to 10 ml with water. The solutions were heated at 50ºC for 16 minutes. Samples were cooled to RT and absorption was measured at 765nm using a UV visible spectrophotometer (Perkin Elmer, Lamda35) with water as blank. Each sample was analyzed in triplicate. Sample absorbance was compared with standard graph obtained for gallic acid from which concentration of polyphenol present in each of the samples were calculated.

3. RESULTS AND DISCUSSION

Factors affecting the polyphenol content are a) drying method b) temperature of extraction c) duration of extraction and d) extraction solvent. Accordingly, orange peels were subjected to various drying techniques and dried peels were extracted at different temperatures and durations using two different of solvents. Total Polyphenols content in orange peel was estimated by modified FolinCiocalteu method and calculated equivalent to Gallic acid. The total polyphenol content obtained for all samples are given in Table 2. Color of the peel changed drastically during sun drying compared to oven drying. The color change and odor change were minimal on the freeze dried samples which even retained the characteristic odor of orange. Weight of the dried orange peel reduced from 50grams to 13.2 g, 13.6 g and 13.4 g for freeze drying, oven drying and sun drying respectively [Table1]. Irrespective of the drying technique, the samples lost around 70% of weight which could be attributed to loss in water content. However, different drying techniques do have an effect on the polyphenol content as evinced from the fact that sun dried samples (3.07% @ 90^0C) gave significantly higher values across temperatures followed by oven dried samples, freeze dried and undried (0.6% @ 90^0C) in decreasing order. Both the solvents exhibited very similar extraction properties for each of the different temperatures for all the samples. The total polyphenol content differed significantly with extraction temperatures. With increase in extraction temperature from 30ºC to 90ºC, the concentration of extracted polyphenol content increased between a range of 40 to 85 % irrespective of the solvent. Hence extraction temperature has a direct correlation to the total polyphenol content extracted. An attempt to reduce the duration of extraction to 1 hour showed that the amount of total polyphenol is significantly reduced while increasing to 5 hours had no significant effect indicating those 3 hours to be the optimum duration.

Table.1.Drying condition and loss on drying results of orange peel

Name	Drying condition	Initial weight(a)	weight of sample after drying(b)	Loss on drying= (a-b)*100/a
Sundried materials	Dried in sun for 2 days	50.0 g	13.6 g	72.8%
Oven dried material	Dried at 50 ºC for 36 hours	50.0 g	13.4 g	73.2%
Freeze dried materials	Dried at -50ºc for 36 hours	50.0 g`	13.5 g	73.0%

Of the four properties studied, the effect of solvent was minimal while the effect of drying, extraction temperature and duration were significant. From an industrial point of view, drying of the peel is recommended as it would reduce the bulk of the material for easy handling and enable efficient extraction. Since the results from oven drying was better next to sun drying, oven drying could still be preferred as the drying conditions can be more controlled compared to sun drying. As either of the solvents gave similar results, aqueous methanol which is easier to handle and recycle could be preferred compared to acidified aqueous methanol. Since the total polyphenol content was highest at 90ºC, optimization of the temperature between the ranges of 50ºC to 90ºC combined with other extraction techniques like soxhlet extraction etc is under the scope of future study to develop a more viable, cost effective and energy saving process in industry.

Table.2.Polyphenol content of orange peel (n=9) at different extraction temperature, time and extraction solvents

Extraction temperature^{o} C	Extraction solvents	Extraction time	Polyphenol content in mg/g			
			Freeze dried sample(FD)	Sundried sample (SD)	Oven dried sample (OD)	Un dried sample(UD)
90	Aqueous methanol	Three hours	1.74	3.07	2.57	0.57
70	Aqueous methanol	Three hours	1.59	2.71	2.00	0.49
50	Aqueous methanol	Three hours	1.28	2.69	1.63	0.40
30	Aqueous methanol	Three hours	1.09	2.15	1.42	0.34
90	Acidified aqueous methanol	Three hours	1.96	3.26	2.22	0.57
70	Acidified aqueous methanol	Three hours	1.56	2.80	2.03	0.51
50	Acidified aqueous methanol	Three hours	1.20	2.55	1.66	0.39
30	Acidified aqueous methanol	Three hours	1.34	2.16	1.59	0.40
90	Aqueous methanol	One hour	1.01	1.94	1.47	0.34
90	Acidified aqueous methanol	One hour	1.64	2.83	1.85	0.32
90	Aqueous methanol	Five hours	1.82	3.30	2.56	0.60
90	Acidified aqueous methanol	Five hours	2.10	3.31	2.53	0.62

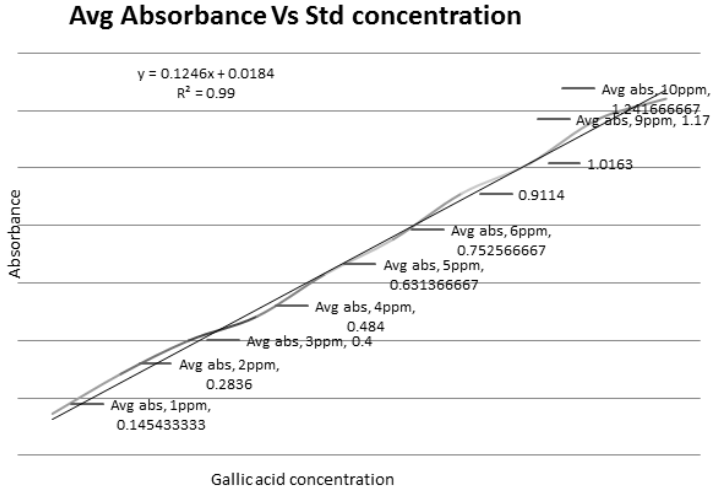
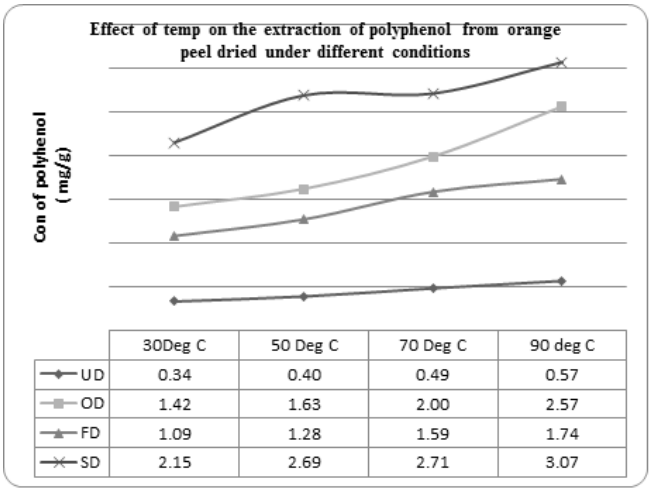
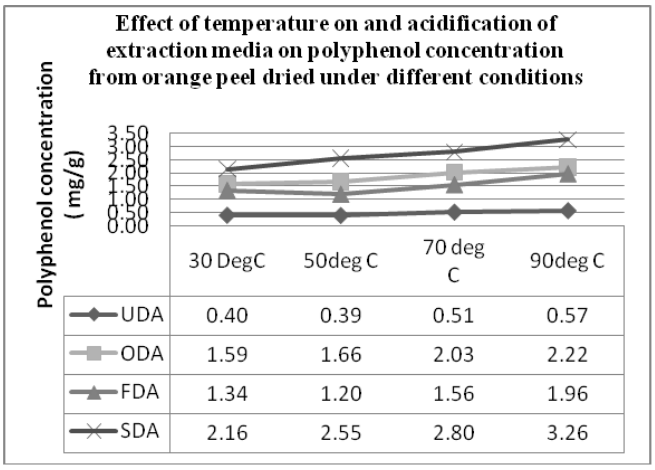


Figure.1.Gallic acid standard graph (mean SD, n=3).Gallic acid standard solution were prepared from 1 ppm to 10ppm and analysed by applying Folin Ciocalteu test method and the absorbance was read at 765nm using water as blank



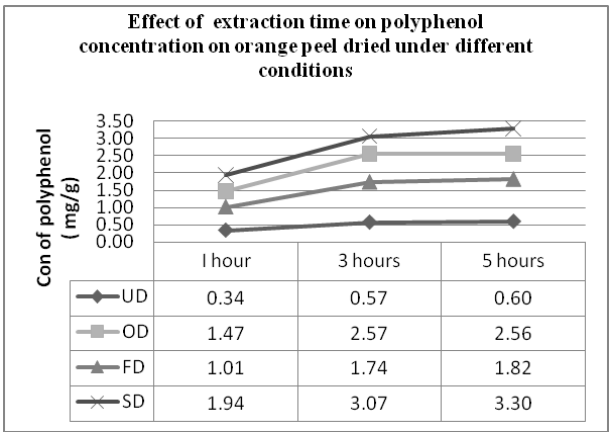
	30Deg C	50 Deg C	70 Deg C	90 deg C
UD	0.34	0.40	0.49	0.57
OD	1.42	1.63	2.00	2.57
FD	1.09	1.28	1.59	1.74
SD	2.15	2.69	2.71	3.07

Figure.2.Total Polyphenol content of orange peel (mean SD, n=9).Orange peel dried under different conditions were extracted under different temperature using aqueous methanol Extracted samples were analyzed for polyphenol content by using FolinCiocalteu method and the content of polyphenol obtained are represented inline graph.



	30 DegC	50deg C	70 deg C	90deg C
UDA	0.40	0.39	0.51	0.57
ODA	1.59	1.66	2.03	2.22
FDA	1.34	1.20	1.56	1.96
SDA	2.16	2.55	2.80	3.26

Figure.3.Total Polyphenol content of orange peel (mean SD, n=9).Orange peel dried under different conditions were extracted under different temperature using acidified aqueous methanol .Extracted samples were analyzed for polyphenol content by using FolinCiocalteu method and the content of polyphenol obtained are represented in line graph.



	I hour	3 hours	5 hours
UD	0.34	0.57	0.60
OD	1.47	2.57	2.56
FD	1.01	1.74	1.82
SD	1.94	3.07	3.30

Figure.4.Total polyphenol content of orange peel (n=9). Orange peel dried under different conditions was extracted at different time points using aqueous methanol. Extracted samples were analyzed for polyphenol content by using FolinCiocalteu method and the content of polyphenol obtained are represented in line graph.

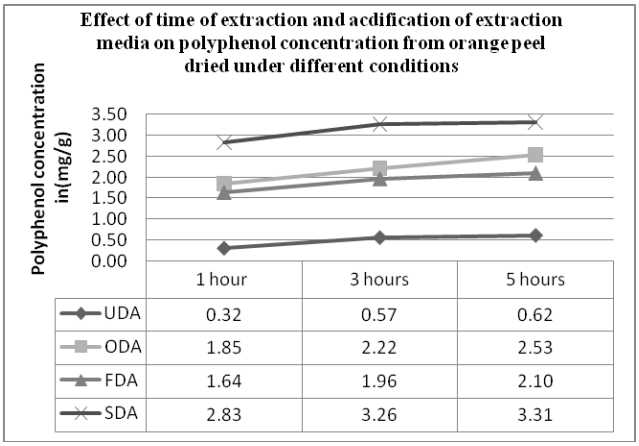


Figure.5.Total polyphenol content of orange peel (n=9). Orange peel dried under different conditions was extracted at different time points using acidified aqueous methanol. Extracted samples were analyzed for polyphenol content by using FolinCiocalteu method and the content of polyphenol obtained are represented in line graph

4. CONCLUSION

It has been established that polyphenols could be extracted from throw away orange peels. While extraction solvents used in the study had very minimal effect on the total polyphenol content, drying technique and extraction temperature and duration has been shown to have a pronounced effect. To arrive at commercial feasible extraction method, further study is being done incorporating the above findings with other extraction techniques.

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