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Quantification of Flavonoids from different Parts of Grapefruit (Citrus x Paradisi) from different Extraction Methods

Aarzoo Jangra[1] and Dr. Bimla Pawar[2]

[1]Ph.D. Scholar, Department of Food Technology GJUS&T HISAR (HARYANA)
[2]Assistant Professor, SRCASW, University of Delhi
E-mail: [1]aarzoo.jangra00@gmail.com

Abstract—*In this work flavonoid content of different parts of grapefruit extracted from different extraction techniques were compared. The grapefruit peel, pulp and seed were dried in hot air oven and ground to fine powder. Dried grapefruit powder was extracted with solvent (methanol) to determine the flavonoid content in the grapefruit peel, pulp and seed. The hplc was applied for determination of flavonoids under 2 types of extraction methods i.e; sonication and soxhelet extraction from different parts of grapefruit i.e peel, pulp and seed. Different flavonoids i.e, epicatechin, rutin, hesperidin, daidzein, quercetin, apigenin and naringin were studied. It was found that pulp, peel and seed of grapefruit were a rich source of bioactive compounds including flavonoids. All the extraction strategies including traditional (SAE) and present day (UAE) at the upgraded states of extraction were observed appropriate for evaluation of different flavonoids. UAE was found most suitable for maximum extraction of individual flavonoids. UAE take less time, less solvent and less utilization of heat so UAE was more suitable for extraction. UAE showed the advantage in terms of high efficacy and short time was used for extraction.*

Keywords*: Grapefruit, Flavonoids, Extraction, Soxhelet, Ultrasonication*

1. INTRODUCTION

Bioactive compounds are accepting increasingly being particles from natural sources. These can be utilized as an additive in a substantial number of items in various industries like food and cosmetics (USDA data 2004). Natural bioactive mixes are also in huge demands as individuals are more worried about their wellbeing and wellbeing giving diets (Hertog *et al*., 1993).

They have been found to have numerous helpful properties like anti-inflammatory, (Toshio *et al*., 2002), anti-allergic (Cottiglia *et al*., 2001), oestrogenic activity (Bohm et al., 1998; Manach et al., 2005) anti-cancer activity, antitumour action, protein restraint movement, and oestrogen.

Citrus by-products are a vital source of flavonoids. The principle flavonoids found in citrus species are hesperidin, quercetin and naringin (Schieber *et al*., 2001). Surprisingly consideration is focussing on the extraction of pure flavonoids from these by-products. (Baghurst, 2003; Manthey and Guthrie, 2002; Middleton et al., 2000).

High Potential Liquid chromatography (HPLC) has been being used for quantitative analysis and measurement of plant auxiliary metabolites for so many years. HPLC combined with a PDA locator has been the most broadly utilized instrument in the field of agribusiness Ross et al. (2000). Ultra performance liquid chromatography (UPLC) is the special version of HPLC where resolution, speed and sensitivity are very high as compared to the HPLC (Chawla and Ranjan, 2016).

Peel residues from sweet and bitter grapefruits have observed to be an important source of phenolic acids and flavonoids, chiefly polymethoxyflavones (pmfs), flavanones, and glycosylated flavanones (Gorinstein *et al.,* 2001). These bioactive compounds are strongly related to therapeutic properties including antiallergenic, antiatherogenic, anti-inflammatory, antimicrobial, anticarcinogenic, antithrombotic, cardioprotective, and vasodilatory effects. (Middleton *et al.,*2000).

A comparison of commonly used extraction techniques and extraction solvents for the extraction of grapefruit's bioactive compounds (flavonoids) and responses of individual compounds to different techniques was the main aim of this investigation. Extracts obtained at optimized conditions using methanol, in case of soxhlet-assisted extraction (SAE), ultrasound-assisted extraction (UAE) were subjected to UPLC for quantification of flavonoids and phenols after clean-up.

2. COLLECTION OF SAMPLES

Grapefruit that was analyzed in this study were procured from local market of Dehradun (Uttrakhand), India in the month of December (2017) and January (2018). In order to investigate

the flavonoids content from different plant parts like the seed, pulp, peel. Sample collected according to availability.

Selected grapefruits were subjected to drying in open air for removal of water droplets from the outer surface and further analysis was carried out as follows.

2.1 Preparation of sample

Peel, pulp and seed of grapefruit were separated and stored separately for further work. For initial oven drying and freeze-drying, 1 kg of grapefruit was taken. After that with the help of knife seed, pulp and peel of grapefruit were separated.

2.2.1 Tray drying method

Grapefruits (seed, pulp and peel) were dried in tray dryer (Oven 300, NSW 354) at 45°C for 3 days. Dried samples were ground using a commercial grinder and were stored in air-tight glass containers separately at 4°C for further analysis.

2.3 Preparation of extract

For extraction, Ultrasonicator and solvent extractor type of extraction techniques were used (Zhishen, et al. 1999) method was used. After extraction filtration was done and combined crude were stored in amber bottles in refrigerated conditions to prevent oxidative damage until further analysis.

2.3.1 Ultrasonicator

One gram powder was accurately weighed and placed in the sealed vessel by adding 70 ml of 90% methanol solvent, and then the vessel was placed in an ultrasonic cleaning bath (model no Power-Sonic 410) for extraction for 60 min at (40° C). After extraction, The mixture was filtered through Whatman filter paper no. 1 for the removal of peel, pulp and seed particles. The extracts were filtered and evaporated to dryness by the oven at 40^0c. The extract was stored in amber coloured glass bottles at refrigerated temperature for further analysis.

2.3.2 Solvent extractor

Powder of the sample (1 gm) was accurately weighed and placed in a thimble. The 90% methanol solvent poured, followed by extraction for 2 hrs by Solvent Extractor (VELP Scientifica SER148 Solvent Extractor) and The mixture was filtered through Whatman filter paper no. 1 for the removal of peel, pulp and seed particles. The residue was re-extracted twice to ensure complete extraction. The extracts were filtered and evaporated to dryness by the oven at 40^0c.the extract was stored in amber coloured glass bottles at refrigerated temperature for further analysis.

3. RESULTS AND DISCUSSION

	PEEL		PULP		SEED	
Flavonoids	SAE Mean ± SD	UAE Mean ± SD	SAE Mean ± SD	UAE Mean ± SD	SAE Mean ± SD	UAE Mean ± SD
Epicatechin	2.32±0.085	0.8±0.008	1.23±0.015	2.64±0.030	0.82±0.032	7.36±0.292
Rutin	2.72±0.105	0.51±0.008	1.72±0.043	3.67±0.112	0.48±0.006	4.35±0.160
Hesperidin	7.39±0.017	3.02±0.052	0.240±0.006	0.50±0.020	1.81±0.0468	1.91±0.043
Daidzein	0.89±0.028	0.12±0.002	0.68±0.029	1.58±0.038	0.040±0.001	2.7±0.061
Quercitin	3.94±0.117	1.32±0.027	0.346±0.014	1.05±0.038	1.07±0.027	2.37±0.090
Apigenin	9.940±0.089	Nd	1.41±0.016	5.97±0.014	Nd	1.71±0.039
Naringin	5.03±0.208	0.35±0.001	4.81±0.086	10.08±0.436	0.18±0.007	9.37±0.413

Note: Here (SAE) soxhletndassisted extraction, (UAE) ultrasound assisted extraction, (ND) not detected and the values are mean of triplicate analysis. All results are in mg/gm.Apigenin is natural flavonoid and mainly present in flavours, natural products, herbs and vegetables. Maximum apigenin content was Peel 9.94 mg/g estimated in Peel (SAE) followed by pulp (UAE) 5.97 mg/g Seed (UAE): 1.71, Pulp (SAE): 1.41mg/g. Apigenin was not detected in the extract of Peel (UAE) and Seed (SAE).A similar type of study was carried out by Zhang *et al*., 2007 and Xian *et al.*, 2017. For peel SAE found more effective than UAE because high porosity products like peel are more prone to alternating compression and expansion cycle produces by ultrasonic waves (Gallego-Juarez, 1998). Peel residues from sweet and bitter grapefruits were observed to be an important source of phenolic acids and flavonoids, chiefly polymethoxyflavones (pmfs), flavanones, and glycosylated flavanones (Gorinstein *et al*., 2001). In SAE the solvent transfers into the samples and extracts the compounds by permeation and solubilisation under higher temperature. Hence little destruction of the microstructure of sample occurs and a few slight ruptures took place on the surface of the sample (Biesaga, 2011)

Naringin content was found varying from 0.19 to 10.8 mg/g in different parts of grapefruit. It was found that maximum in pulp (10.8 mg/g) of UAE. Significance difference was observed in among UAE (10.8 mg/g) and (4.81 mg/g). In UAE, in peel, the mechanical effects of ultrasound provided a greater penetration of solvent into cellular materials, via

cavitation effects, and improved the release of chemical substances into the solvent. The ultrasounds induce subsequent changes on the surface and number of pits appeared on the plant surface. These changes could cause the plant to crumble and rupture more readily. The surface of the sample after UAE was obviously destroyed. In high porosity products, small intracellular space is found which require high internal resistance to water transfer rate Ross et al. (2000).

According to UPLC results highest (7.39mg/g), Hesperidin content was observed in peel (SAE) while the lowest content was observed in Pulp (SAE). SAE has resulted in better extraction. Hesperidin in case of Peel only whereas UAE has shown its efficacy for better extraction of Hesperidin from Pulp and Seed (i.e 0.50 and 1.91 mg/g) in comparison to SAE (0.24 and 1.81 mg/g) respectively (Peterson et al., 2006).. The peels are richer in flavonoids than seeds De-Castroetal. (2006).

Rutin is also called rutoside is used for the treatment of different disorder like mucositis and haemorrhoids (Tripoli *et al*., 2007). The highest amount of rutin was detected in (UAE) Seed 4.35mg/g and Pulp (UAE) 3.67 mg/g whereas in Peel maximum extraction was detected (SAE) 2.72 mg/g.

In grapefruit, catechin was not detected. Among flavonoids catechins are arranged into flavanols family they just present in substances which are plant made also found that in the extraction of grapefruit catechin was not found(Dugo et al., 2005).

Less amount of daidzein was found in grapefruit when compared with different flavonoids i.e.; in Seed (UAE) it was found maximum 2.70mg/gm whereas in (SAE) it was 0.04. Daidzein extraction was found least in Peel (UAE) in comparison to (SAE) 0.89 mg/g likewise pulp extraction was more in (UAE) 1.58 mg/gas compare to (SAE) 0.68 mg/g.

Among various flavonoids, quercetin is an effective cancer prevention agent and found in various fruits and vegetables. It gives different appealing colours to organic products, vegetables and their different parts. Also from different researches, it has been observed that quercetin has anti-inflammatory, anticancer and antihistamine exercises (Tripoli *et al.,* 2007). For Peel SAE (3.94 mg/g) quercetin was distinguished highest among all concentrate techniques. Qin *et al.,* (2010) also studied and found that during ultrasound extraction of various flavonoids only quercetin showed degradation in (UAE). Amount of quercetin content in the peel, pulp and seed of grapefruit is as follows: in Peel (UAE) degradation was showed 1.32mg/g whereas for Pulp and Seed (UAE) 1.05 and 2.37 mg/g has resulted in better extraction as a comparison to (SAE) 0.37 and 1.07 mg/g. Amount of quercetin was observed less in fruits when compared with onion varieties. Results were in accordance with the finding of Citrus peels are the primary waste fraction of citrus fruits and have been used as a source for molasses, pectin, cold-pressed oils and limonene. Citrus peels also contain numerous biologically active compounds including natural antioxidants such as phenolic acids and flavonoids (Manthey and Grohmann, 1996,).

The highest amount of epicatechin 7.36 mg/g was detected in Seed (UAE) in comparison to (SAE) 0.82 mg/g it was observed that extraction in pulp and seed was more in UAE. Pulp efficacy was more in UAE 2.65 mg/gas compare to (SAE) 1.28 mg/g likewise in Peel epicatechin was found more in (SAE) 2.32 mg/gas compared to (UAE) 0.80mg/g. Epicatechin helps in lowering circulatory strain, lifting weights, bring down cholesterol, enhanced safe framework reaction, enhanced memory (Banks *et al.,* 2018).

The extraction rate of flavonoids increases with repetition of the method for the same sample. With the increase of extraction temperature, the extraction rate of flavonoids from grapefruit seeds increased.

4. CONCLUSION

Peel and seeds of citrus fruits are richer in phenolic compounds such as phenolic acids and flavonoids. synthetic molecules are suspected to cause negative health effects. Therefore, the present study was intended with the objectives of the study of different flavonoid content in the peel, pulp and seed of grapefruit. It was found that pulp, peel and seed of grapefruit was a rich source of bioactive compounds including flavonoids. All the extraction strategies including traditional (SAE) and present day (UAE) at the upgraded states of extraction were observed appropriate for evaluation of different flavonoids and Phenols both Oven drying and Freeze drying methods were observed extremely helpful in the extraction of flavonoids. UAE was found most suitable for maximum extraction of individual flavonoids. UAE take less time, less solvent and less utilization of heat so UAE was more suitable for extraction. Findings of the above results show that the extraction method and drying methods do impact the measure of different flavonoids in grapefruit seed, pulp and peel.

REFERENCES

[1] Baghurst, K. 2003. The health benefits of citrus fruit. Hort. Australia. Sydney.

[2] Baydar N G, Ozkan G and Sagdic O Total phenolic contents and antibacterial activities of grape (Vitis vinifera L.) extracts. Food Chem 2004, 15: 335-39.

[3] Biesaga, m. Influence of extraction methods on the stability of flavonoids. J. Chromatogr. (2011), 1218, 2505–2512. 70.

[4] Bleve M, Ciurlia L, Erroi E, Lionetto G, Longoc L and Rescica L An innovative method for the purification of anthocyanins from grape skin extracts by using liquid and sub-critical carbon dioxide. Sep Purif Technol 2008, 64:192-97.

[5] Bohm, H., H. Boeing, J. Hempel, B. Raab, and A. Kroke. 1998. Fla- vonols, flavanes and anthocyanins as native antioxidants of food and their possible role in the prevention of chronic diseases. Zeitschrift Fur Ernahrungswissenschaft 37:147–163.

[6] Chawla and Ranjan, 2016. Principle, Instrumentation, and Applications of UPLC: A Novel Technique of Liquid Chromatography Open Chemistry Journal, 2016, 3, 1-16.
[7] De-Castro, W.V., S. Mertens-Talcott, A. Rubner, V. Butterweck, and L. Derendorf. 2006. Variation of flavonoids and furanocoumarinsingrape- fruit juices: A potential source of variability in grapefruit juice-drug interaction studies. J. Agr. Food Chem. 54:249–255.
[8] Dugo, P., M.L. Presti, M. Öhman, A. Fazio, G. Dugo, and L. Mondello. 2005. Determination of flavonoids in citrus juices by micro-HPLC- ESI/MS. J. Separation Sci. 28:1149–1156.
[9] Gorinstein, s., zachwieja, z., folta, m., barton, h., piotrowicz, j., zemser, m., weisz, m., trakhtenberg, s. & màrtín-belloso, o. Comparative contents of dietary fiber, total phenolics, and minerals in persimmons and apples. Journal of agricultural and food chemistry.(2001), 49, 952-957.
[10] Hertog mg, kromhout d, aravanis c, et al. Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. Arch intern med (1995);155:381–6.
[11] Manach, C., A. Mazur, and A. Scalbert. 2005. Polyphenolsansprevention of cardiovascular diseases. Current Opinion Lipidology 16:77–84.
[12] Manthey, j. A. & grohmann, k. Concentrations of hesperidin and other orange peel flavonoids in citrus processing byproducts. Journal of agricultural and food chemistry. (1996) 44, 811-814
[13] Manthey, J.A. and N. Guthrie. 2002. Antiproliferative activities of citrus flavonoids against six human vanver cell lines. J. Agr. Food Chem. 50:5837–5843
[14] Middleton ej. Effect of plant flavonoids on immune and inflammatory cell function. Adv exp med boil. (1998); 439:175–82.
[15] Middleton jr, e. Effect of plant flavonoids on immune and inflammatory cell function flavonoids in the living system. Springer.(1998)
[16] Middleton, Jr., E., C. Kandaswami, and T.C. Theoharides. 2000. The effectsofplantflavonoidsonmammaliancells: Implicationsforinflam- mation, heart disease, and cancer. Pharmacol. Rev. 52:673–751.
[17] Nahar L and Sarker S D Supercritical fluid extraction. Nat Prod Iso 2005,20: 47-76.
[18] Palma M and Taylor L T Extraction of polyphenolic compounds from grape seeds with near critical carbon dioxide. J Chromatogr A 1999,849:117-24.
[19] Peterson, J.J., G.R. Beecher, S.A. Bhagwat, J.T. Dwyer, S.E. Gebhardt, D.B. Haytowitz, and J.M. Holden. 2006. Flavanones in grapefruit, lemons, and limes: A complilation and review of the data from the analytical literature. J. Food Comp. Anal. 19:S74–S80.
[20] Qin, c.; li, y.; niu, w.; ding, y.; zhang, r.; shang, x. Analysis and characterisation of anthocyanins in mulberry fruit. Czech j. Food sci. (2010), 28, 117–126.
[21] Ross, S., D.S. Ziska, K. Zhao, and W.A. Elsohly. 2000. Variance of common flavonoids by brand of grapefruit juice. Fitoterapia 71:154–161
[22] Tripoli, e., guardia, m. L., giammanco, s., majo, d. D. & giammanco, m. a. < i> citrus</i> flavonoids: molecular structure, biological activity and nutritional properties: a reviewfood chemistry (2007), 104, 466-479.
[23] Usda, NATIONAL AGRICULTURRAL STATISTICS SERVICE, CITRUS FRUITS, 2004 SUMMARY. WWW.USDA.GOV
[24] Yousof s, mohd ghazali h and swee king g naringin content in local citrus fruits.food chem(1990) 37:113-21
[25] Zhang 2007. Flavonoids inGrapefruit and Commercial Grapefruit Juices: Concentration, Distribution, and Potential Health Benefits. Proc. Fla. State Hort. Soc 120:288–294.