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Effects of Grilling on Luteolin (3',4',5,7-tetrahydroxyflavone) Content in Sweet Green Bell Pepper (*Capsicum annuum*)

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Abstract: The content of luteloin in green bell pepper (*Capsicum annuum*) produced in Turkey were determined by RP-HPLC with DAD detection. The luteloin (3',4',5,7-Tetrahydroxyflavone) content of green pepper samples were 46.00±0.76 mg kg^{-1} f.w. (average). The alterations of luteloin concentrations with heating process (grilling, *közleme*) and the loss of luteloin amount were also determined. Luteolin contents of grilled peppers were found as 29.96±0.96 mg kg^{-1} f.w. The method was objective and reproducible for accurate detection of luteloin in green pepper and other pepper varieties.

Key words: Luteolin, green pepper, HPLC-DAD, grilling

INTRODUCTION

Interest in especially plant food polyphenols has increased owing to their role as antioxidants, antimutagens and scavengers of free radicals and their implication in the prevention of pathologies such as cardiavascular disease and cancer. Epidemiological researches have confirmed a correlation between an increased consumption of phenolic antioxidants and a reduced risk of cardiovascular diseases and certain types of cancer (Hertog *et al*., 1993; Hertog, 1996; Holman and Katan, 1999; Tokuşoğlu, 2001, 2006).

Several food flavonoids show different kinds of positively biological activity *in vivo* and *in vitro* (Rice-Evans *et al*., 1997; Harborne and Williams, 2000; Tokuşoğlu *et al*., 2003, 2005a, b).

The phenolic compound luteolin (3',4',5,7-Tetrahydroxyflavone) (Fig. 1) has been shown to have strong antioxidant (Shimoi *et al*., 1994), anti-inflammatory/antiallergic (Yamamoto *et al*., 1998) activities, antitumorigenic (Yasukawa *et al*., 1989) and to be an inhibitor of protein kinase C (Ferriola *et al*., 1989) and lipoxygenase (Yamamoto *et al*., 1998). Luteolin monoglucuronide and free luteolin were also detected in human serum after ingestion of luteolin (Shimoi *et al*., 1998).

Pepper, genus *Capsicum*, belongs to the great family of tropical plants Solanaceae (Somos, 1984) and this taxon includes both sweet cultivars eaten mainly as vegetables and hot cultivars often used as a spice. Green bell peppers (*Capsicum annuum*) are considered to be a good sources

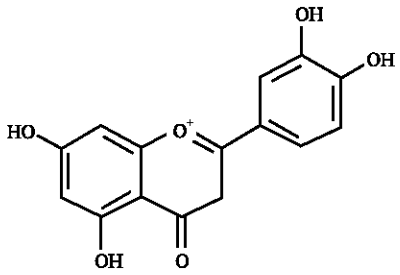

Fig. 1: Luteolin

of various beneficial phytochemicals, such as carotenoids (β-carotene, lutein and zeaxanthin), flavonoids and ascorbic acid (vitamin C) (Crosby *et al*., 2004; Tokuşoğlu, 2006). Especially the yellow, orange and red colors in peppers originate from the 30 different carotenoid pigments produced in the fruit ripening. Quercetin (3,3',4',5,7-penta hydroxy flavone) which is a food-derived flavonoid has been act anticarcinogen and antimutagen *in vitro* and *in vivo*. Moreover, its strong antioxidant activity protects LDL from oxidation, reducing the risk of heart disease. Luteolin (3',4',5,7-Tetrahydroxyflavone) may help prevent breast cancer by reducing excess estrogen formation (Crosby *et al*., 2004; Tokuşoğlu, 2006).

Bell peppers are best when they are thick walled and juicy, so they should feel heavy for their size. If a green bell pepper shows streaks of red, it will be slightly sweeter than a totally green one (Crosby *et al*., 2004; Tokuşoğlu, 2006). Sweet bell peppers are generally consumed as baking or microwaving of whole stuffed peppers with rice and tomato slices.

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Research on flavonoid, especially flavonoid aglycons as quercetin, luteolin and flavonoid glycosides, phenolic acid content in pepper fruit is scarce. Flavonoid aglycons have been determined after acid hydrolysis (Hertog *et al*., 1992; Lee *et al*., 1995; Howard *et al*., 2000).

In Turkey, some vegetables is consumed after grilling (*közleme*) process by using an electrical grill or barbeku at 150-200°C. Grilling process can provide enhanced flavor to some vegetables such as green pepper, aubergine etc.

The objectives of this study was to determine the quantification of luteolin in green bell peppers grown in Manisa and İzmir area in Turkey using Reversed-Phase High Performance Liquid Chromatography (RP-HPLC) with diiod array (DAD) detection and to detect the alterations with grilling effects.

MATERIALS AND METHODS

Chemicals: Luteolin (3',4',5,7-Tetrahydroxyflavone) (Fig. 1) standard was purchased from Sigma Chemical Company, (Poole, Dorset). Absolute ethanol (HPLC grade), methanol (HPLC grade), perchloric acid, phosphoric acid, formic acid, ethyl acetate were purchased from E. Merck co. (Darmstadt, Germany). Sodium acetate and all organic solvents were of analytical grade and obtained from Merck (Darmstadt, Germany). Double-distilled water was purchased from LabScan (Turkey).

Sampling: Five different group of green bell peppers with same maturity were prepared for analysis. Three group of green peppers (*Capsicum annuum*) were purchased from 3 different greengrocers in the local markets at a period of their most frequent consumption and two group of samples were collected from growing area in İzmir and Manisa, Turkey. (Each 5 kg as five units and harvesting season: 2005 fall). The edible parts of samples were used. After purchasing, the samples were immediately cleaned and scrubbed well due to the some bell peppers had waxed and the some samples had possible soiled etc. The seeds of pepper samples were removed and samples chopped into small pieces. Then, pieces of pepper samples were squeezed with blender and homogenized under N_2 atmosphere. Final samples stored at -18°C prior to subsequent analysis.

Proximate analysis: The moisture content was determined by drying a representative 2 g sample at 100-105°C for 40 h Total ash was determined by incineration of a representative 2 g sample at 450°C for 30 h. Total lipids of samples were determined according to described method by Tokuşoğlu and Ünal (2003) regarding edible algae lipids. Crude protein was determined by Kjeldahl protein units (Gerhardt incineration apparatus and Gerhardt Vapodest 20 distillation apparatus) and protein was calculated as nitrogen (%)×6.25 (AOAC, 1990). Available carbohydrate was determined by the anthrone spectrophotometric method (Osborne, 1986). All proximate chemical determinations were done in triplicate. The energy content of the green bell pepper was determined by multiplying the values obtained for crude protein, total carbohydrate and total lipid by 4.00, 3.75 and 9.00, respectively and summing the results. Final results were multiplied with 4.18 and energy content was obtained as kJoule. Dietary fiber content of peppers was determined based on AOAC (1990) whereas ascorbic acid levels were determined according to Yıldırım *et al*. (2002).

Heating process (grilling, *közleme*): Electrical grill was heated to 150°C before process. Green bell peppers were grilled during 7-8 min at 150°C by using electrical grill.

Extraction conditions for luteolin analysis: Twenty five milliliter of acidified ethanol containing 1% perchloric acid was added to same volume of sample extract. To this extract, 2 mL of 1.2 M perchloric acid was added in order to obtain aglycon form of lutein. Final extract was dissolved with 5 mL of ethyl acetate and reaction mixture was stirred at ice bath for ½ h. Final extract was filtered through a 0.5 μm (Acrodisc) filter prior to filtration 10 μL of filtrate was injected into the HPLC.

HPLC conditions: Green pepper samples were analyzed by RP-HPLC using UV detection. Five micrometer Inertsil ODS-3V column (150×4.6 mm I.D.) [Phenomenex, CAL, USA] using methanol/phosphate buffer (20 mM, pH 3.0)/formic acid (55: 40: 5 v/v/v) as mobile phase with a flow rate of 1.0 mL min^{-1}. UV Absorbance detector was set 254 nm., detector sensitivity was 0.05 AUFS and column oven was set at 35°C. The samples were injected as 10 μL. All determinations were performed from 3 separate extractions and each was injected in triplicate (n = 3).

Analytical precision of method: The standard were dissolved to 500 μg mL^{-1} with methanol (HPLC grade). After the preparation of stock solutions, they were stored at -28°C. For calibration, all standards were prepared as 25 μL/1 mL methanol from above-mentioned stock solutions. Calibration curves of this thorough the origin and their R^2-values was 0.9999. The quantitative amounts of luteolin in green pepper samples were determined using these curves. The linearity plotting at 254 nm was ($y = 187.762x + 30.855$) ($R^2 = 0.9999$). The assay recoveries

Table 1: The analytical parameters of luteolin

Analytical parameters	Luteolin
Interday precision (n = 3)	
mg kg^{-1} f.w. sample	45.2±0.72
RSD (%)	1.65
Recovery (%) n = 3	99.98
Detection limit	
mg kg^{-1} f.w. sample	0.45
μg mL^{-1} assay	0.05

for luteolin ranged from 98.96 to 99.99%. The inter-day precision (RSD) for luteolin was 45.2±0.72 mg kg^{-1} f.w. sample. The analytical parameters of luteolin in Table 1. The Detection Limits (DL) of luteolin was 0.45 mg kg^{-1} while the Limits Of Quantification (LOQ) was 0.05 μg mL^{-1}.

Determination of total flavonoids: Total flavonoids were analyzed according to procedure described by Zhishen (1999). One milliliter of extract was placed in a 10 mL volumetric flask. Five milliliter of bidistilled and 0.3 mL of $NaNO_2$ were added and mixed. About 0.6 mL of 10% $AlCl_3.6H_2O$ was added after 5 min. Two millilitre of 1 M NaOH was added 5 min later and the solution mixture, vigorously. The absorbance of final solution was measured at 510 nm, immediately. Total flavonoid contents were calculated using a standard calibration curve, prepared from (+) catechin.

Statistical analysis: Statistical analysis was performed using Student's t-test. The differences between the all groups with a value of $p<0.01$ was considered significant.

RESULTS AND DISCUSSION

The proximate chemical composition, dietary fiber, vitamin C (ascorbic acid) and energy values of green bell peppers grown in country (as per cent) as shown in Table 2 ($p<0.01$). Dry matter (%) was found as 9.92±0.24 (Table 2). Total fat, total protein and total available carbohydrates of peppers were 0.33±0.08, 0.99±0.03 and 10.63±0.05 g, respectively ($p<0.01$). Energy values were determined as 46.79±1.03 cal (195.58±4.29 kjoule) (Table 2). Dietary fiber content and vitamin C (ascorbic acid) levels of peppers were found as 2.76±0.10 g and 133.00±1.06 mg (Table 2).

Luteloin was perfectly separated with the HPLC-DAD procedure used and Fig. 2 shows the baseline separation of luteloin in green bell peppers (Fig. 2). Peak identity was confirmed when peak retention times (avg. RT 17.2 min) were identical to that of the pure standard in mobile phase. Table 3 shows the luteloin content in sweet green bell samples. In present study, the luteloin concentration ranged 18.53-48.96 mg kg^{-1} (n = 12).

Table 2: The proximate chemical composition, dietary fiber amounts, vitamin C levels and energy values of analyzed green bell peppers ($p<0.01$)

Chemical composition	
Dry matter (%)	9.92±0.24
Total fat (g)	0.33±0.08
Protein (g)	0.99±0.03
Carbohydrate (g)	10.63±0.05
Dietary fiber (g)	2.76±0.10
Vitamin C (mg)	133.00±1.06
Calories (cal)	46.79±1.03
Energy (kj)	195.58±4.29

Data are based on dry wt. (%), Mean±SD (n = 3)

Table 3: The luteolin composition of raw and grilled (köz) green bell peppers ($p<0.01$)

Green bell pepper	Dry matter (g 100 g^{-1})	Luteolin (mg kg^{-1} f.w.)	Total phenolics (mg kg^{-1} f.w.)
Raw	9.92±0.24	46.00±0.76	132.48±2.10
Grilled	4.97±0.96	29.96±0.96	92.83±1.74

Values are means of triplicate determinations. All analysis in duplicate

Andrea *et al.* (2003) determined 10.7 mg kg^{-1} f.w. of luteolin and 20.1 mg kg^{-1} of total flavonoids in green bell peppers grown in Hungary. Luteloin content of some green bell peppers in our research was in accordance with the studies described by Andrea *et al.* (2003).

Lee *et al.* (2005) determined that the developments of flavonoids in pepper (*Capsicum* sp.) depends on the genetic variety and environmental variation. The researchers found that especially cultivar effects were significant for luteolin and some phytochemicals at each location including a greenhouse at College Station, Texas, USA. According to their study, Fidel (37 μg g^{-1}) and Banana Supreme (21.5 μg g^{-1}) were the best sources of luteolin. Luteloin data in our study was higher than the study described by Lee *et al.* (2005).

Luteolin contents in field-grown fruits may also be affected by a wide scale of environmental influences containing temperature, soil, nutritional condition of plant and light levels (Tokuşoğlu, 2006).

Arabi Paola *et al.* (2004) detected the flavonoids in vegetable foods commonly consumed in Brasil. Based on their findings, luteolin was found in green pepper as 2.1±0.2 mg/100 g f.w. (*Capsicum annuum* L. var. *annuum*) at second semester of 2001 and as 1.2±0.2 mg/100 g f.w. at first semester of 2002. Total flavonoid was 6.2 mg/100 g f.w. at second semester of 2001 while 3.0 mg/100 g f.w. at first semester of 2002. It has been showed that seasonal variations affected the lutein concentration of green peppers in the study described by Arabi Paola *et al.* (2004).

Our luteolin concentration findings are in agreement with the study reported by Arabi Paola *et al.* (2004).

Ioku *et al.* (2005) indicated that luteolin content was 8.15±4.10 mg/100 g of f.w. green pepper in Japan. This level was higher than our data. Based on their findings,

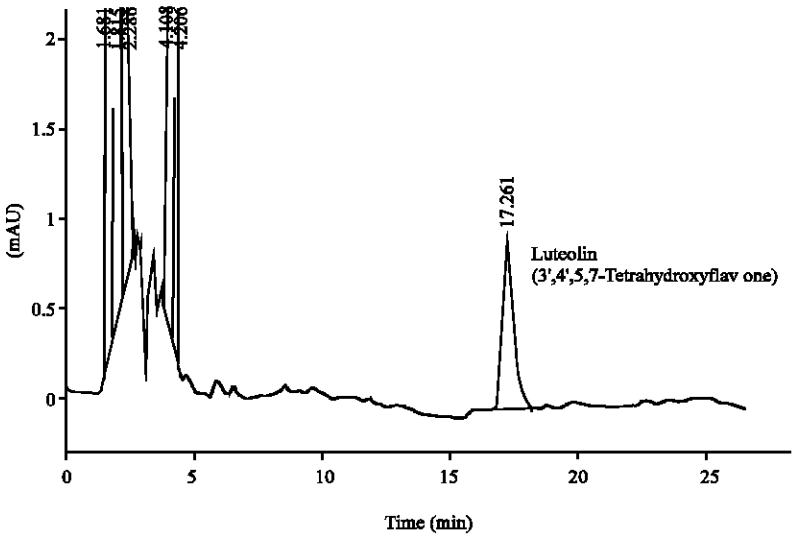

Fig. 2: Luteolin in green bell pepper (*Capsicum annuum*)

flavonoid contents were increased in October to December and the tendency to decrease in May to June.

Miean and Mohamed (2001) determined the 33.0 mg kg^{-1} of luteolin in green chili and Crosby *et al.* (2004) detected that several paprika and cayenne types have recently been confirmed as excellent sources of the flavonoid compounds quercetin and luteolin, with levels between 260 and 400 ppm.

Luteolin contents of grilled peppers were found as 29.96±0.96 mg kg^{-1} f.w. and total phenolic substances were 92.83±1.74 mg kg^{-1} f.w. ($p<0.01$) (Table 3). No study could be found concerning luteoin concentration and total phenolic levels in grilled green peppers. In this context, this data gave important results.

It may be concluded that Turkish green bell peppers are a good source of luteolin. It should be utilize the good candidate parents for the breeding program to develop novel pepper varieties with increased health benefits and enhanced beneficial phytochemicals such as luteolin. The study regarding genetic varietal effects of green bell peppers is also in progress.

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