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Ideal extraction temperature for antioxidants from holy basil and bunching onion

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Summary. This study aimed to determine ideal temperature for antioxidants from holy basil and bunching onion. Holy Basil *(Ocimum Tenuiflorum)* and Onion *(Allium Fistulosum)* were extracted with various temperatures ranging from 75 – 100 °C with two solvents i.e. methanol and water (room temperature & boiling). Total phenolic contents (TPC) and total flavonoid contents (TFC) were determined in the extracts by using the Folin-Ciocalteau and Aluminum chloride complex formation assays respectively. Extracts were analyzed in triplicates statistically compared using one-way analysis of variance (ANOVA) and the difference between the mean was ascertained at 95% confidence interval ($P<0.05$) using Tukey›s honest significance test. In both holy basil and bunching onion, the TPC and TFC values for methanolic extracts were significantly ($P<0.01$, $P<0.001$) higher than the water extracts. The best temperature among the various temperature used was 85°C where maximum TPC and TFC were observed in the extracts. This study shows that using optimum temperature helps in extraction of maximum antioxidants with methanol.

Keywords: culinary herbs, extraction temperature, antioxidants concentration

Introduction

The interest in the potential uses of natural antioxidants as food preservatives and for health reason has shown an increasing trend (1). Over the past 20 years, synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytolulene (BHT) and tert-butyl hydroquinone (TBHQ) are being used as food preservatives due to their low cost. However, several researchers have found that there are possible unhealthy effects of synthetic antioxidants and developed countries like Japan, Europe and Canada have banned the use of synthetic antioxidants used in food (2). There are numerous types of natural antioxidants which act as reducing agents to stabilize the free radicals in human body (3) e.g. polyphenols (4). Phytosterols, (5-7), flavonoids (8), vitamin C (1, 9-12). Apart from health benefits, antioxidants also have many industrial uses, such as preservatives in cosmetics and to prevent the degradation of rubber and gasoline (12). However, synthetic antioxidants used in the food and pharmaceutical industries may be toxic (2) compared to natural sources (13). Holy basil *(Ocimum tenuiflorum or Ocimum sanctum)* is an aromatic plant that is native to Eastern World. It is also called tulsi in India and is considered as a holy religious plant among Hindus. Apart from this, it is used as therapeutic ingredients for various purposes (14-16). Furthermore, commonly used as a condiment and garnishing agent in variety of cuisines (17). According to (18), the antioxidants

found in herbs have been identified to have multiple biological effects, including antioxidant activity. The most important chemical constituents in O. tenuiflorum extract (mainly in leaf) are eugenol, carvacrol, tannins, methyl eugenol and caryophyllene (19-22). However, other researchers have observed that other essential antioxidants, such as ascorbic acid, carotenoids, tocopherol, tocotrienols, glutathione, phenolic compounds (like flavonoid) and cichoric acid are also present in it (1, 10, 23). Furthermore, O. tenuiflorum also has showed to exhibit a hepatic protective effect and can be used in the treatment of hepatic disorders anti-stress, immune modulator, anti-inflammatory, mast cell stabilization, anti-histamine (14) and (24). Onion (A. fistulosum) also possesses antioxidants which are characterized by its higher contents of thiosulfunates (allicin), an antioxidant useful for disease condition (25, 26). Allicin also has anti-bacterial, antiviral, and anti-fungal properties (9, 25, 27, 28). Various studies report that cooking temperature may affect antioxidants concertation/activity (1, 3, 13, 29-33). The optimum release depends on the types and nature of the material and antioxidants (34). Various methods of extraction are used but little attention has been paid to have ideal temperature for extractions (35). Similarly, aqueous and organic solvents would also have positive or negative effect on extraction (36). Therefore, it is would be excellent to have optimal solvent and ideal temperature for extraction of antioxidants (37)

Despite of the wide uses of these two common herbs in cooking, the optimum temperature that would optimize the retention of antioxidant contents of these herbs in cooking is still unknown. Thus, there is a need to study the effect of temperature to identify the suitable temperature that can maximize the retention of the antioxidants in the extract.

Materials and Methods

This study involved two famous Asian culinary herbs, namely holy basil and bunching onion. Both herbs were purchased from the local market in Kuantan, Pahang, Malaysia. The herbs were then cleaned with distilled water, dried and ground. After grinding, extracted separately with methanol at various temperatures (65, 75, 85, 95 and 100°C), water (room temperature) and hot water (65, 75, 85, 95 and 100°C). Two assessments were performed namely Folin-Ciocalteau and Aluminium chloride complex formation for total phenolic contents (TPC) and total flavonoid (TFC) contents. For phenolic contents, a calibration curve of the Gallic acid standards (5, 2.5, 1, 0.5, 0.1 mg/l) was prepared; the absorbance was measured at 760 nm using UV-Vis spectrophotometer and using pure methanol as a blank as described previously by (38). The concentration of phenolic contents in samples was estimated using the formula below:

Phenol content mg GAE/g = [(Slope x absorbance) + c] / Sample concentration
*c is the y-intercept

Total flavonoid contents were determined by Aluminium chloride complex formation as described by (39). A calibration curve of the quercetin standards (5, 2.5, 1, 0.5, 0.1 mg/l) was prepared and the absorbance was measured at 425 nm using UV-Vis spectrophotometer and using pure methanol as a blank. The concentration of total flavonoid in samples was estimated using the formula below:

Flavonoid contents (QE mg/g) = [(Slope x absorbance) + c] /Sample concentration
*c is the y-intercept

Statistical analysis

Statistical analysis of the data was performed by using SPSS (Version 12.1), statistically compared using one-way analysis of variance (ANOVA) and the difference between the mean was ascertained at 95% confidence interval ($P<0.05$) using Tukey's honest significance test.

Results and Discussion

The present study demonstrated some interesting findings for the antioxidant concentration of extracts with different temperatures. As mentioned earlier, the antioxidant concentrations of these two herbs were determined in the form of total phenolic contents (TPC)

and total flavonoid contents (TFC) and extracted with two different solvent namely methanol and water. The TPC of three different procedure extracts of the two herbs i.e. are presented in the Figure 1 & 2 and Table 1.

Highest ($P < 0.001$) concentration was obtained for the total phenolic contents at 85°C for both herbs compared to the rest of the temperatures used. From these results, it can be deduced that hot water extracts at 85°C exhibits higher content of TPC (Fig. 1). General concept of the effect of the temperature on the antioxidant's concentration is that higher temperature would lead to lower antioxidant concentration (40). The results of this study reveal that TPC at 85°C was higher than at 65, 75, 95 and 100 °C which might be the suitable temperature. Furthermore, increase in temperature beyond 85 °C would lead to a decrease in TPC yield as it has been observed at 95 and 100°C (Fig 1). Similar, effect has been suggested by (13) that an increase in temperature will lead to an increase in TPC to the maximum concentration whereas there will be a decrease at further increase of temperature and that is what we observed (85 °C). The present study reveals that the effect of tem-

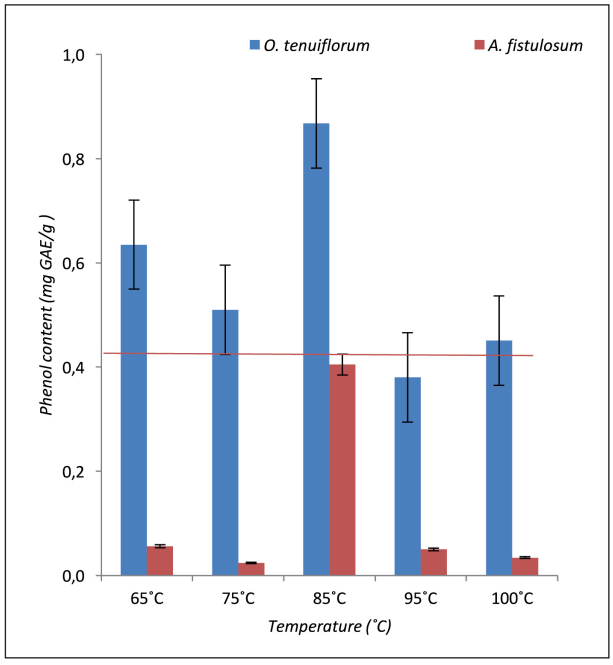

Figure 1. Alteration in cooking temperature affects total phenol contents of holy basil and onion. Each bar represents the mean values ± standard deviation, where n = 3.

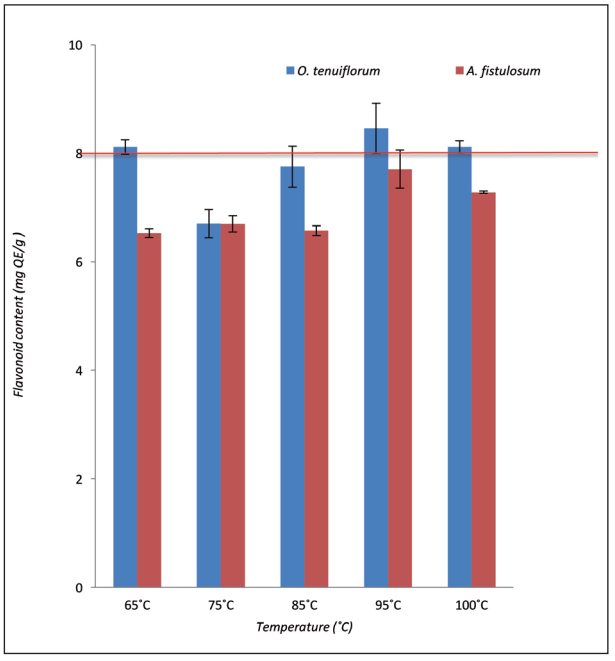

Figure 2. Alteration in cooking temperature affects total flavonoid contents of holy basil and onion. Each bar represents the mean values ± standard deviation, where n = 3.

Table 1. The phenolic contents of these extracts were estimated using a standard curve of Gallic acid and expressed as milligrams of Gallic acid equivalents (GAE), the flavonoid contents that were projected using a standard curve of quercetin and expressed as mg of Quercetin equivalents (QE) over weight of sample in gram and

Holy Basil Extracts			Onion Extracts		
Methanol	Water (room Temperature)	Hot Water	Methanol	Water (room Temperature)	Hot Water
Total Phenol Contents (TPC) (mgs GAE/g)					
0.83 ± 0.17^{a}	0.02 ± 0.04^{b}	0.11 ± 0.01^{b}	4.16 ± 0.63^{c}	0.01 ± 0.03^{b}	0.11 ± 0.03^{b}
Total Flavonoid Contents (TFC) (mg of Quercetin equivalents (QE)					
5.04 ± 0.06^{a}	1.57 ± 0.01^{c}	3.77 ± 0.05^{b}	4.91 ± 0.12^{b}	2.14 ± 0.04^{c}	4.81 ± 0.11^{a}

Each value represents the mean ± standard deviation, where n = 3. The vales with different superscripts are significantly ($P<0.05$, $P<0.01$) different.

perature on the antioxidant contents of Holy basil and Onion were different from one temperature to another which is due to the thermal effects which varies depending on the type of plants, species (33, 41, 42).

The TFC was higher at 95 and 100°C of hot water extracts as compared to other temperatures for both herbs (Figure 2). Similar result was found by (10) who reports that heating at a higher temperature gave a higher flavonoid content compared to heating at a lower temperature. This has been attributed to the hydroxyl structure of flavonoids that is effectively extracted at 95°C (33). Surprisingly, the results of this study showed that the highest content of TFC was obtained at higher temperature compared to TPC. This was similar to (13) who found that the highest yield of TFC higher temperature (63°C) while the highest yield of TPC were observed at lower temperature (53°C). These authors attribute the results to the differences in the hydroxyl group in the phenolic compounds which responsible for responding differently towards thermal treatment. The specific hydroxyl structure in flavonoids and are acting in a specific different mechanism (43). The variation may be due to the differences in cultivar which may be influenced by genetic factors (41) and varied from one region to another (44).

From Figure 1 Holy basil expressed the highest phenolic contents at temperature of 85°C. As suggested by (44), the total phenolic content which mainly affects the antioxidant activity is a main parameter to determine the overall concentration and activity of antioxidants in the herbs. Therefore, it is considerably valid to conclude that the temperature which resulted in the highest phenolic compounds was the optimum temperature for retention of the antioxidant contents in the tested samples. Figure 1 has revealed that 85°C was the optimum temperature for the optimization of antioxidants concentration in Holy basil.

These results show that both tested herbs possess significant amount of phenolic compounds. The content of TPC was highest in methanol compared to cold and hot water extract similar to the other studies of Holy basil (45) and Onion (44). It can be observed from these results that methanol extracts of both herbs yielded the highest concentration of TFC compared to hot and cold water extracts respectively. This has been reported elsewhere in a study conducted by a group of (46), who found that the phenolic compounds yield 30% more with methanol compared to the other forms of solvents. The content of holy basil and onion show that both herbs are important for antioxidant activities (10). However, the concentration of the TFC is varied depending on the type of extraction solvent. Similar to the TPC, the highest concentration of TFC was observed in the methanol extracts of Holy basil and Onion. A similar observation has been reported for spices with methanol to be the most effective extraction agent for TFC compared to the other solvents (10).

Phenolic compounds are good sources of antioxidants and have excellent potential in elucidating antioxidants scavenging activity (18, 44, 45). However, the concentration of the phenolic compounds varies depending on the type of extraction (46, 47) and (10). The observed concentration TPC and TFC may have the antioxidant activity and free radical-scavenging capacity in the tested herbs (44, 48, 49) and (44). In fact, the higher the phenolic contents, the higher the free radical-scavenging activity and antioxidant capacity of the sample, and vice versa (49, 50). In the present study, it was found that extraction solvent also influenced the antioxidant contents of these two herbs. Methanol possessed highest yields of total phenols as compared to hot and cold water. Interestingly, the present study revealed that the effect of temperature on the antioxidant contents of both herbs were unique from one temperature to another. Hot water extracts at 85°C had the highest phenolic compounds followed by 65, 95, 100 and 75°C for Holy basil while for Onion; the phenolic compounds yield ranged from highest to lowest was at 85, 65, 75, 95 and 100°C. In fact, the antioxidant activity elucidated by Holy basil of hot water extracts at all manipulated temperatures was considerably higher except for 95°C. Thus, it appears that 85°C was the optimum temperature for the optimization of antioxidants contents for both herbs.

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References

1. Charles DJ. Antioxidant Properties of Spices, Herbs and Other Sources. New York: Springer Science Business Media, 2013.
2. Juntachote T, Berghofer E, Bauer F, and Siebenhandl S. The application of response surface methodology to the production of phenolic extracts of lemon grass, galangal, O. tenuiflorum and rosemary. International Journal of Food Science and Technology 2006; 41, 121–133.
3. Sies and Helmut. Oxidative stress: Oxidants and antioxidants. Experimental physiology 1997; 82 (2), 291–295.
4. Duthie GG, Duthie SJ, and Kyle JA. Plant polyphenols in cancer and heart disease: implications as nutritional antioxidants. Nutritional Research Rev. 2000; 13, 79-106.
5. Calpe-Berdiel L, Escola-Gil JC, Blanco-Vaca, F. New insights into the molecular actions of plant sterols and stanols in cholesterol metabolism. Atherosclerosis 2009; 203, 18-31.
6. Jones PJH, and Abu Mweis SS. Curr Opin Clinical Nutrition Metabolic Care 2009; 12, 147-151.
7. Bouic P.J. Sterols and sterolins: new drugs for the immune system Drug Discovery Today 2002; 7, 775-778.
8. Parvu M, Rosca-Casian O, Puscas M, and Groza G. Antifungal activity of Allium fistulosum L. Contribution Botanical 2009; 44, 125-129.
9. Parvu M, Parvu AE, Rosca-Casian O, Vlase L, and Groza G. Antifungal activity of Allium obliquum. Journal of Medicinal Plants Research 2010; 4, 138-141.
10. Settharaksa S, Madaka F, Sueree L, Kittiwisut S, Sakunpak A, Moton C, and Charoenchai L. Effect of solvent types on phenolic, flavonoid contents and antioxidant activities of syzygium gratum (wight) S.N. International Journal of Pharmacy and Pharmaceutical Sciences 2012; 6, 2.
11. Pourmorad F, Hosseinimehr SJ, and Shahabimajd N. Antioxidant Activity, Phenol and Flavonoid Contents of Some Selected Iranian Medicinal Plants. African Journal of Biotechnology 2006; 5 (11), 1142-1145.
12. Dabelstein W, Reglitzky A, Schütze A, and Reders K. Automotive fuels. Ullmann's Encyclopedia of Industrial Chemistry 2007. Retrieved from http://onlinelibrary.wiley.com
13. Vidović SS, Zeković ZP, Lepojević ZD, Radojković MM, Jokić SD, and Anačkov GT. Optimization of the ocimum basilicum l. extraction process regarding the antioxidant activity. Faculty of Technology Novy Sad 2012; 1450-7188, 43, 315-323.
14. Prakash P, and Gupta N. Therapeutic uses of Ocimum sanctum. with a note on eugenol and its pharmacological actions: A short review. Indian Journal of Physiology and Pharmacology 2005; 49, 125-131.
15. Uhl SR. Spices, seasonings, & flavorings. Technomic Publishing Company, Lancaster 2000.
16. Samuelsson G. Drugs of Natural Origin: A Textbook of Pharmacognosy. Swedish Pharmaceutical Press: Stockholm, Sweden 1999.
17. Jang HW, Ka MH, and Lee KG. Antioxidant Activity and Characterization of Volatile Extracts of Capsicum annuum L. and Allium spp. Flavour and Fragrance Journal 2008; 23, 3, 178-184.
18. Kahkonen MP, Hopia AI, Vuorela HJ, Rauha J, Pihlaja K, Kujala TS, and Heinonen M. Antioxidant Activity of Plant Extracts Containing Phenolic Compounds. Journal of Agriculture and Food Chemistry 1999; 47, 3954-3962.
19. Kelm MA, Nair MG, Strasburg GM, DeWitt DL. (). Antioxidant and cyclooxygenase inhibitory phenolic compounds from Ocimum sanctum Linn. Phytomedicine 2000; 7, 7-13.
20. Raj KJ, Richa M, and Singh B. Anti-convulsant potential of Basil O.sanctum Linn and its cultures. Indian Journal of Experimental Biology 2003; 41, 1329–1333.
21. Anul Hakkim F, Gowri Shankar C, and Girija S. Chemical composition and antioxidant property of O. tenuiflorum (Ocimum sanctum L.) leaves stems, and inflorescence and their in vitro callus cultures. Journal of Agriculture and Food Chemistry 2007; 55, 9109-9117.
22. Lukmanul Hakkim F, Girija A, and Boopathy R. Antioxidant property of selected Ocimum species and their secondary metabolite content. Journal of Medicinal Plants Research 2008; 2(9), 250–257.
23. Lee J, and Scagel CF. Chicoric acid found in basil (Ocimum basilicum L.) leaves. Food Chemistry 2009; 115, 2, 650-656.
24. Sridevi G, Gopkumar P, Ashok S, and Shastry CS. Pharmacological basis for antianaphylactic, antihistamine and mast cell stabilization activity of Ocimum sanctum. Internet Journal of Pharmacology, 2009; 7.
25. Newall CA, Anderson AA, and Philipson JD. Herbal Medicines. A guide for Health-care Professionals. The pharmaceutical Press, London, UK 1996.
26. Benkeblia N, and Lanzotti V. Allium Thiosulfinates: Chemistry, Biological Properties and their Potential Utilization in Food Preservation. Food, Global Science Books 2007; 1(2), 193-201.
27. Stajner D, Milic N, Canadanovic-Brunet J, Kapor A, Stajner M, and Propovic BM. Antifungal activity of Allium obliquum. Phytotherapy Research 2006; 20, 581-584.
28. Hedges and Lister. Health Attributes of Allium species. Crop and Food Research Report 2007, 1814. Retrieved from http://www.vegetables.co.nz/resources
29. Reblova Z. Effect of temperature on the antioxidant activity of phenolic acids. Czech Journal of Food Science 2012; 30, 171-177.
30. Gulcin I. Antioxidant activity of food constituents: an overview. Arch. Toxicology 2012; 86, 345-391.
31. Carlsen MH, Halvorsen BL, Holte K, Bøhn SK, Dragland S, Sampson L, Willey C, Senoo H, Umezono Y, Sanada C, Barikmo I, Berhe N, Willett WC, Phillips KM, Jacobs DR, and Blomhoff R. The total antioxidant content of more than 3100 foods, beverages, spices, herbs and supplements used worldwide. Nutrition Journal 2010; 9, 3.
32. Nilsson J, Stegmark R, and Akesson B. Total antioxidant capacity in different pea (Pisum sativum) varieties after blanching and freezing. Food Chemistry 2004; 86, 501-507.
33. Yanishlieva NV. Antioxidants in Food – Practical Applications. Woodhead Publishing, Cambridge 2001; 22-70.

34. Pisoschi AM, Pop A, Cimpeanu C, and Predoi G. Antioxidant Capacity Determination in Plants and Plant-Derived Products: A Review. Oxid Med Cell Longev. 2016: 9130976.Published online 2016 Dec 4.
35. Xu D, Li Y, Meng X, Zhou T, Zhou Y, Zheng J, Zhang J, and Li1 H. Natural Antioxidants in Foods and Medicinal Plants: Extraction, Assessment and Resources. Int J Mol Sci. 2017; 18(1): 96.,
36. Tchabo W, Ma Y, Kwaw E, Xiao L, Wu M, and Apaliya MT. Impact of extraction parameters and their optimization on the nutraceuticals and antioxidant properties of aqueous extract mulberry leaf, International Journal of Food Properties 2018; 21:1, 717-732.
37. Dailey A, and Vuong QV. Optimization of Aqueous Extraction Conditions for Recovery of Phenolic Content and Antioxidant Properties from Macadamia (Macadamia tetraphylla) Skin Waste. Antioxidants 2015; 4, 699-718.
38. Velioglu YS, Mazza G, Gao L, and Oomah BD. AnTioxidant Activity and Total Phenolics in Selected Fruits, Vegetables and Grain Products, Journal of Agricultural and Food Chemistry 1998; 46(10): 4113-4117.
39. Zhishen J, Mengcheng T, and Jianming W. The Determination of Flavonoid Contents in Mulberry and Their Scavenging Effects on Superoxide Radicals, Food Chemistry 1999; 64(4):555-559.
40. Pokorny J. Addition of antioxidants for food stabilization to control oxidative rancidity. Czech Journal of Food Sciences 1986; 4, 299-307.
41. Horváthová J, Suhaj M, and Šimko P. Effect of thermal treatment and storage on antioxidant activity of some spices. Journal of Food and Nutrition Research 2007; 46, 1, 20-27.
42. Marinova EM, and Yanishlieva NV. Antioxidant activity and mechanism of action of some phenolic acids at ambient and high temperatures. Food Chemistry 2003; 81, 189-197.
43. Shams El-Din MHA, Madiha MAK, Makhlouf SK, and Mohamed OSS. Effect of Some Cooking Methods on Natural Antioxidants and Their Activities in Some Brassica Vegetables. World Applied Sciences Journal 2013; 26 (6), 697-703.
44. Chang TC, Chang HT, Chang ST, Lin SF, Chang YH, and Jang HD. A Comparative Study on the Total Antioxidant and Antimicrobial Potentials of Ethanolic Extracts from Various Organ Tissues of Alliums spp. Food and Nutrition Sciences 2013; 4, 182-190.
45. Wangcharoen W, and Morasuk W. Antioxidant capacity and phenolic content of holy basil Songklanakarin J. Sci. Technol. 2007; 29(5): 1407-1415.
46. Casazza AA, Bahar Aliakbarian, Mantegna S, Cravotto G, Perego P. Extraction of phenolics from Vitis vinifera wastes using non-conventional techniques. Journal of Food Engineering 2010; 100, 50-55.
47. Turkmen N, Sari F, and Velioglu YS. The effect of cooking methods on total phenolics and antioxidant activity of selected green vegetables. Food Chemistry 2005; 93, 713-718.
48. Tung YT, Wu JH, Kuo YH, and Chang ST. Antioxidant activities of natural phenolic compounds from Acacia confuse bark. Bioresource Technology 2007; 98, 5, 1120-1123.
49. Jang HD, Chang KS, Chang TC, and Hsu CL. Antioxidant potentials of Buntan Pomelo (Citrus Grandis Osbeck) and its ethanolic and acetified fermentation products. Food Chemistry 2010; 118, 3, 185-191.
50. Huang Z, Wang B, Eaves DH, Shikany JM, and Pace RD. Total phenolics and antioxidant capacity of indigenous vegetables in the Southeast United States: Alabama collaboration for cardiovascular equality project. International Journal of Food Sciences and Nutrition 2009; 60, 2, 100-108.

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