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Effect of microwave treatment on bioactive compounds of *Spinacia oleracea*(spinach) and *Anethum graveolons*(dill) leaves .

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Abstract: The aim of the present study was to evaluate the effect of microwave heating, (for one, two and three minutes) on some bioactive compounds of *Spinacia* oleracea(spinach) and (dill) *Anethum graveolens* leaves blended in water. The effect of microwave drying process of spinach caroteoids and color indices were determined in this study. According to the obtained results, dill leaves contain higher total phenols, antioxidant activity and carotenoids (1287.00 mg as gallic acid / 100g, 48.14% and 45.98mg/kg, respectively) than spinach leaves (1031.39 mg as gallic acid/100g, 40.10% and 40.00mg/ kg, respectively). Total phenols, antioxidants activity and carotenoid contents of spinach and dill heated samples significantly ($p<0.05$) increased after one minute of heating. Then gradual decrease was observed in the same parameters after 2 and 3 minutes in both spinach and dill. Significant decrease in all tested parameters was observed in microwave dried samples compared to the fresh state. Antioxidant activity decreased 20% in dried spinach and 30.3% in dried dill compared to fresh samples. This work indicated that the tested bioactive compounds of spinach and dill was stable only after one minute of microwave heating, however, after 3min of heating 32.3 to 80% decrease was observed in these parameters.

Keywords: M*icrowave, Spinacia oleracea, carotenoids, Anethum graveolens, gallic acid*

1 Introduction

Recently special attention has been paid towards edible plants that are rich in secondary metabolites (frequently called phytochemicals) and there is now increasing interest in antioxidant activity of such phytochemicals present in the diet. Phenolic compounds are broadly distributed in the plant kingdom and are the most abundant secondary metabolites of plants, with more than 8,000 phenolic structures currently known, ranging from simple molecules such as phenolic acids to highly polymerized substances such as tannins. Plant phenolics are generally involved in defense against ultraviolet radiation or aggression by pathogens, parasites and predators, as well as contributing to plants' colors. They are ubiquitous in all plant organs and are therefore an integral part of the human diet. Phenolics are widespread constituents of plant foods (fruits, vegetables, cereals, olive, legumes, chocolate, etc.) and beverages (tea, coffee, etc.), and partially responsible for the overall organoleptic properties of plant foods. Antioxidants are important in prevention of pollution damage of plants, disease prevention in both plants and animals and play a very important role in the body defense system and reactive oxygen species (Krinsky, 1989). The bioactive compounds including polyphenolic and photosynthetic pigments like chlorophylls and carotenoids have been shown to have possible health benefits with antioxidative, anticarcinogenic, antihypertensive, antimutagenic antioxidative, anticarcinogenic, antihypertensive, antimutagenic, and antimicrobial activities. Based on the scientific arguments, supplementation of diet with various herbs is recommended among individual consumers, both for its healing properties and nutritive value (Cieslik *et al.,* 2006). The biological importance of carotenoids lies is the fact that carotenoids can also serve as antioxidants, and many reports indicated that carotenoids may possess some anticarcinogenic properties, which may be related to their ability to interact with and quench various radical species that can be generated within cells (Sun *et al.,* 2002). Deep-coloured vegetables and fruits are known to be good sources of phenolics, including flavonoid, anthocyanin, and carotenoids (Oboh, 2005). Green leafy vegetables are popularly used for food in many countries of the world, being a rich source of s-carotene, ascorbic acid, minerals and dietary fiber [5-8]. The popular thinking is that fresh fruits and vegetables are better for us than cooked ones nutrition wise. Despite this thinking, most vegetables are usually cooked before consumption. These cooking processes could bring many changes in physical characteristics and chemical composition of vegetables (Pellegrini *et al.,* 2010, Gorinstein *et al.,* 2009). However, processing can also lead to disruption of the food matrix, increasing the inaccessibility of many phytochemicals and thus improving the nutritional quality of vegetables (Hassanein *et al.,* 2003). Moreover, the attention should be also paid to the processing methods in order to preserve the desirable antioxidant properties of foods (Burfoot *et al.,* 1990). The effects of microwave on food constituents (Yoshida *et al.,* 1990), including in lipid fraction of animal fats, vegetable oils and some raw and cooked vegetables [23-26] have been studied. The objectives of this study were to confirm the effect of different microwave heating times on the total phenols, antioxidant activity, carotenoids, chlorophylls of two green leafy vegetables, spinach and dill, are popularly used in many countries of the world as spices to flavor food and also in medicinal purposes. The effect of microwave drying process on the same parameters has been studied also on the same parameters.

2 Materials and Methods

Sample preparation for microwave heating

Fresh plant materials of spinach and dill were purchased from local market in Ahmednagar. Samples were washed under tap water and inedible parts and stems were removed. The edible parts of the vegetables (3g) were homogenized with 50 ml distilled water in a laboratory blender, Poured in 100 ml glass beaker (four for each plant). One beaker left as control (corresponding to 0 min) and the second, third and fourth beakers were heated in microwave oven at full power (750 W) for 1, 2 and 3 min, respectively. The samples were then cooled for a few minutes at room temperature, filtered and adjusted to 100ml.

Microwave drying process

The edible parts of spinach and dill leaves were dried using a microwave-oven. The characteristic parameters of drying program were as follows: rotation speed - 6 rpm; product mass per load-3 g; drying time-1 min for dill and 2min for spinach (Table 1).

Determination of total content of phenolic compounds

The total content of phenolic compounds (TPC) in samples was determined according to the method reported by Boyer and Hai Liu, 2004. One ml of extract was mixed with 5 ml of 10% Folin-Ciocalteu reagent in distilled water and 4 ml of 7.5% sodium carbonate solution. After incubation of samples with reagent at room temperature for 30 min with periodical mixing, the absorbance at 765 nm was measured. The calibration curve was constructed within the concentration range 0.075-0.6 mg/ml of Gallic acid. Mean values were calculated from three parallel analyses. Results were calculated as Gallic Acid Equivalents (GAE) in mg/100 g of dry plant material using the following equation:

$$C = a\times \gamma\times (V/m) \times 100,$$

Where: C - total amount of phenolic compounds, mg GAE/100g; a - dilution number; γ - concentration obtained from calibration curve, dilution number; γ - concentration obtained from calibration curve, mg/ml; V - volume of aqueous ethanol used for extraction, m; m-weight of sample (g).

DPPH free radical scavenging ability

The free radical scavenging ability of samples against DPPH (1, 1-diphenyl-2 picryl hydrazyl) free radical was evaluated as described by Zhang and Hamauzu (Gorinstein *et al.,* 2009). One ml extracts was mixed with 1ml of 0.4 mmol l-1 methanolic solution containing DPPH radicals. The mixture was left in the dark for 30 min and the absorbance was measured at 516 nm. The DPPH free radical scavenging ability was subsequently calculated with respect to the reference (which contains all the reagents without the test sample).

Chlorophylls and carotenoids content

Following the procedures described by Mosquera *et al.* 1991. The chlorophyll fraction was measured in a UV spectrophotometer at 670 nm and the carotenoid fraction at 470 nm.

Statistical analysis

All determinations were conducted in triplicate. Differences between means of data were compared by least significant difference (LSD) calculated using the SAS for Windows 2000 Version 8.2 (Turkmen *et al.,* 2005).

3 Results and Discussion

Effect of microwave heating on total phenols, antioxidants activity, chlorophyll and carotenoid contents of spinach and dill leaves

The total phenols content of the liquor from microwaved spinach and dill leaves were presented in table 2. The data revealed that total phenols in spinach leaves increased from 1031.39mg/100g prior to microwave heating to 1371.84mg/100g after one min. Dramatic decrease to 482.81and 204.60 mg/100g was recorded after two and three min, respectively. Total phenols in dill leaves increased from 1287.00 to 1636.80 after one min then decreased to 981.75 and 861.63 mg/100g after two and three min, respectively Data in same table showed that antioxidants activity of spinach and dill leaves significantly ($p < 0.05$) increased from 40.10 to 51.83% and from 48.14 to50.71%, respectively after 1min of heating. However, gradual decrease was recorded after 2 and 3 min of heating in antioxidant activity. Heating or cooking in water seems to cause a leakage of vegetable antioxidants like phenols into the cooking water. Then degradation of these antioxidant compounds may take place with prolonged time to microwave heating. Gahler *et al.* 2003 reported an improvement in the antioxidant activity of tomatoes after heat treatment than in raw tomato. These results are in agreement with Wachtel-Galor *et al.* 2008 who concluded that microwaving led to a greater loss of antioxidants into the liquor than did boiling. Our results in a line with Turkmen *et al.* 2005 who found that after 1.5 min microwaving the antioxidant activity increased by 15.90% and 16.68% in some fresh vegetables. Carotenoids content increased after one min then decreased about 27% in spinach and 76% after 3min in dill leaves. The increase after one min may be due to the heat treatment that enhances the liberation and the bioavailability of carotenoids as investigated by Rock et al. [35]. In the same table chlorophyll contents decreased significantly ($p < 0.05$) after, 2 and 3min in spinach and after 3min in dill. The same findings were stated by Pellegrini *et al.* 2010 who found

Table 1: Different methods of drying

	Spinach	**Dill**
Weight of sample	5	5
Microwave (minute)	2	2
Moisture %	6.65	5.93

Table 2:Effect of microwave heating on TPC , antioxidant, chlorophyll, carotenoid content.

Treatment	Total phenols	Antioxidant%	Chlorophyll (mg/kg)	Carotenoids (mg/kg)
Spinach(0 min)	1031.49	40.10	32.47	40.00
Spinach (1 min)	1371.81	51.83	28.83	43.41
Spinach(2 min)	282.61	36.90	28.12	35.83
Spinach (3min)	204.60	24.78	11.96	29.02
Dill (0 min)	1287.60	48.14	33.97	45.98
Dill (1 min)	1636.80	50.71	34.6	48.52
Dill (2 min)	981.75	33.54	30.8	36.11
Dill (3min)	861.63	21.76	20.58	11.46

that total chlorophyll content of raw fresh broccoli, was significantly decreased by all the cooking methods include microwaving except for oven steaming.

Effect of microwave drying process on total phenols, antioxidants activity, chlorophyll and carotenoid contents of spinach and dill

Data in table 4 revealed that total phenols were affected obviously with the microwave drying process in both spinach and dill leaves. Significant decrease ($P>0.05$) was observed in total phenols content of both microwave dried samples compared to the fresh state (12.73% in spinach and 12.18% in dill). Antioxidant activity decreased 20% in dried spinach and 30.3% in dried dill compared to fresh samples. This decrease may due to the decrease in total phenols and the thermal labile components content of samples such as carotenoids, (table 4). Data in the same table showed a noticeable decrease in chlorophyll content in dried spinach and dill compared to fresh samples (on dried weight). This data are in parallel and ensure the data above. The same results were obtained by Annamalai (2011) who found a significant reduction in antioxidant property for microwave dried plant material when compared to other drying treatments. Bejar et al. studied the effect of microwave drying on orange peel and leaves and stated that microwave drying decreased the total phenol content of the dried leaves compared to the fresh one.

4 Conclusions

The present work showed that microwave heating induces significant loss in some bioactive components and their activities in spinach and dill. However the appearance quality as brightness and greenness does not extremely affected. More research should be investigated in more foods in order to better guide food preparation methods that preserve food of their rich bioactive components and antioxidant capacity

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