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E-mail: medsci@tubitak.gov.tr

TÜBİTAK

ORIGINAL ARTICLE

Effects of Cooking Techniques on Antioxidant Enzyme Activities of Some Fruits and Vegetables

B. İmge ERGÜDER
Aslıhan AVCI
Erdinç DEVRİM
İlker DURAK

Department of Biochemistry,
Faculty of Medicine,
Ankara University,
06100 Ankara -TURKEY

Aim: The aim of this study was to investigate possible effects of cooking techniques on antioxidant enzyme activities in broccoli, tomato, red cabbage, parsley, carrot, green pepper, lemon, onion, and garlic, which are consumed frequently in our daily diet.

Materials and Methods: Superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) activities were measured in fresh and thermally treated (boiling, microwaving and baking) fruits and vegetables.

Results: When compared to raw vegetables, SOD, CAT and GSH-Px activities were found to diminish in most of the thermally treated vegetables except tomato, in which GSH-Px and CAT activities were found to increase. Our results show that antioxidant enzyme activities decrease in most of the fruits and vegetables after they are thermally treated, except in the tomato, in which increases are observed. It is possible that this property of the tomato is one of the reasons for its increased bioavailability after being thermally treated. We think that lycopene transfers from trans form to cis form, which is a more bioactive form, after being thermally treated.

Conclusions: We suggest that fruits and vegetables should be consumed raw if possible and tomatoes after thermal treatment.

Key Words: Antioxidant enzymes, influence of cooking

Bazı Meyve ve Sebzelerin Antioksidan Enzim Aktiviteleri Üzerinde Pişirme Tekniklerinin Etkileri

Amaç: Bu çalışmanın amacı günlük diyetimizde sık olarak tükettiğimiz brokoli, domates, kırmızı lahana, maydonoz, havuç, yeşil biber, limon, soğan ve sarımsak gibi bazı meyve ve sebzelerde pişirme tekniklerinin antioksidan enzim aktiviteleri üzerine olası etkilerini incelemekti.

Yöntem ve Gereç: Çiğ ve pişirilmiş (kaynatma, mikrodalga ve fırın) meyve ve sebzelerde süperoksit dismutaz (SOD), glutatyon peroksidaz (GSH-Px) ve katalaz (KAT) enzim aktivitelerinin ölçümleri yapıldı.

Bulgular: Çiğ değerleriyle karşılaştırıldığında, SOD, KAT ve GSH-Px aktiviteleri, domates hariç, pişirilmiş diğer meyve ve sebzelerin tamamına yakınında azalmış olarak bulundu. Pişirilmiş domateste ise GSH-Px ve KAT aktiviteleri artmış olarak bulundu. Sonuçlarımız göstermiştir ki pişirme işlemleri bu yiyeceklerdeki antioksidan enzimlerde aktivite azalmasına yol açmaktadır, fakat domatesin pişirilmesi antioksidan enzimlerde aktivite artışına neden olmaktadır. Bunun nedeninin likopenin ısıyla muamele sonrasında trans formundan biyoyararlanımı daha yüksek olan cis formuna dönüşmesi olabileceğini düşünmekteyiz.

Sonuç: Sonuç olarak meyve ve sebzelerin mümkünse çiğ olarak tüketilmesinin, domatesin ise pişirme sonrası tüketilmesinin daha yararlı olacağını önerebiliriz.

Anahtar Sözcükler: Antioksidan enzimler, pişirmenin etkileri

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Correspondence
B. İmge ERGÜDER
Department of Biochemistry,
Faculty of Medicine,
Ankara University,
06100 Sihhiye, Ankara - TURKEY

imgeerguder@yahoo.com

Introduction

Epidemiological studies show that consumption of fruits and vegetables is associated with reduced risk of chronic diseases (1). Nutritional recommendations to reduce the risk of coronary heart disease have focused largely on the intake of nutrients that affect the risk factors, including plasma lipid and lipoprotein levels, blood pressure, and body weight, etc. Over the past two decades, considerable evidence has been gathered in support of the hypothesis that free-radical-mediated oxidative processes and specific end products play a key role in atherogenesis. This may involve effects on intimal proliferation, fibrosis, calcification, endothelial function, vasoreactivity, plaque rupture, and thrombosis. Antioxidants constitute a diverse group of compounds with different properties. They operate by inhibiting oxidant formation, intercepting oxidants once they have formed, and repairing oxidant-induced injury. In terms of the coronary heart disease process, several points of antioxidant intervention have been proposed. Inhibition of low density lipoprotein (LDL) oxidation is the most well-known. Antioxidants create resistance to the oxidants. Although there is an effective antioxidant defense mechanism to protect the body against oxidant attacks, sometimes it cannot cope with oxidant load in the body, and additional dietary antioxidants are needed. Among them, vitamin C, vitamin E, flavonoids, and carotenes have received the greatest attention with regard to coronary heart disease prevention. A number of other dietary constituents are also proposed to act as antioxidants and to protect against coronary heart disease (2-11). Antioxidant properties of carotenoids are thought to be at least partly responsible for the protective effects of fruits and vegetables rich in carotenoids against colon cancer (12). Beta-carotene has been shown to enhance immune functions in humans (13).

Green leafy vegetables, fruits and vegetable oils are excellent sources of antioxidant components. Most vegetables undergo a cooking process rather than being eaten raw. Cooking practices may affect the antioxidant content and properties in vegetables (14); however, little is known in this regard.

Therefore, this study was carried out to determine the effects of domestic cooking practices such as boiling, microwaving and baking on antioxidant enzyme activities. We investigated activities of antioxidant enzymes, namely superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT), in fresh and thermally treated fruits and vegetables, including lemon, tomato, red cabbage, parsley, carrot, green pepper, broccoli, onion and garlic.

Materials and Methods

All fruits and vegetables (lemon, broccoli, tomato, red cabbage, parsley, carrot, green pepper, onion, garlic) used in this study were purchased from a market and immediately cleaned, washed, sliced and homogenized in distilled water (2 g vegetable in 10 ml distilled water). Fruits and vegetables were then allocated to four groups as raw and thermally treated (boiling, microwaving and baking). Of the three thermally treated groups, one was boiled for 20 minutes, one microwave-baked for one minute (only lemon for 30 sec.) at 360 watt and the third classical-oven baked for 10-20 minutes at 200°C. Cooking times were determined according to common cooking techniques. All samples were filtered using filter paper. GSH-Px activity was measured by following changes in NADPH absorbance at 340 nm (15). CAT activity was determined by measuring decrease in H_2O_2 absorbance at 240 nm (16). In the activity calculations, extinction coefficients of H_2O_2 and NADPH were used for CAT and GSH-Px, respectively. SOD activity was measured by the method based on nitro blue tetrazolium (NBT) reduction rate (17). One unit for SOD activity was expressed as the enzyme amount causing 50% inhibition in NBT reduction rate. Activity results were expressed as unit / g weight of related vegetable. In this study, all measurements were carried out three times and their mean values were used in the calculations.

Statistics

One sample t test was used in the statistical evaluation of enzyme activities between raw and thermally treated vegetables. P values lower than 0.05 were considered as significant.

Results

Mean enzyme activities are given in Figures 1-3 and percent changes in thermally treated fruits and vegetables relative to their raw forms are given in Tables 1-3. When compared to raw forms, SOD, CAT and GSH-Px activities were found to diminish significantly in most of the thermally treated fruits and vegetables. GSH-Px and CAT activities, however, were found to increase in the thermally treated tomato.

Discussion

Reactive oxygen species (ROS) have been implicated in tissue injury. The main ROS to be considered are superoxide anion (O_2^-), which is predominantly generated by the mitochondria; hydrogen peroxide (H_2O_2) produced from O_2^- by the action of SOD, and peroxynitrite ($ONOO^-$), generated by the reaction of O_2^- with nitric oxide (NO). These ROS produced are scavenged by SOD, GSH-Px and

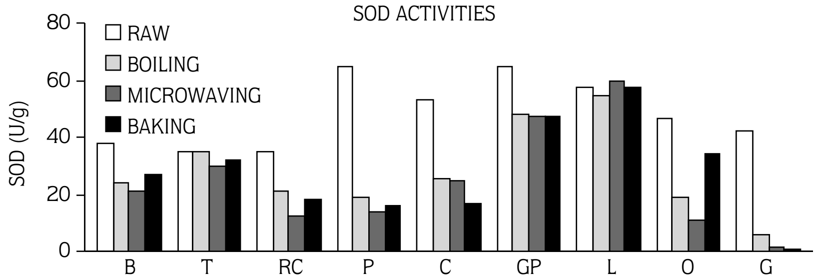

Figure 1. Mean SOD activities in raw and thermally treated fruits and vegetables.

B: Broccoli.	T: Tomato.	RC: Red cabbage.
P: Parsley.	C: Carrot.	GP: Green pepper.
L: Lemon.	O: Onion.	G: Garlic.

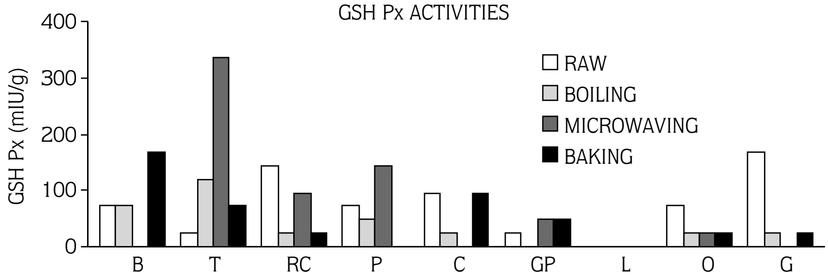

Figure 2. Mean GSH-Px activities in raw and thermally treated fruits and vegetables.

B: Broccoli.	T: Tomato.	RC: Red cabbage.
P: Parsley.	C: Carrot.	GP: Green pepper.
L: Lemon.	O: Onion.	G: Garlic.

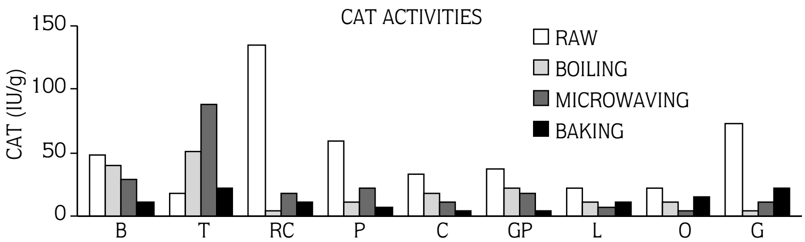

Figure 3. Mean CAT activities in raw and thermally treated fruits and vegetables.

B: Broccoli.	T: Tomato.	RC: Red cabbage.
P: Parsley.	C: Carrot.	GP: Green pepper.
L: Lemon.	O: Onion.	G: Garlic.

CAT. Under some circumstances, these endogenous antioxidant defenses are likely to be perturbed as a result of overproduction of oxygen radicals, inactivation of detoxification systems, consumption of antioxidants, and failure to adequately replenish antioxidants in tissue. It has been demonstrated in numerous studies that ROS are directly involved in oxidative damage of cellular macromolecules such as lipids, proteins, and nucleic acids in tissues (18).

Table 1. Percent changes in SOD activities of thermally treated fruits and vegetables relative to their raw forms.

	Broccoli	Tomato	Red cabbage	Parsley	Carrot	G pepper	Lemon	Onion	Garlic
Boiling	-36.51[a]	0.46	-39.01[a]	-70.40[a]	-51.47[a]	-25.66[a]	-4.78	-60.11[a]	-85.57[a]
Microwaving	-45.17[b]	-13.92[b]	-64.84[b]	-78.16[b]	-53.13[b]	-26.99[b]	3.35	-77.32[b]	-96.22[b]
Baking	-28.81[c]	-8.32[c]	-46.81[c]	-75.43[c]	-68.03[c]	-26.74[c]	-0.01	-26.38[c]	-89.59[c]

[a]: $P < 0.005$; One sample t-test against raw vegetables.

[b]: $P < 0.005$; One sample t-test against raw vegetables.

[c]: $P < 0.005$; One sample t-test against raw vegetables.

Table 2. Percent changes in GSH-Px activities of thermally treated fruits and vegetables relative to their raw forms.

	Broccoli	Tomato	Red cabbage	Parsley	Carrot	G pepper	Lemon	Onion	Garlic
Boiling	0	400 [a]	-83.33 [a]	-33.33 [a]	-75 [a]	-100 [a]	0	-66.67[a]	-85.71[a]
Microwaving	-100 [b]	1300 [b]	-33.33 [b]	100 [b]	-100 [b]	100 [b]	0	-66.67[b]	-100 [b]
Baking	133 [c]	200 [c]	-83.33 [c]	-100 [c]	0	100 [c]	0	-66.67[c]	-85.71[c]

[a]: $P < 0.005$; One sample t-test against raw vegetables.

[b]: $P < 0.005$; One sample t-test against raw vegetables.

[c]: $P < 0.005$; One sample t-test against raw vegetables.

Table 3. Percent changes in CAT activities of thermally treated fruits and vegetables relative to their raw forms.

	Broccoli	Tomato	Red cabbage	Parsley	Carrot	G pepper	Lemon	Onion	Garlic
Boiling	-15.38 [a]	180 [a]	-97.30 [a]	-81.25 [a]	-44.44[a]	-40 [a]	-50.00[a]	-50.00[a]	-95 [a]
Microwaving	-38.46 [b]	380 [b]	-86.49 [b]	-62.50 [b]	-66.67 [b]	-50 [b]	-66.67 [b]	-83.33 [b]	-85 [b]
Baking	-76.92 [c]	20 [c]	-91.89 [c]	-87.50 [c]	-88.89[c]	-90 [c]	-50.00[c]	-33.33[c]	-70 [c]

[a]: $P < 0.005$; One sample t-test against raw vegetables.

[b]: $P < 0.005$; One sample t-test against raw vegetables.

[c]: $P < 0.005$; One sample t-test against raw vegetables.

Domestic cooking practices, such as boiling, microwaving and frying, provoke a partial loss of the flavonols (19). There is considerable cooking loss of vitamins, and information on vitamin contents of cooked foods is essential for assessing the adequacy of vitamin intakes. The cooking losses were very high in case of ascorbic acid, thiamine, riboflavin, beta-carotene and folic acid (20). In one study, cooking of zucchini, beans and carrots with smaller amounts of water resulted in significantly higher content of phenolic phytochemicals in the vegetables compared to cooking with larger water volumes (21). Lycopene is a bioactive carotenoid present in many fruits and vegetables. Tomatoes constitute the major dietary source of lycopene. Subjecting tomato juice to cooking temperatures in the presence of corn oil resulted in the formation of the cis form, which was considered to be more bioavailable (22). In addition to lycopene, tomato is a rich source of beta-carotene, folate, potassium, vitamin C, flavonoids, and vitamin E. The processing of tomatoes may significantly affect the bioavailability of these nutrients. Homogenization, heat treatment, and the incorporation of oil in processed tomato products lead to increased lycopene bioavailability, while some of the same processes cause significant loss of other nutrients (23). Onion is a major source of flavonoids and is cooked in various ways throughout the world. The major flavonoids in onion are two quercetin glycosides, quercetin 4'-O-beta-glucoside (Q4'G) and quercetin 3,4'-O-beta-diglucosides (Q3,4'G), which are recognized as bioactive substances that are good for our health. Boiling, frying with oil and butter, and microwave cooking were examined in one study. The investigators reported that various cooking methods do not consider the degradation of quercetin conjugates when cooking onion. Microwave cooking without water better retains flavonoids and ascorbic acid. Frying does not affect flavonoid intake. The boiling of onion leads to about 30% loss of quercetin glycosides, which transfers to the boiling water (24).

As far as we know, this is the first study investigating the possible effects of cooking techniques on antioxidant enzyme activities in the fruits and vegetables consumed frequently in our daily diet. The results of the present study showed that all antioxidant enzyme activities decrease in the vegetables/fruits, except tomato, when they are thermally treated (boiling, microwaving and baking). However, we observed that GSH-Px and CAT activities increase in the thermally treated tomato (boiling, microwaving and baking), and SOD activity increases in the boiled tomato whereas it decreases in the microwaved and baked tomato. Additionally, we determined that lemon has no GSH-Px activity in raw and thermally treated forms; this finding may be due to its acidic pH. On the other hand, GSH-Px activity was found to increase in microwaved parsley and in microwaved and baked green pepper.

Our results suggest that thermal treatments lead to loss in the activities of the antioxidant enzymes in most fruits and vegetables, which may cause decreases in their antioxidant potentials. However, the tomato appears to exhibit special properties in this regard, which indicate that thermal treatments cause significant increases in its antioxidant enzyme content activities. It is possible that this property of the tomato is one of the reasons for its increased bioavailability after cooking, which was also observed in a previous study (23). Hence, thermal treatments may lead to an increase in the antioxidant power of tomato, in contrast to observations in most other fruits and vegetables.

In conclusion, we suggest that it is better to consume most fruits and vegetables in their raw forms. Tomato is an exception in this regard, because the antioxidant property of tomato seems to increase after cooking.

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