Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 99 (2006) 121–128
Food Chemistry
www.elsevier.com/locate/foodchem

Antioxidant activity of cherry laurel fruit (*Laurocerasus officinalis* Roem.) and its concentrated juice

C.M. Liyana-Pathirana [a], F. Shahidi [a,b], C. Alasalvar [c,*]

[a] *Department of Biology, Memorial University of Newfoundland, St. John's, Nfld, Canada A1B 3X9*
[b] *Department of Biochemistry, Memorial University of Newfoundland, St. John's, Nfld, Canada A1B 3X9*
[c] *Faculty of Health, Life, and Social Sciences, Food Research Centre, University of Lincoln, Brayford Pool, Lincoln LN6 7TS, UK*

Received 23 August 2004; received in revised form 7 June 2005; accepted 7 June 2005

Abstract

Cherry laurel fruit and its concentrated juice (pekmez) were examined for their antioxidant activities using different free-radical scavenging activity tests [hydrogen peroxide, superoxide radical, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical], together with reducing power and inhibition of oxidation of human low-density lipoprotein cholesterol. On a fresh weight basis, pekmez exhibited a significantly ($P < 0.01$) higher antioxidant activity than that of cherry laurel fruit in most cases. However, on a dry weight basis hydrogen peroxide and DPPH radical scavenging activities, and reducing power were significantly higher ($P < 0.01$) in cherry laurel fruit than in its pekmez, with some exceptions, thus indicating possible destruction of antioxidative compounds during pekmez production. This was also partly due to the moisture content of these two samples. On the basis of the results presented, it is suggested that the intake of cherry laurel fruit and pekmez rich in phenolics would have beneficial effects in improving amelioration of degenerative diseases caused by oxidative stress. Therefore, both cherry laurel fruit and pekmez might be considered as functional food ingredients and nutraceuticals.

Keywords: Cherry laurel fruit; *Laurocerasus officinalis* Roem.; Pekmez; Antioxidant activity; Free-radical scavenging; Reducing power; Inhibition of LDL oxidation

1. Introduction

Cherry laurel (*Laurocerasus officinalis* Roem.) belongs to the Rosaceae family and is a popular fruit (dark purple or black when mature), mainly distributed in the coasts of the Black Sea region of Turkey and is locally called "*Taflan*" or "*Karayemiş*" (Alasalvar, Al-Farsi, & Shahidi, 2005). It is mostly consumed as fresh fruit in local markets but may also be dried, pickled, and processed into pekmez, jam, marmalade, and fruit juice products. Besides its use for food, both fruit and seeds of cherry laurel are well known as traditional medicines in Turkey and have been used for many years for the treatment of stomach ulcers, digestive system complaints, bronchitis, eczemas, haemorrhoids, and as a diuretic agent, among others (Baytop, 1984).

The concentrated juice of cherry laurel fruit produced by boiling/heating (pekmez) has traditionally been used for centuries in Turkey. Fresh or dried fruits such as grapes, are mainly used for the production of pekmez, but other sugar-rich fruits such as apples, pears, plums, mulberries, cherry laurels, watermelons, and apricots can also be used in pekmez production (Aksu & Nas, 1996; Tosun & Ustun, 2003). Among these fruits, pekmez from cherry laurel is becoming

* Corresponding author. Tel.: +44 1522 886024; fax: +44 1522 886026.
E-mail address: calasalvar@lincoln.ac.uk (C. Alasalvar).

0308-8146/$ - see front matter © 2005 Published by Elsevier Ltd.
doi:10.1016/j.foodchem.2005.06.046

increasingly popular because of its potential and perceived health benefits (Alasalvar et al., 2005; Batu, 1993).

The plant-derived edible and non-edible products contain a wide range of phenolic compounds (such as phenolic acids, flavonoids, anthocyanins, tannins, lignans, and catechin, among others) that possess antioxidant activities. These phenolics provide protection against harmful free-radicals and have been known to reduce the risk of certain types of cancer, coronary heart disease (CHD), cardiovascular disease (CVD), stroke, atherosclerosis, and other degenerative diseases associated with oxidative stress (Hertog, Feskens, Hollman, Katan, & Kromhout, 1993; Mazza, Fukumoto, Delaquis, Girard, & Ewert, 1999; Ness & Powles, 1997; Shahidi & Naczk, 2004; Surh, 2003; Temple, 2000; Watson, 2003). The antioxidant activity of these phenolics is mainly due to their redox properties, which allow them to act as reducing agents or hydrogen-atom donors. Thus, natural antioxidants function as free-radical scavengers and chain breakers, complexers of prooxidant metal ions and quenchers of singlet-oxygen formation (Pratt, 1992).

Evaluation of antioxidant activity of fruits, vegetables, and other plant products cannot be performed accurately by any single method due to the complex nature of phytochemicals present (Chu, Chang, & Hsu, 2000). Numerous methods have been proposed to evaluate/estimate the antioxidant potential of natural sources of antioxidants (Amarowicz, Naczk, Zadernowski, & Shahidi, 2000; Amarowicz, Pegg, Rahimi-Moghaddam, Barl, & Weil, 2004; Shahidi & Naczk, 2004; Siriwardhana & Shahidi, 2002). Of these, at least two different methods should be employed in order to evaluate the antioxidant activity of the products of interest.

As part of a parallel study, compositional characteristics and antioxidant components [such as $ORAC_{FL}$ (oxygen radical absorbance capacity) using fluorescein (FL), phenolic acids, and total contents of phenolics, anthocyanins, and carotenoids] of cherry laurel varieties and pekmez were investigated (Alasalvar et al., 2005). However, relatively little or no information is available on free-radical scavenging activities, reducing power, and inhibition of oxidation of human low-density lipoprotein (LDL) cholesterol of cherry laurel fruit (Kolayli, Küçük, Duran, Candan, & Dinçer, 2003) and its pekmez. Therefore, detailed information about health-promoting components of cherry laurel fruit and its juice could lead to a better understanding and their full utilisation as functional food ingredients and nutraceuticals, pharmaceuticals, and medicines. The objective of this research was to compare the potential health-benefits of cherry laurel fruit and its pekmez as reflected in their free-radical scavenging, reducing power, and inhibition of oxidation of human LDL cholesterol.

2. Materials and methods

2.1. Preparation and storage of cherry laurel fruit and pekmez

Kiraz variety of cherry laurel (*Laurocerasus officinalis* Roem.) fruits was harvested in fully ripe state in the Giresun province of Turkey in July 2003. Fruits were packed in polyethylene bags (250-g portions), frozen, and stored at −20 °C until used. Pekmez was produced by the traditional method using deseeded cherry laurel fruits which were manually squeezed and then filtered through two layers of cheesecloth. Afterwards, combined filtered juice was boiled/heated inside a copper container (at an open wooden fire) until reaching pekmez consistency (about 3–4 h, depending upon the amount of juice used). It was stirred frequently during boiling/heating. After cooling, pekmez was poured into 250-ml bottles, sealed, and stored at 5 °C in the dark. Cherry laurel fruits were placed inside a polystyrene box with cooling gels (pre-frozen to −20 °C) and dispatched by DHL Express to the Department of Biochemistry, Memorial University of Newfoundland (St. John's, NL, Canada) within 2 days. Pekmez was also dispatched to the same place at the same time without cooling gel (it is stable at ambient temperature for a long period). Upon arrival in the laboratory, cherry laurel fruits and pekmez were stored at −20 and 5 °C, respectively, until analysed.

2.2. Chemicals

All chemicals and solvents were purchased from Sigma–Aldrich, Canada, Ltd. (Oakville, Ont., Canada) and from Fisher Scientific Company (Nepean, Ont., Canada), respectively.

2.3. Determination of hydrogen peroxide scavenging activity

The hydrogen peroxide scavenging assay was carried out following the procedure of Ruch, Cheng, and Klaunig (1989). Samples (0.47 mg/ml assay solution for 400 ppm concentration cherry laurel fruit and pekmez) were dissolved in 3.4 ml of 0.1 M phosphate buffer (pH 7.4) and mixed with 0.6 ml of a 43 mM solution of hydrogen peroxide prepared in the same buffer. The reference antioxidant compound used was catechin. [Catechin is a universal standard used in evaluation of antioxidant activity. Therefore, we have retained catechin for tests of antioxidant activity although chlorogenic acid was earlier reported to be the main phenolic acid in free form (Alasalvar et al., 2005).] Final concentrations of the sample/standard were 100, 200 or 400 ppm (0.118, 0.235 or 0.47 mg/ml assay solution, respectively). The absorbance (at 234 nm) of the

reaction mixture was recorded for 40 min at 10 min intervals. A separate blank sample devoid of hydrogen peroxide was also used for background subtraction. The concentration of hydrogen peroxide in the assay medium was determined using a standard curve. Hydrogen peroxide scavenging activity of sample/standard was calculated using the following equation:

Hydrogen peroxide scavenging activity (%)
= 100 − [(hydrogen peroxide concentration of medium containing the additive of concern)/(hydrogen peroxide concentration of the control medium)] × 100.

2.4. Determination of superoxide radical scavenging activity

Superoxide radical was generated with an enzymatic reaction according to a modified version of the procedure detailed by Nishikimi, Appaji, and Yagi (1972). The reaction mixture contained 1 ml of a 3 mM hypoxanthine, 1 ml of xanthine oxidase (100 mIU), 1 ml of a 12 mM diethylenetriaminepentaacetic acid, 1 ml of a 178 mM nitro blue tetrazolium, and 1 ml of the sample/standard (2.0 mg/ml assay solution for 400 ppm concentration). Final concentration of starting material in the reaction mixture was 100, 200 or 400 ppm (0.5, 1.0 or 2.0 mg/ml, respectively). Catechin was used as the reference antioxidant compound. The absorbance (at 560 nm) of the medium was recorded for 60 min at 10 min intervals. The absorbance values were corrected by subtracting 0 min readings from those obtained subsequently. Superoxide radical scavenging activity (at 10 min) of additives was calculated using the following equation:

Superoxide radical scavenging activity (%)
= 100 − [(absorbance of medium containing the additive of concern)/(absorbance of the control medium)] × 100.

2.5. Determination of DPPH radical scavenging activity

The method described by Kitts, Wijewickreme, and Hu (2000) was used with slight modifications (Liyana-Pathirana & Shahidi, 2005) in order to assess the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of cherry laurel fruit and pekmez. A 0.135 mM DPPH solution in ethanol (1.0 ml) was mixed with various amounts (0.2, 0.4 or 0.8 mg/ml assay solution for 100, 200 or 400 ppm final concentration, respectively) of cherry laurel and pekmez samples and vortexed thoroughly. The absorbance of the mixtures at ambient temperature was recorded for 60 min at 10 min intervals. Catechin was used as the reference antioxidant compound. The absorbance of the remaining DPPH radicals was read at 519 nm using a diode array spectrophotometer (model 8452A, Agilent Technologies Canada Inc., Mississauga, Ont., Canada). The scavenging of DPPH radical was calculated according to the following equation:

DPPH radical scavenging activity (%)
$= [(\mathrm{Abs}_{\mathrm{contol}} - \mathrm{Abs}_{\mathrm{sample}})/(\mathrm{Abs}_{\mathrm{control}})] \times 100,$

where $\mathrm{Abs}_{\mathrm{contol}}$ is the absorbance of DPPH radical + methanol; $\mathrm{Abs}_{\mathrm{sample}}$ is the absorbance of DPPH radical + sample extract/standard.

2.6. Determination of reducing power

The reducing power of samples was determined using the method of Oyaizu (1986) with some modifications. The assay medium contained 2.5 ml of sample/standard in a 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. After incubation at 50 °C for 20 min, 2.5 ml of 10% trichloroacetic acid were added to the mixture followed by centrifugation at 1750*g* for 10 min. One millilitre of the supernatant was mixed with 2.5 ml HPLC-grade water and 0.5 ml of 0.1% ferric chloride, and the absorbance of the resultant solution was read at 700 nm. A standard curve was prepared using various concentrations of ascorbic acid and reducing power was expressed as μM ascorbic acid equivalents.

2.7. Determination of the effects of hydrolysis on oxidation of human LDL cholesterol

The procedure described by Hu and Kitts (2001) and Liyana-Pathirana and Shahidi (2005) was employed in this study. LDL was dialysed in 10 mM PBS (phosphate buffered saline) (pH 7.4) at 4 °C in the dark for 24 h. LDL (0.2 mg LDL/ml) was mixed with different amounts of cherry laurel and pekmez samples (0.2 or 0.4 mg/ml of assay solution for 100 and 200 ppm final concentration). Catechin was used as the reference antioxidant compound. The reaction was initiated by adding a solution of $CuSO_4$ (10 μM) and subsequent samples were incubated at 37 °C for 22 h. The formation of conjugated dienes was recorded at 234 nm using a diode array spectrophotometer (Agilent Technologies Canada Inc.). The inhibitory effect of cherry laurel and pekmez extracts on the formation of conjugated dienes (% $\mathrm{inhibition}_{\mathrm{CD}}$) was then calculated using the equation given below; a separate blank that contained all of the reagents except LDL was used for each extract:

$$\%\ \mathrm{Inhibition}_{\mathrm{CD}} = [(\mathrm{Abs}_{\mathrm{oxidative}} - \mathrm{Abs}_{\mathrm{sample}})/(\mathrm{Abs}_{\mathrm{oxidative}} - \mathrm{Abs}_{\mathrm{native}})] \times 100,$$

where Abs_{sample} is the absorbance of LDL + $CuSO_4$ + sample extract/standard; Abs_{native} is the absorbance of LDL + PBS; $Abs_{oxidative}$ is the absorbance of LDL + $CuSO_4$ + PBS.

Using percentage values, the amount of LDL (μg) that can be protected against copper-mediated oxidation by 1 g sample was obtained.

2.8. Statistical analysis

Results were expressed as mean values ± SD ($n = 3$) on both fresh weight basis (FWB) and dry weight basis (DWB). Statistical significance (*t*-test: two-sample equal variance, using two-tailed distribution) was determined using Microsoft Excel statistical fractions. Differences at $P < 0.05$ were considered to be significant.

3. Results and discussion

3.1. General

Cherry laurel fruit and its pekmez were examined for their antioxidant activities using different free-radical scavenging activity tests (hydrogen peroxide, superoxide radical, and DPPH radical), together with reducing power and inhibition of oxidation of human LDL cholesterol. As part of a parallel study, Alasalvar et al. (2005) reported the compositional characteristics and antioxidant components of two native fresh varieties of cherry laurel, namely kiraz and findik, and pekmez made from kiraz variety. The main acids were: chlorogenic acid in the free form, caffeic acid and gallic acid in the bound form in alkaline-hydrolysis and acid-hydrolysis, respectively, for all samples. The total antioxidant activity measured by $ORAC_{FL}$ ranged from 3363 to 19981 μmol of Trolox equivalents/g, total anthocyanins ranged from 9.3 to 123.8 mg cyanidin 3-glucoside equivalents/100 g, and total phenolics ranged from 454 to 1444 mg ferulic acid equivalents/100 g on a FWB, respectively, among cherry laurel fruit (kiraz variety) and pekmez (Alasalvar et al., 2005). The $ORAC_{FL}$ value and total content of phenolics were higher in pekmez than cherry laurel fruit. In contrast, a significant ($P < 0.01$) proportion of anthocyanins were lost (92.5%) during heat processing in the production of pekmez (Alasalvar et al., 2005).

Kolayli et al. (2003) studied the chemical and antioxidant properties of *L. officinalis* Roem. (Cherry laurel) fruit grown in the Black Sea region. Although they used antioxidant activity determination methods (thin-layer chromatography plate and ferric thiocyanate) that were different from those used in this study, they found that cherry laurel fruit provided a rich source of protective antioxidant compounds. The antioxidant and radical scavenging activities of the aqueous extract of cherry laurel fruit were comparable to or higher than those of the reference antioxidants such as butylated hydroxytoluene (BHT), vitamin C, and Trolox (Kolayli et al., 2003).

Free-radicals possess an unpaired electron, which makes them highly reactive. Antioxidants neutralise free-radicals by donating a hydrogen atom (Siriwardhana & Shahidi, 2002). Therefore, attempts have been made to evaluate the effectiveness of antioxidants in scavenging free-radicals, together with reducing power and inhibition of oxidation of human LDL cholesterol.

3.2. Hydrogen peroxide scavenging activity

The scavenging activity of hydrogen peroxide by cherry laurel samples and a reference antioxidant compound (catechin) was measured spectrophotometrically at 234 nm (Table 1). Compared to catechin, both cherry laurel fruit and pekmez showed a weak hydrogen peroxide scavenging activity. Even at 400 ppm concentration, both samples demonstrated poor activity ranging from 2.8% to 3.7% (FWB) and from 5.9% to 16.3% (DWB). However, catechin exhibited excellent hydrogen peroxide scavenging activity ranging from 93.5% to 100%, even at 100 ppm level. Hence, cherry laurel fruit and pekmez investigated in this assay did not show direct reaction with hydrogen peroxide and did not serve as effective scavengers.

In vivo, hydrogen peroxide is generated by several oxidase enzymes (Halliwell, Murcia, Chirico, & Aruoma, 1995). In general, it may act directly or indirectly as a messenger molecule causing synthesis and activation of several anti-inflammatory mediators (Sprong et al., 1998). Hydrogen peroxide is only mildly reactive by itself, but it may be converted to HO by transition metal ions, especially iron and copper (Knight, 1999).

3.3. Superoxide radical scavenging activity

The superoxide radical is a powerful oxidising agent that can react with biological membranes and induce tissue damage (Yoshikawa, Naito, & Kondo, 1997). It may also decompose to singlet oxygen, hydroxyl radical, or hydrogen peroxide (Niki, 1997). The superoxide radical scavenging activities of cherry laurel samples and catechin, as measured by the xanthine–xanthine oxidase system, are presented in Table 1. The decrease of absorbance at 560 nm with the presence of antioxidants indicates the consumption of superoxide anions in the reaction mixture. On a FWB, the activity of both cherry laurel fruit and pekmez increased with increasing concentrations. Pekmez as such, demonstrated superior activity compared to cherry laurel ($P < 0.01$) at concentrations tested, except at 400 ppm (DWB) for

Table 1
Free-radical scavenging activity tests and reducing power of cherry laurel fruit and its concentrated juice[a]

	FWB			DWB		
	100 ppm	200 ppm	400 ppm	100 ppm	200 ppm	400 ppm
Hydrogen peroxide scavenging						
Kiraz	0.8 ± 0.0^{c}	0.8 ± 0.1^{c}	3.7 ± 0.1^{c}	3.5 ± 0.1^{c}	3.5 ± 0.4^{c}	16.3 ± 0.5^{c}
Pekmez	1.0 ± 0.1^{d}	1.2 ± 0.2^{d}	2.8 ± 0.2^{d}	2.1 ± 0.2^{d}	2.5 ± 0.5^{d}	5.9 ± 0.4^{d}
Catechin[b]	93.5 ± 1.2^{e}	96.0 ± 1.0^{e}	100.0 ± 0.0^{e}	93.5 ± 1.2^{e}	96.0 ± 1.0^{e}	100.0 ± 0.0^{e}
Superoxide radical scavenging						
Kiraz	18.7 ± 0.2^{c}	22.3 ± 0.2^{c}	25.1 ± 0.2^{c}	82.3 ± 1.1^{c}	98.2 ± 0.7^{c}	100.0 ± 0.0^{c}
Pekmez	61.9 ± 0.7^{d}	79.7 ± 0.6^{d}	92.1 ± 0.5^{d}	100.0 ± 0.0^{d}	100.0 ± 0.0^{d}	100.0 ± 0.0^{c}
Catechin	90.7 ± 0.6^{e}	94.0 ± 0.5^{e}	100.0 ± 0.0^{e}	90.7 ± 0.6^{e}	94.0 ± 0.5^{e}	100.0 ± 0.0^{c}
DPPH radical scavenging						
Kiraz	14.0 ± 0.1^{c}	20.7 ± 0.1^{c}	23.4 ± 0.1^{c}	61.6 ± 0.4^{c}	91.1 ± 0.5^{c}	100.0 ± 0.0^{c}
Pekmez	13.9 ± 0.7^{c}	25.4 ± 0.9^{d}	50.8 ± 0.7^{d}	29.2 ± 1.4^{d}	53.4 ± 2.0^{d}	100.0 ± 0.0^{c}
Catechin	97.0 ± 0.8^{d}	100.0 ± 0.0^{e}	100.0 ± 0.0^{e}	97.0 ± 0.8^{e}	100.0 ± 0.0^{e}	100.0 ± 0.0^{c}
Reducing power						
Kiraz	7.0 ± 0.1^{c}	11.8 ± 0.1^{c}	20.7 ± 0.1^{c}	30.8 ± 0.2^{c}	51.9 ± 0.5^{c}	91.1 ± 0.4^{c}
Pekmez	6.2 ± 0.2^{d}	17.2 ± 0.5^{d}	39.3 ± 0.7^{d}	13.0 ± 0.5^{d}	36.2 ± 1.0^{d}	82.7 ± 1.4^{d}
Catechin	622 ± 0.0^{e}	622 ± 0.0^{e}	622 ± 0.0^{e}	622 ± 0.0^{e}	622 ± 0.0^{e}	622 ± 0.0^{e}

[a] Data are expressed as means ± SD ($n = 3$) on both FWB and DWB. Scavenging activities of hydrogen peroxide, superoxide radical, and DPPH radical, expressed as percentage. Reducing power, expressed as μM ascorbic acid equivalents.
[b] Reference antioxidant compound.
[c–e] Means ± SD followed by the same letter for each experiment (FWB and DWB separately), within a column, are not significantly different ($P > 0.05$).

which both samples showed 100% scavenging of superoxide radicals. Therefore, pekmez was most effective for scavenging superoxide radical even at 100 and 200 ppm. Kolayli et al. (2003) found that aqueous extract of cherry laurel fruit exhibited a higher superoxide scavenging activity compared to ascorbic acid as the reference compound ($P < 0.001$).

In general, during food processing and storage natural antioxidants may be degraded considerably. On the other hand, the chemical reactions that occur among different food components may lead to the formation of secondary antioxidants (Nicoli, Anese, & Parpinel, 1999). Maillard reaction, that occurs between free amino groups of a protein or an amino acid with reducing sugars during food processing, cooking or storage, may lead to the formation of a complex mixture of reaction products (Ledl & Schleicher, 1990). Using different test systems, antioxidant activity of Maillard reaction products (MRP) have been studied extensively (Kawashima, Itoh, & Chibata, 1977; Monti et al., 1999; Wijewickreme & Kitts, 1997). MRP have been shown to serve as natural antioxidants to be added to food materials susceptible to oxidative deterioration (Smith & Alfawaz, 1995). This could be one of the reasons why pekmez would have higher antioxidant capacity than its starting material, as some of the endogenous antioxidant compounds may have been degraded upon subjecting to high temperatures, MRP are formed at the same time. Thus, on balance, the overall changes occurring contribute to enhanced antioxidative properties of the treated samples.

3.4. DPPH radical scavenging activity

The DPPH radical scavenging assay is commonly employed to evaluate the ability of antioxidants to scavenge free-radicals. The use of the DPPH free-radical is advantageous in evaluating antioxidant effectiveness because it is more stable than the hydroxyl and superoxide radicals (Siriwardhana & Shahidi, 2002). The antioxidant potential of cherry laurel samples was evaluated using the stable DPPH radical. This method has been used extensively to predict antioxidant activity because of the relatively short time required for analysis (Chen, Wang, Rosen, & Ho, 1999). The DPPH scavenging activities of the cherry laurel fruit and pekmez, together with that of catechin at 100, 200, and 400 ppm concentrations are shown in Table 1. Catechin scavenged DPPH radicals efficiently/completely at all concentrations, except at 100 ppm. Thus, phenolic compounds present may have acted as free-radical scavengers by virtue of their hydrogen-donating ability (Castelluccio, Bolwell, Gerrish, & Rice-Evans, 1996). On the other hand, both cherry laurel fruit and pekmez exhibited moderate DPPH radical scavenging activity compared to catechin at all concentrations, except at 400 ppm concentration (DWB) for which cherry laurel fruit and pekmez scavenged all of the existing radicals.

3.5. Reducing power

The antioxidant activity of cherry laurel samples as reflected in their reducing power is presented in Table 1.

Table 2
Inhibition of oxidation of human LDL cholesterol of cherry laurel fruit and its concentrated juice[a]

Samples	FWB		DWB	
	100 ppm	200 ppm	100 ppm	200 ppm
Kiraz	9.7 ± 0.1^{c}	13.3 ± 0.1^{c}	42.7 ± 0.5^{c}	58.5 ± 0.3^{c}
Pekmez	23.4 ± 0.6^{d}	36.5 ± 2.4^{d}	49.2 ± 1.2^{d}	76.8 ± 5.1^{d}
Catechin[b]	100.0 ± 0.0^{e}	100.0 ± 0.0^{e}	100.0 ± 0.0^{e}	100.0 ± 0.0^{e}

[a] Data are expressed as means ± SD ($n = 3$) on both FWB and DWB. Inhibition of oxidation of human LDL cholesterol, expressed as percentage.
[b] Reference antioxidant compound.
[c–e] Means ± SD followed by the same letter (FWB and DWB separately), within a column, are not significantly different ($P > 0.05$).

Catechin demonstrated superior reducing power at all concentrations compared to cherry laurel fruit and pekmez. Thus, the reducing power of the extracts as reducing agents for terminating free-radical chain reactions by electron donation was less than that of catechin (Amarowicz, Naczk, & Shahidi, 2000). Duh (1998) also stated that reductones are efficient reducing agents and their efficiency is attributed to their hydrogen-donating ability. The cherry laurel samples examined in this study demonstrated poor reducing capacity compared to catechin at 100 and 200 ppm concentrations. The results of reducing power demonstrate the electron donor properties of cherry samples thereby neutralizing free-radicals by forming stable products. The outcome of the reducing reaction is to terminate the radical chain reactions that may otherwise be very damaging (Yen & Chen, 1995).

3.6. Inhibition of oxidation of human LDL cholesterol

Dietary antioxidants that prevent LDL from oxidation are of great importance in protection against atherosclerosis (Esterbauer, Gebicki, Puhl, & Jürgens, 1992). The inhibition of copper-induced LDL oxidation by cherry laurel fruit and pekmez is summarised in Table 2. The formation of conjugated dienes due to copper-induced LDL oxidation was completely inhibited by catechin at concentrations examined. Results of the current study indicated that pekmez was more effective than cherry laurel fruit in inhibiting human LDL oxidation ($P < 0.05$).

Dietary intake of antioxidants is associated with a reduced risk of atherosclerosis and, in turn, CVD, which is possibly due to their ability to prevent oxidation of LDL cholesterol (Dittrich et al., 2003). Numerous studies have reported the antioxidant activities of various crude plant extracts in, in vitro, LDL models (Emmons, Peterson, & Paul, 1999; Heinonen, Meyer, & Frankel, 1998; Singh, Murthy, & Jayaprakasha, 2002). In general, oxidation of LDL follows a radical chain reaction that generates conjugated diene hydroperoxides as its initial products. It has been reported that inhibition of human LDL oxidation may arise due to free-radical scavenging and/or metal ion chelation (Decker, Ivanov, Zhu, & Frei, 2001). In addition, Natella, Nardini, DiFelice, and Scaccini (1999) reported that inhibition of copper-catalysed oxidation represents the association of both chelation of metal ions and scavenging of free-radical species in the LDL system.

4. Conclusion

Different assays used for examining free-radical scavenging activity, reducing power, and inhibition of oxidation of human LDL cholesterol of cherry laurel fruit and pekmez revealed that both samples did not perform similarly. On a FWB, pekmez exhibited significantly ($P < 0.01$) higher antioxidant activity than cherry laurel fruit in most cases, whereas on a DWB hydrogen peroxide and DPPH radical scavenging activities, and reducing power were significantly ($P < 0.01$) higher in cherry laurel fruit than in its pekmez, with some exceptions. This was partly due to the moisture content of these two samples. Moreover, the difference in antioxidant activity between the two samples tested may be attributed to the increase in concentration of phenolic and polyphenolic compounds occurring after preparing pekmez; the concentrate of juice. Heat processing may lead to the release of and/or destruction of some of the phenolics involved, but may also bring about the formation of MRP which are known to be antioxidative in nature. Compared to catechin, both cherry laurel fruit and pekmez showed strong scavenging activities for superoxide and DPPH radicals, and exhibited a good inhibition of oxidation of human LDL cholesterol on a DWB. Therefore, both cherry laurel fruit and pekmez might be considered as functional food ingredients and nutraceuticals.

Acknowledgements

We thank the Giresun International Society of Education for their financial support. The authors are also grateful to Mr. Salih Alasalvar and Mr. Mehmet Külekci for providing cherry laurel sample and pekmez, respectively.

References

Aksu, M. İ., & Nas, S. (1996). Dut pekmezi üretim tekniği ve çesitli fiziksel - kimyasal özellikleri (Mulberry pekmez manufacturing technique and physical and chemical properties). *Gida, 21*, 83–88 (in Turkish).

Alasalvar, C., Al-Farsi, M., & Shahidi, F. (2005). Compositional characteristics and antioxidant components of cherry laurel varieties and pekmez. *Journal of Food Science, 70*(1), S47–S52; erratum *70* (5), pp. ix.

Amarowicz, R., Naczk, M., & Shahidi, F. (2000). Antioxidant activity of various fractions of non-tannin phenolics of canola hulls. *Journal of Agricultural and Food Chemistry, 48*, 2755–2759.

Amarowicz, R., Naczk, M., Zadernowski, R., & Shahidi, F. (2000). Antioxidant activity of condensed tannins of beach pea, canola hulls, evening primrose, and faba bean. *Journal of Food Lipids, 7*, 195–205.

Amarowicz, R., Pegg, R. B., Rahimi-Moghaddam, P., Barl, B., & Weil, J. A. (2004). Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. *Food Chemistry, 84*, 551–562.

Batu, A. (1993). Kuru üzüm ve pekmezin insan sağliği ve beslenmesi açisindan önemi (The importance of raisin and "pekmez" on human health and nutrition). *Gida, 18*, 303–307 (in Turkish).

Baytop, T. (1984). *Therapy with medicinal plants in Turkey (past and present)*. Istanbul: Istanbul University Publication No. 3255.

Castelluccio, C., Bolwell, G. P., Gerrish, C., & Rice-Evans, C. (1996). Differential distribution of ferulic acid to the major plasma constituents in relation to its potential as an antioxidant. *Biochemical Journal, 316*, 691–694.

Chen, Y., Wang, M., Rosen, R. T., & Ho, C.-T. (1999). 2,2-Diphenyl-1-picrylhydrazyl radical-scavenging active components from *Polygonum multiflorum* Thunb. *Journal of Agricultural and Food Chemistry, 47*, 2226–2228.

Chu, Y.-H., Chang, C.-L., & Hsu, H.-F. (2000). Flavonoid content of several vegetables and their antioxidant activity. *Journal of the Science of Food and Agriculture, 80*, 561–566.

Decker, E. A., Ivanov, V., Zhu, B.-Z., & Frei, B. (2001). Inhibition of low-density lipoprotein oxidation by carnosine and histidine. *Journal of Agricultural and Food Chemistry, 49*, 511–516.

Dittrich, R., El-Massry, F., Kunz, K., Rinaldi, F., Peich, C. C., Beckmann, M. W., et al. (2003). Maillard reaction products inhibit oxidation of human low-density lipoproteins in vitro. *Journal of Agricultural and Food Chemistry, 51*, 3900–3904.

Duh, P.-D. (1998). Antioxidant activity of Burdock (*Arctium lappa* Linné): its scavenging effect on free-radical and active oxygen. *Journal of the American Oil Chemists' Society, 75*, 455–461.

Emmons, C. L., Peterson, D. M., & Paul, G. L. (1999). Antioxidant capacity of oat (*Avena sativa* L.) extracts. 2. In vitro antioxidant activity and contents of phenolic and tocol antioxidants. *Journal of Agricultural and Food Chemistry, 47*, 4894–4898.

Esterbauer, H., Gebicki, J., Puhl, H., & Jürgens, G. (1992). The role of lipid peroxidation and antioxidants in oxidative modification of LDL. *Free Radical Biology and Medicine, 13*, 341–390.

Halliwell, B., Murcia, M. A., Chirico, S., & Aruoma, O. I. (1995). Free radicals and antioxidants in food and in vivo: what they do and how they work. *Critical Reviews in Food Science and Nutrition, 35*, 7–20.

Heinonen, I. M., Meyer, A. S., & Frankel, E. N. (1998). Antioxidant activity of berry phenolics on human low-density lipoprotein and liposome oxidation. *Journal of Agricultural and Food Chemistry, 46*, 4107–4112.

Hertog, M. G. L., Feskens, E. J. M., Hollman, P. C. H., Katan, M. B., & Kromhout, D. (1993). Dietary antioxidant flavonoids and risk of coronary heart disease: The Zutphen Elderly Study. *Lancet, 342*, 1007–1011.

Hu, C., & Kitts, D. D. (2001). Free radical scavenging capacity-related antioxidant activity and ginsenoside composition of Asian and North American ginseng extracts. *Journal of the American Oil Chemists' Society, 78*, 249–255.

Kawashima, K., Itoh, H., & Chibata, I. (1977). Antioxidant activity of browning products prepared from low molecular carbonyl compounds and amino acids. *Journal of Agricultural and Food Chemistry, 25*, 202–204.

Kitts, D. D., Wijewickreme, A. N., & Hu, C. (2000). Antioxidant properties of a North American ginseng extract. *Molecular and Cellular Biochemistry, 203*, 1–10.

Knight, J. A. (1999). *Free radicals, antioxidants, aging, and disease*. Washington, DC: AACC Press.

Kolayli, S., Küçük, M., Duran, C., Candan, F., & Dinçer, B. (2003). Chemical and antioxidant properties of *Laurocerasus officinalis* Roem. (Cherry laurel) fruit grown in the Black Sea region. *Journal of Agricultural and Food Chemistry, 51*, 7489–7494.

Ledl, F., & Schleicher, E. (1990). New aspects of the Maillard reaction in foods and in the human body. *Angewandte Chemie International Edition in English, 29*, 565–594.

Liyana-Pathirana, C. M., & Shahidi, F. (2005). Antioxidant activity of commercial soft and hard wheat (*Triticum aestivum* L.) as affected by gastric pH conditions. *Journal of Agricultural and Food Chemistry, 53*, 2433–2440.

Mazza, G., Fukumoto, L., Delaquis, P., Girard, B., & Ewert, B. (1999). Anthocyanins, phenolics, and color of Cabernet Franc, Merlot, and Pinot Noir wines from British Colombia. *Journal of Agricultural and Food Chemistry, 47*, 4009–4017.

Monti, S. M., Ritieni, A., Graziani, G., Randazzo, G., Mannina, L., Segre, A. L., et al. (1999). LC/MS analysis and antioxidative efficiency of Maillard reaction products from a lactose–lysine model system. *Journal of Agricultural and Food Chemistry, 47*, 1506–1513.

Natella, F., Nardini, M., DiFelice, M., & Scaccini, C. (1999). Benzoic and cinnamon acid derivatives as antioxidants: structure–activity relation. *Journal of Agricultural and Food Chemistry, 47*, 1453–1459.

Ness, A. R., & Powles, J. W. (1997). Fruit and vegetables, and cardiovascular disease: a review. *International Journal of Epidemiology, 26*, 1–13.

Nicoli, M. C., Anese, M., & Parpinel, M. (1999). Influence of processing on the antioxidant properties of fruit and vegetables. *Trends in Food Science and Technology, 10*, 94–100.

Niki, E. (1997). Free radicals in chemistry and biochemistry. In M. Hiramastu, T. Yoshikawa, & M. Inoue (Eds.), *Food and free radicals* (pp. 1–10). New York: Plenum Press.

Nishikimi, M., Appaji, R. N., & Yagi, K. (1972). The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. *Biochemical and Biophysical Research Communications, 46*, 849–854.

Oyaizu, M. (1986). Studies on products of browning reaction-antioxidative activities of products of browning reaction prepared from glucosamine. *Japanese Journal of Nutrition, 44*, 307–315.

Pratt, D. E. (1992). Natural antioxidants from plant material. In M.-T. Huang, C.-T. Ho, & C. Y. Lee (Eds.), *Phenolic compounds in food and their effects on health. Antioxidants and cancer prevention. ACS symposium series 507* (Vol. II, pp. 54–71). Washington, DC: American Chemical Society.

Ruch, R. J., Cheng, S.-J., & Klaunig, J. E. (1989). Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis, 10*, 1003–1008.

Shahidi, F., & Naczk, M. (2004). *Phenolics in food and nutraceuticals*. Boca Raton, FL: CRC Press.

Singh, R. P., Murthy, K. N. C., & Jayaprakasha, G. K. (2002). Studies on the antioxidant activity of pomegranate (*Punica granatum*) peel and seed extracts using in vitro models. *Journal of Agricultural and Food Chemistry, 50*, 81–86.

Siriwardhana, S. S. K. W., & Shahidi, F. (2002). Antiradical activity of extracts of almond and its by-products. *Journal of the American Oil Chemists' Society, 79*, 903–908.

Smith, J. S., & Alfawaz, M. (1995). Antioxidative activity of Maillard reaction products in cooked ground beef, sensory and TBA values. *Journal of Food Science, 60*, 234–236, 240.

Sprong, R. C., Winkelhuyzen-Janssen, A. M. L., Aarsman, C. J. M., van Oirschot, J. F. L. M., van der Bruggen, T., & van Asbeck, B. S. (1998). Low-dose *N*-acetylcysteine protects rats against endotoxin-mediated oxidative stress, but high-dose increases mortality. *American Journal of Respiratory and Critical Care Medicine, 157*, 1283–1293.

Surh, Y. J. (2003). Cancer chemoprevention with dietary phytochemicals. *Nature Reviews Cancer, 3*, 768–780.

Temple, N. J. (2000). Antioxidants and disease: more questions than answers. *Nutrition Research, 20*, 449–459.

Tosun, I., & Ustun, N. S. (2003). Nonenzymic browning during storage of white hard grape pekmez (*Zile pekmezi*). *Food Chemistry, 80*, 441–443.

Watson, R. R. (2003). *Functional foods and nutraceuticals in cancer prevention*. Oxford: Blackwell Publishing.

Wijewickreme, A. N., & Kitts, D. D. (1997). Influence of reaction conditions on the oxidative behaviour of model Maillard reaction products. *Journal of Agricultural and Food Chemistry, 45*, 4571–4576.

Yen, W. J., & Chen, B. H. (1995). Isolation of xanthophylls from Taiwanese orange peels and their effects on the oxidation stability of soybean oil. *Food Chemistry, 53*, 417–425.

Yoshikawa, T., Naito, Y., & Kondo, M. (1997). Free radicals and diseases. In M. Hiramastu, T. Yoshikawa, & M. Inoue (Eds.), *Food and free radicals* (pp. 11–19). New York: Plenum Press.