

THE EFFECT OF PICKLING ON TOTAL PHENOLIC CONTENTS AND ANTIOXIDANT ACTIVITY OF 10 VEGETABLES

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Abstract:

Epidemiological evidence suggests the critical role of vegetable consumption in preventing chronic degenerative diseases. Considering that pickle is a widely consumption type of vegetable in Turkish diet the objective of the present study was to assess the total phenol content and antioxidant capacity of pickled vegetables. For this purpose total antioxidant capacity of 10 fresh and pickled vegetables was analysed by DPPH (2,20-diphenyl-1-picryl hydrazyl) radical scavenging activity and Trolox equivalent antioxidant capacity (TEAC) methods and total phenolic content (TPC) using Folin–Ciocalteu reagent. Following the pickling in 15th day there was a significant ($P<0.05$) decrease in TPC of all veg-

etables, in contrast this TPC increased significantly after 30th day. Also at 60th day of pickling the TEAC values of all vegetables are increased significantly ($P<0.05$), but DPPH values of green pepper, cauliflower, cucumber and sneak melon decreased compared with fresh state. Our findings suggest that, pickling process is relatively a good method for the preservation of phenolic acids in vegetables, and most of the antioxidant capacities remained after 30th day of fermentation.

Keywords:

Total phenol content, total antioxidant capacity, pickling

Introduction

Pickle is a traditional fermented food made of vegetables such as cabbage, cucumber, carrot, green tomato, pepper, eggplant and beans. Also, pickling is one of the oldest preservation methods of food by fermentation. The pickling is basically, conversion of sugar to acid by microorganisms that are lactic acid bacteria (LAB) (Nurul and Asmah, 2012). The salt also plays an important role in fermentation by drowning out water and nutrients from vegetable and become substrates for lactic acid bacteria grow. As sugar convert to the lactic acid the condition become acidic and inhibits the growth of pathogens and other nonacidic tolerant microorganisms' especially aerobic spoilage microorganisms. As a result from pickling, the vegetable will have a longer shelf life, translucent appearance, firm texture and pickle flavour. Fermentation of fruits and vegetables can occur spontaneously by the natural LAB that placed surface of vegetable, such as *Lactobacillus* spp., *Leuconostoc* spp., and *Pediococcus* spp. (Karovičová et al., 1999).

Pickled products by LAB fermentation have unique flavour and great healthful effects (Choi et al., 2013). LAB fermented vegetables helps to enhance human nutrition with the attainment of balanced nutrition, providing vitamins, minerals, and carbohydrates (Yamano et al., 2006), besides, they contain pigments such as flavonoids, lycopene, anthocyanin, β -carotene, and glucosinolates. This phytochemicals act as antioxidants in the body by scavenging harmful free radicals implicated in degenerative diseases like cancer, arthritis, and ageing (Kaur and Kapoor, 2001). Fruit and vegetables are good sources of natural antioxidants such as vitamins, carotenoids, flavonoids and other phenolic compounds (Takebayashi et al., 2013; Isabelle et al., 2010). Protecting these nutrition values of plant foods is a growing scientific field. Then, a common way to maintain and improve the nutritional and sensory features of vegetables is pickling or lactic acid fermentation (Demir et al., 2006; Cagno et al., 2013).

In Turkey, pickling is an important way of consume vegetable. It is preferred not only as a good way to keep vegetables fresh but also it is a popular taste in Turkish cuisine. Although there is an industrial production of vegetable pickles mostly pickling still a domestic process. There are studies

demonstrating that fermentation increased the antioxidant capacity of vegetables like soybean (Moktan et al., 2008) but some plant foods showed decrease in antioxidant capacity like olive (Othman et al., 2009). So, the effect of pickling on antioxidant properties of vegetables is change by various factors like vegetable kind, microorganisms, time, temperature and pH. Literature is scanty on the effects of domestic pickling procedure that has a wide consumption in diet. Therefore, in the present study, total phenol content (TPC) and antioxidant capacity (AC) of pickled vegetables, using different antioxidant methods (DPPH and TEAC) were examined. The pickled vegetables should be fermented and riped between 15 and 30 days before eating. So, the specific aim was to analyse the change of antioxidant capacity by pickling and after waiting for one month.

Materials and Methods

Chemicals and reagents

6-hydroxyl-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,20-azino-bis-(3 ethylbenzothiazoline-6-sulfonate) (ABTS⁺), and Folin-Ciocalteu phenol reagent were purchased from Sigma (St. Louis, MO). All other chemicals used were of analytical grade. Fresh vegetables were obtained from market in Konya, Turkey, on September 2014.

Pickle preparation and Sampling

The vegetables were washed clean under tap water and were placed in a clean jar. Before the vegetables dried chickpeas (40 g) added on the bottom of the jar due to accelerate the fermentation. Pickling salt, grape vinegar and water mixture (80g, 0,10 L and 1L respectively) prepared and added to the jar. The lid of the jar was closed and fermented for 15 days at room temperature (20 ± 2 °C).

The first samples were taken on the day of pickling and represents the fresh sample in the present study. Other samples were taken on 15th, 30th, and 60th days after the jar closed. At sample preparation process ten grams of samples were homogenized in 250 mL 80% (v/v) methanol at room temperature. The mixture was shaken using shaking bath at 200 rpm for 120 min at 20°C. The mixture was then centrifuged at 1500 rpm for 15 min at room temperature and the supernatant was taken.

This supernatant was stored at -20°C until analysis.

Determination of total phenolic content

Total phenolic content (TPC) was measured using the Folin–Ciocalteu colorimetric method described previously (Wojdylo et al., 2007). 0.2 mL of Sample extracts prepared for total phenolic content were mixed with 4.8 mL of distilled water and 0.5 mL of 1:3 diluted Folin–Ciocalteu reagent added and then incubated at room temperature for 30 min. Following the addition of 1 mL of 35% sodium carbonate to the mixture, total polyphenols were determined after 1 h of incubation at room temperature. The absorbance of the resulting blue colour was measured at 765 nm with UV-visible spectrophotometer (Hitachi U 2000 Japan). Gallic acid was used as the standard for a calibration curve, and the results were expressed as mg of gallic acid equivalents (GEA) mg GAE/100g fresh weight (FW) of fruit. All determinations were performed in triplicate (n=3).

Determination of DPPH radical scavenging activity

DPPH radical scavenging activity was determined according to Yu et al. (2002). This method is based on the ability of the antioxidant to scavenge the DPPH cation radical. Briefly, 100 mL of sample extract or standard was added to 0.9 mL buffer (3.0276g trisHCl in water) and 2 mL of DPPH reagent (0.0394g in methanol) and vortexed vigorously. It was incubated in dark for 30 min at room temperature and the discolouration of DPPH radical was measured against blank at 517 nm. Ethanol (100%) was used as control.

Inhibition (%) of DPPH absorbance = $(A_{\text{control}} - A_{\text{test}}) \times 100 / A_{\text{control}}$.

Trolox was used as a reference standard, and the results were expressed as $\mu\text{molTE}/100\text{g}$ FW of fruit or vegetable. All determinations were performed in triplicate (n=3).

Determination of Trolox equivalent antioxidant capacity (TEAC) activity

The TEAC assay was performed according to the method established previously (Re et al., 1999) with minor modifications. Briefly, the ABTS stock solution was prepared from 7mmol/L ABTS and 2.45 mmol/L potassium persulfate in a volume ratio of 1:1, and then incubated in the dark at room temperature for 16 h and used within 2 days. A 100 mL of the tested sample was mixed with 3.8 mL ABTS working solution and the absorbance was taken at 734 nm after 6 min of incubation at room temperature. The percent of inhibition of absorbance at 734 nm was calculated and the results were expressed as $\mu\text{mol TE}/100\text{g}$ FW of pickled vegetable. All determinations were performed in triplicate (n = 3).

Statistical analysis

All data were presented as means $\pm$ standard deviations of 3 determinations. Non-parametric Kruskal Wallis analysis was used to test whether there is a significant difference in total phenolic, antioxidant capacity of vegetables between fresh and pickled forms. Pearson Correlation Coefficient was used to determine the correlation between the parameters studied in fresh and pickled vegetables. Statistical significance was set at $p < 0.05$. Data were analysed using SPSS for windows version 16.0.

Results and Discussion

Total phenolic content

Table 1 shows mean TPC of vegetables in fresh and pickled form. The TPC of fresh vegetables in the range of $107,21 \pm 11,2$ and $16,51 \pm 2,17$ mgGAE/100g. Fresh chili pepper and garlic had the best TPC ($107,21 \pm 11,2$ and $102,65 \pm 8,56$ mgGAE/100g respectively). Following the pickling in 15 days there was a significant ($P < 0.05$) decrease in TPC of all vegetables, in contrast TPC increased significantly after 30 days. At the end of 60 days TPC decreased but despite this analysis of variance revealed a significant ($P < 0.05$) different in TPC between fresh and pickled vegetables in favour of pickled ones. At 30th day of pickling green beans, garlic and chili have showed the maximum enhancement at TPC.

Table 1. Alteration of total phenolic content of vegetables by pickling.

Vegetables		TP value (mgGAE/100gFW)			
		Fresh	15 th day	30 th day	60 th day
Green beans	<i>Phaseolus vulgaris</i>	39.58 ± 5.46 ^a	18.32 ± 2.11 ^b	51.85 ± 7.56 ^c	49.56 ± 5.78 ^c
Pepper (green)	<i>Capsicum annuum</i>	26.43 ± 0.82 ^a	20.98 ± 3.41 ^b	41.71 ± 6.23 ^c	42.82 ± 4.23 ^c
Chili pepper	<i>Capsicum esculentum</i>	107.21 ± 11.2 ^a	64.25 ± 7.31 ^b	132.25 ± 12.59 ^c	130.34 ± 10.98 ^c
White Cabbage	<i>Brassica oleracea var. capitata</i>	65.58 ± 9.01 ^a	48.25 ± 6.82 ^b	78.68 ± 21.03 ^c	76.38 ± 8.15 ^c
Cauliflower	<i>Brassica oleracea var. botrytis</i>	81.24 ± 5.24 ^a	49.56 ± 7.52 ^b	108.35 ± 18.65 ^c	106.32 ± 11.85 ^c
Cucumber	<i>Cucumis sativus</i>	16.51 ± 2.17 ^a	12.23 ± 0.98 ^b	28.24 ± 2.36 ^c	26.84 ± 3.14 ^d
Sneak melon	<i>Cucumis flexuosus</i>	20.35 ± 1.98 ^a	15.36 ± 1.25 ^b	25.63 ± 3.24 ^c	26.63 ± 2.54 ^c
Tomato	<i>Lycopersicon esculentum</i>	37.94 ± 0.13 ^a	20.02 ± 2.15 ^b	55.23 ± 8.16 ^c	56.35 ± 6.84 ^c
Carrot	<i>Daucus carota</i>	18.21 ± 5.12 ^a	14.18 ± 1.32 ^b	42.28 ± 2.58 ^c	40.35 ± 5.62 ^c

Data are expressed as means ± SE of triplicate experiments. Mean values in a row with different letters are significantly different at $p < 0.05$.

Table 2. Correlation antioxidant capacity and total phenol content of vegetables.

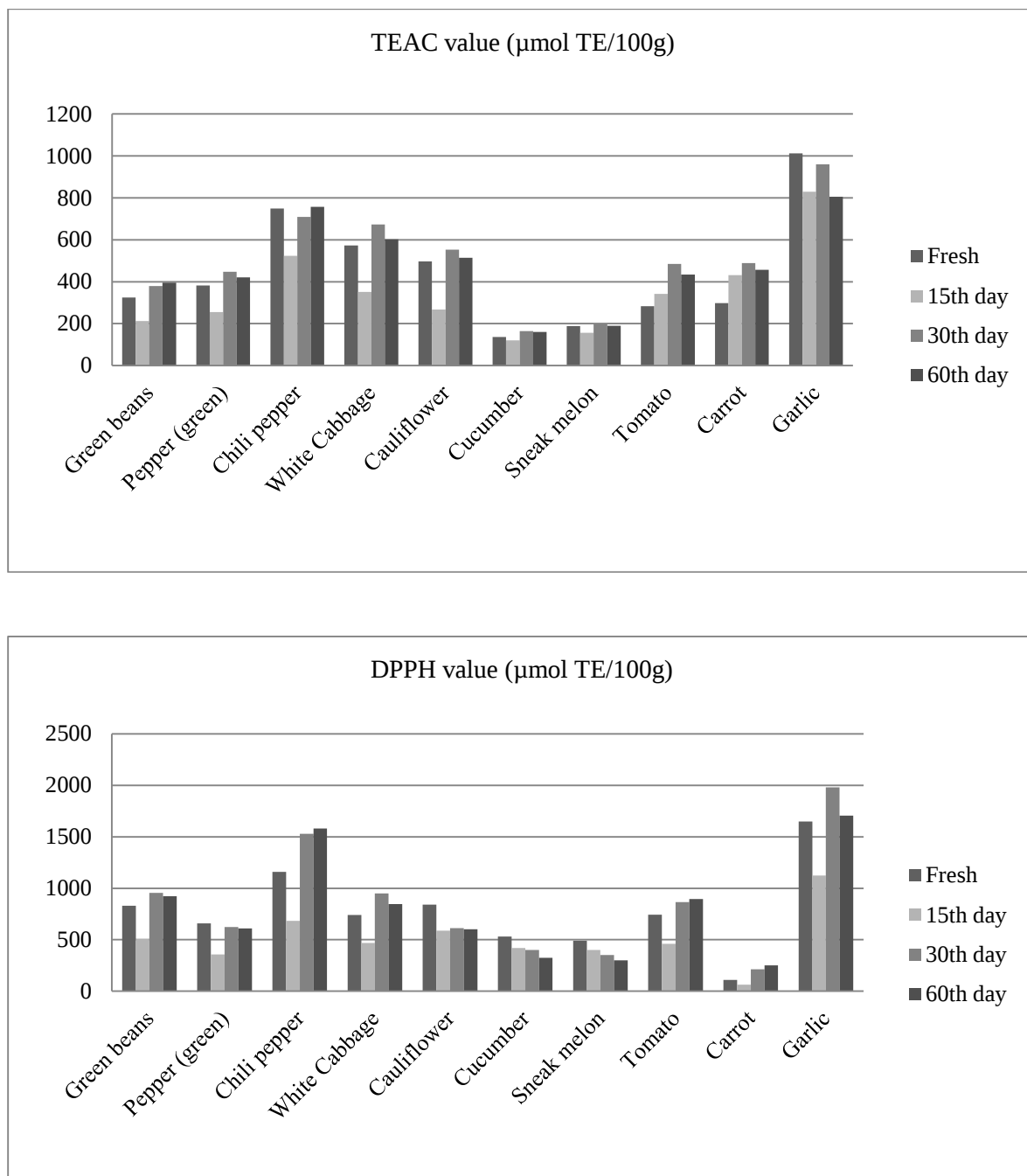
Correlation		r	r ² (%)
Fresh vegetable	TPC-DPPH	0.91	0.83
	TPC-TEAC	0.84	0.72
	TEAC-DPPH	0.86	0.64
Pickled vegetables	TPC-DPPH	0.78	0.62
	TPC-TEAC	0.83	0.70
	TEAC-DPPH	0.74	0.55

TPC, Total phenol content, DPPH, DPPH radical scavenging capacity, TEAC, Trolox equivalent antioxidant capacity.

Previous study found that a number of polyphenols increased after the lactic acid fermentation of vegetables. Soybean is an example of this; fermented soybean foods contain more aglycones as the predominant isoflavone structures compared with unfermented soybean; (Tsangalis et al., 2002). Thus, the conversion of glucosides into their aglycone form by fermentation is a way of increasing TPC of plant based foods. pH is one of the most important environmental parameter affecting the amount and the structural changes of phytochemicals during fermentation. For example, anthocyanin exhibit the highest stability around pH 1–2 (Nielsen et al., 2003) and the retention of anthocyanin is also largely affected by changes in pH (from 2 to 7.5) (Tagliazucchi et al., 2010). Another phenol affected from pH is catechins that rapidly diminish by 70-80% at alkaline pH. Thus, we can say that pH changes during fermentation could change the contents and structure of the phenolic compounds.

Antioxidant Capacity

The AC of pickled vegetable extracts were evaluated according to the DPPH and TEAC methods. Following the 15th day of pickling, the AC of 10 vegetables decreased significantly ($P < 0.05$), while, at the 30th day AC significantly ($P < 0.05$) increased. After the pickling process, garlic had the highest AC among the 10 vegetable pickles, followed by chili pepper, white cabbage and green beans. In this study, garlic showed the highest AC before and after pickling. Through at 60th day of pickling the TEAC values of all vegetables are increased significantly ($P < 0.05$), but DPPH values of green pepper, cauliflower, cucumber and sneak melon decreased compared with fresh state. Compared with the fresh and 30th day of DPPH value, chili pepper was the vegetable that increased most obviously, and according to TEAC value the most increase seen at tomato.

Figure 1. TEAC and DPPH values of fresh and pickled vegetables.

Studies carried out on pickled plant foods revealed that fermentation enhanced the availability of antioxidants, like blueberry (Su and Chen 2007), mulberry (Perez-Gregorio et al., 2011), apple pomace (Ajila et al., 2011). The effects of pickling on AC could be explained by the release of simple phenolic compounds by acid and enzymatic hydrolysis of polymerised phenolic compounds. Another possible explanation is that lactic acid bacteria themselves possess enzymatic and non-enzymatic antioxidant mechanisms and minimize the generation of reactive oxygen species to harmless levels for the cell (Lee et al., 2006). On the contrary, fermentation caused a decrease in antioxidant activity of olive (Othman et al., 2009) and potherb mustard (Fang et al., 2008). Also, as a result of fermentation, tea catechins were significantly reduced by the transformation to theaflavins and thearubigins, resulting in the loss of the total soluble phenolic content and antioxidative activity (Kim et al., 2011). So, a number of studies have addressed that the effect of pickling on antioxidant capacity of foods is variable. This may be caused by the compounds during fermentation like the microorganisms, cultivation medium, times, temperature, pH and atmosphere (Hur et al., 2014).

In general, there was a good correlation between the TP content and AC (as assessed by DPPH and TEAC) among the vegetables and pickles studied (Tables 2). A significant correlation ($p < 0.01$) was observed between TP content and AC both in pickles (r values being 0.78 and 0.83 respectively with DPPH and TEAC respectively) and fresh vegetables (r values 0.81 and 0.85 with DPPH and TEAC respectively) (Tables 2). These findings suggest that TP may be a contributor to the AC of vegetables studied here and are in agreement with the literature (Isabelle et al., 2010; Sreeramulu et al., 2010; Kevers et al., 2007). Studies which did not report similar correlation suggest this lack of correlation could be due to the presence of non-phenolic antioxidants in the vegetables, or presence of phenolics having strong radical scavenging activity (Wu et al., 2004; Mariko et al., 2005).

Conclusion

Among fresh vegetables tested, chili pepper had the highest amount of phenolics and garlic had the strongest antioxidant capacity. Also among pickles tested, garlic, chili and white cabbage respectively had the highest AC and TPC. So it is evaluated that vegetables are rich in total phenolic showed strong antioxidant activity at the same time.

The present study indicates that pickling has an improving effect on the levels of bioactive components and antioxidant capacities of vegetables. Also, pickling process is relatively a good method for the preservation of phenolic acids in vegetables, and most of the antioxidant capacities remained after 30th day of fermentation. First probable reason of this is induce of fermentation the structural breakdown of plant cell walls and leading to the liberation or synthesis of various antioxidant compounds. We can affirm that domestically prepared pickle is not only a delicious vegetable product, but also a good source of antioxidants.

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