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Physical and chemical characteristics of the ripe pepino (*Solanum muricatum*) fruit grown in Turkey

Osman Kola
Department of Food Engineering, Faculty of Engineering, Sakarya University, 54040-Sakarya, Turkey.
e-mail: okola@sakarya.edu.tr

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Abstract

The pepino (*Solanum muricatum* Aiton, Solanaceae) is a little-known crop from the tropical and subtropical regions esteemed for its edible fruits, which are aromatic, juicy, scented, mild sweet, and may have great variation in size, shape and colour depending on the cultivar. Organic acids, sugar fractions, total phenolics and some quality characteristics (titratable acidity, pH, soluble solids, colour, etc.) of pepino fruits (cultivar Miski) obtained from Akyazı, Sakarya, Turkey, were determined. High-performance liquid chromatographic methods were used to identify and quantify non-volatile organic acids and sugars. Pepino fruits (cv. Miski) were egg-shaped, watery, of 210-370 g/fruit weight, 6-12.5 cm in diameter, 7-14.5 cm long, hollow in the middle with several small seeds attached, and with 82-89% edible part. The juice yield (%) of pepino varied from 60.0 to 68.0. Ripe pepino fruits had the titratable acidity (%) ranging from 0.090 to 0.124, Brix (total soluble solids, SSC) from 4.91 to 5.40, and pH values from 4.72 to 5.22. β-carotene (µg/g DW) content was ranging from 57 to 68 in ripe pepino fruit. Three organic acids (citric, malic and ascorbic acids) and three sugars (sucrose, glucose and fructose) were detected by HPLC. The major organic acid was citric acid (0.053-0.065 g/100 g). Ascorbic acid was the second most abundant organic acid in the pepino fruit and malic acid was the third one. With regard to sugars, fructose was present in the largest amount for pepino fruits cv. Miski (1.49-1.55 g/100 g), accounting for about 39.28% of total sugar.

Key words: Pepino, *Solanum muricatum*, 'Miski', 'El Camino', organic acid, sugar, HPLC, chroma, hue, phenolics.

Introduction

Pepino is an herbaceous plant that has long been grown and originally cultivated in South America [1,2]. Currently, there are several commercial varieties developed in New Zealand and Australia through selection and breeding (e.g. Suma, Miski, Lincoln Long, Golden Litestripe, etc.), while other cultivars (e.g. El Camino and Schmidt) are direct introductions of material grown in Chile [3]. The pepino is usually grown as an annual crop, but in frost-free areas it is sometimes grown as pluriannual [4].

The pepino (*Solanum muricatum* Aiton, Solanaceae) is a little-known crop from the tropical and subtropical Andes esteemed for its edible fruits [5], which are aromatic, juicy, scented, mild sweet, and may have great variation in size, shape and colour depending on the cultivar [1, 2]. The fruit matures 30 to 80 days after pollination. It is visually attractive; the skin is commonly golden yellow and covered with purple stripes [6]. El-Zeftawi *et al.* [7] reported that the fruit often fail to ripen normally, and thus fail to develop their characteristic flavour after harvest. Hence, harvesting pepino fruit at the appropriate stage of maturity is essential for a high pepino quality [8-10].

Sucrose is present in low amounts during the early stages of growth and its accumulation begins when the pepino fruit approaches full size. Starch, present in the immature fruit, is almost absent in the mature fruit [11]. The pepino is said to have some medicinal properties. It is a good antiscorbutic, since it contains vitamin C (25-70 mg/100 ml) at higher levels than normally found in most fruits [12-14].

This work is a first step to identify and quantify organic acids and sugars in pepino fruits (cv. Miski) from Turkey using HPLC methods. The overall objective of the study was to determine the quality characteristics of ripe pepino fruits harvested at golden-yellow stages of ripening and to identify objective indices of the properties of both the fruit and the juice.

Materials and Methods

Material and chemicals: Pepino fruits (cv. Miski) were harvested in the middle of June 2009 in a greenhouse located in Akyazı, Sakarya, Turkey. Pepino fruits were harvested at golden-yellow stages of ripening and kept at temperature of 5°C until analysis.

All chemicals and solvents were obtained from Sigma Chemical Co. (St Louis, MO, USA) and Merck (Darmstadt, Germany).

Determination of quality characteristics: Fruit quality attributes were assessed and analyzed immediately at the time of harvest. Pepino fruits were cut into quarters and each quarter was further separated into core and peel tissues. Samples were thoroughly homogenized in a food processor.

Five pepino fruits were used to determine fresh weight, juice yield, fruit colour, dry matter content, total soluble solids (SSC), pH, titratable acidity (TA), total phenolics, β-carotene content, carbohydrate and sugar fractions. The titratable acidity was determined by titration with sodium hydroxide (0.1 N) to the phenolphthalein end point and expressed as % citric acid as this

is the main acid in pepino flesh [13]. The pH value was measured with a digital pH meter (Hanna pH211). Total soluble solids were measured as °Brix using a refractometer (WYA Abbe refractometer)[15, 16].

Liquid chromatographic analysis of organic acids and sugars: Sugar (glucose, fructose, sucrose) and organic acid (citric, malic, ascorbic) fractions were determined by a previously reported HPLC method [17, 18]. The HPLC analyses were carried out using a Perkin Elmer HPLC system with Totalchrom navigator 6.2.1 software, a pump and UV detector (Perkin Elmer series-200) (Waltham, MA, USA).

Pepino slurries (5 g) were diluted with ultra pure water for individual sugar analysis and with metaphosphoric acid (2.5%) solution for organic acid analysis. The homogenate was centrifuged at 6000 rpm for 5 min. Supernatants were filtered through a 0.45 µm membrane filter (Iwaki Glass) before HPLC analysis, and the mobile phase solvents were degassed before use. All the samples and standards were analyzed in triplicate and the mean values were reported.

Separation and determination of organic acids were carried out by a modified method [18]. The separation was carried out on a SGE Wakosil C18RS 5 µm column (250 x 4.6 mm ID). Detection was performed at 215 nm. Optimum efficiency of separation was obtained using sulphuric acid solution of pH 2.5 (solvent A) and methanol (solvent B). Other parameters adopted were as follows: injection volume, 20 µl; column temperature, 30°C.

Analysis of sugars was performed according to the method described by Bartolome *et al.* [17], using a refractive index (RI) detector (Perkin Elmer). The separation was carried out on a SGE SS Exsil amino column (250 x 4.6 mm ID). The elution solvent used was 80% acetonitrile and 20% deionised water. The column was operated at 30°C with 0.9 ml/min flow rate. Sample injection volume was 20 µl.

Ascorbic acid was determined using HPLC method. A HPLC (Hitachi LaChrom Elite HPLC) was equipped with a DAD detector (L-2455 Diode Array Detector) and an autosampler (L-2200 Autosampler). A Phenomenex Luna 5u C18 column (250 × 4.6 mm ID) was used. A 0.002 M $(NH_4)_2HPO_4$ with 3% methanol (v/v) solution was used as the mobile phase at a flow rate of 1.5 ml/min. Ascorbic acid solution (ascorbic acid 13.837, Merck, Germany) was prepared as the standard. Sample injection volume was 10 µl. Samples were filtered through a 0.45-µm pore size membrane filter before injection.

Determination of total phenolics (TP): Total phenolics content was measured according to Singleton and Rossi [19] procedure. An aliquot of pepino slurry was extracted with a buffer containing acetone, water and acetic acid (70:29.5:0.5 v/v) for 1 h in the dark. Three parallel extracts were obtained from each fruit samples. Then, the extract, Folin-Ciocalteu's reagent and water were kept for 8 min, followed by the addition of 7% sodium carbonate. After 2 h, the absorbance was measured by an automated UV-VIS spectrophotometer (model T60U, PG Instruments) at 750 nm. Gallic acid was used as the standard. The results were expressed as mg gallic acid equivalent in 100 g fresh weight (FW) of pepino.

Analysis of structural carbohydrates: Cell wall extraction for the determination of pectic substances (water-soluble pectin, EDTA-soluble pectin and insoluble pectin) was conducted according to McComb and McCready [20] as modified by Huyskens [21]. The colorimetric determination of the pectin fractions was conducted using metahydroxybiphenyl (Sigma H6527, Germany) as the colour reagent and following the method described by Blumenkrantz and Asboe-Hansen [22]. The galacturonic acid content was measured photometrically (model T60U, PG Instruments) at 520 nm. Analyses were performed with five fruits. The pectic substance content was expressed as mg galacturonic acid/g dry weight (DW).

Determination of colour and β-carotene content: Fruit colour was measured with a Minolta portable chromameter (Minolta, model CR-400) which provided CIE L*, a*, and b* values. Chroma ($C= [(a^*)^2 + (b^*)^2]^{1/2}$) was calculated as described by McGuire [23] and Huyskens-Keil *et al.* [10]. Hue angle (°h), in degrees, was calculated as $h = arctangent(b^*/a^*)$ or $tan^{-1}(b^*/a^*)$.

Colour pigments, i.e. β-carotene content of pepino, were determined as described by Goodwin [24]. The colour extinction of the samples was measured at wavelength of 453 nm (β-carotene) using a UV/VIS-spectrophotometer (Shimadzu Mini UV-1240 Spectrophotometer). The content of pigments was expressed as µg/g dry weight (DW).

Results and Discussion

General composition of the pepino fruit: Pepino fruits (cv. Miski) were egg-shaped, watery, of 210-370 g/fruit weight, 6-12.5 cm in diameter, 7-14.5 cm long, hollow in the middle with several small seeds attached, and with 82-89% edible part. The juice yield (%) of pepino varied from 60.0 to 68.0.

The chemical composition of the pepino fruit is given in Table 1. In this study, ripe pepino fruits had the titratable acidity (%) ranging from 0.090 to 0.124, Brix (total soluble solids, SSC) from 4.91 to 5.40, and pH values from 4.72 to 5.22.

Ahumada and Cantwell [8] reported pepino fruits were round to elongate in shape, can weigh from 100 to 500 g at maturity and contain 6-12% soluble solids. In their study on the quality and physiological behaviour of pepinos, Lizana and Levano [25] reported that typical Chilean pepinos had 8.5% soluble solids when ripe. De Arriola *et al.* [12] reported concentrations of titratable acidity as 0.06%, Brix (total soluble solids) as 9.5%, total sugars as 4.06 g/100 g and ascorbic acid as 34.25 mg/100 g in ripe pepino fruits of a local selection. The fresh-weight composition of ripe 'El Camino' fruits contained 0.1% protein, 4.9-6.4 g/100 g sugars, 48-68 mg/100 g vitamin C, 119-153 mg/100 g organic acids and 52-70 mg/100 g amino acids according to Redgwell and Turner [13].

Table 1. General properties and composition of the pepino fruits harvested at golden-yellow stages of ripening.

Characteristics	Values
Fruit weight (g)	210–370
Juice yield (%)	60-68
Total soluble solids (°Brix)	4.91-5.40
Titratable acidity (%) [1]	0.090-0.124
pH	4.72-5.22
Total phenolic compounds (mg GAE/100 g FW)	480-540
Insoluble pectin (mg galacturonic acid/g DW)	1.10-1.32
Water soluble pectin (mg galacturonic acid/g DW)	9.60-10.10
Chroma [2]	19-21
Hue [2]	-78
β-carotene (µg/g DW)	57-68

[1] As citric acid.
[2] In pericarp of ripe pepino fruits.

Harman *et al.* [26] reported that as the fruit matures, soluble solids, pH and titratable acidity did not vary significantly, but total sugar content increased during maturation and ripening. They considered 'El Camino' and 'Suma' pepino fruits to be good flavoured if they had 10% soluble solids.

Environmental conditions play an important role in pepino fruit quality. For example, high temperatures during ripening are detrimental to sugar content [27]. Therefore, in Mediterranean conditions, the autumn-winter period is recommended for growing pepinos to obtain fruits of good quality. However, in these conditions, soluble solids concentration (SSC) usually does not reach 8 °Brix, which is a low SSC value for European consumer demands [14].

Colour is the most obvious change that occurs in many fruits and is often the major criterion used by consumers to determine whether the fruit is ripe or unripe [28]. It was reported that as ripening progressed, skin ground colour of pepino changed from green to dark yellow; the yellowing of the skin ground colour was indicated by increases in chroma; and the chroma of pepino fruits increased significantly, mainly from the premature to the mature stage [29]. In our study, chroma in pericarp, peel and core tissues was 19-21, 22-24 and 12-17, respectively. Huyskens-Keil *et al.* [10] obtained a different result for the chroma of pepino cv. Golden Globe, reporting an average of 25-30. In our study, hue values were -78, -87 and -82 in pericarp, peel and core tissues, respectively.

One of the most abundant and important carotenoid in most fruits including pepino is β-carotene. It was reported that as the ripening progressed from the premature to the mature stage, the contents of β-carotene increased significantly and remained constant as the fruits reached the ripe stage [29]. In our study, β-carotene (μg/g DW) content was ranging from 57 to 68 in ripe pepino samples. Similarly, β-carotene content of ripe pepino fruit was reported by Huyskens-Keil *et al.* [10] as 45-65 μg/g DW.

The content of total pectic substances in pepino fruits reportedly remained unchanged during ripening [29]. In our study, insoluble and water soluble pectin (mg galacturonic acid/g DW) of ripe pepino cv. Miski was 1.10-1.32 and 9.60-10.10, respectively.

Sugar and organic acid composition of the pepino: The sugar and organic acid compositions of the pepino fruit, which correspond to the three analytical replicates, are presented in Table 2.

Free sugars play an important role in the flavour characteristics of pepino fruit [30]. Sucrose, glucose and fructose were sugar components in pepino fruits (Table 2). In our study, the total amount of sugar was 3.81-3.93 g/100 g. The main portion of carbohydrates in pepino fruits was the three simple sugars; sucrose, glucose and fructose. Fructose was present in the largest amount in pepino cv. Miski (1.49-1.55 g/100 g), accounting for about 39.28% of total sugar content (Table 2). The remaining sugar content in pepino was 32.69% glucose and 28.03% sucrose. Similarly de Arriola *et al.* [12] reported the concentration of total sugars as 4.06 g/100 g in ripe pepino fruits. Fructose, glucose and sucrose constituents of pepino fruits, harvested at two stages of maturity, the first representing the fruit estimated to be 6 weeks from the maturity stage of 3 and the second representing the fruit estimated to be 2 weeks from the maturity stage of 3, were 1.55-1.89, 0.99-1.55 and 0.063-0.84 g/100 g, respectively[13]. Redgwell and Turner [13] and Prono-Widayat *et al.* [29] reported an increase in sucrose as ripening progressed.

Table 2. Sugar and organic acid composition of pepino fruit harvested at golden-yellow stages of ripening.

Constituent	Value (g/100 g)
Glucose	1.210-1.320
Fructose	1.490-1.550
Sucrose	1.050-1.120
Malic acid	0.009-0.010
Citric acid	0.053-0.065
Ascorbic acid	0.031-0.059

Three organic acids were separated and identified in pepino; citric, ascorbic and malic acids. Citric acid was the predominant nonvolatile organic acid in pepino (0.053-0.065 g/100 g) (Table 2). Citric acid levels in fresh pepino fruits from different regions were 0.12-0.15 g/100 g [12]. Ascorbic acid was the second most abundant organic acid in the pepino fruit (31-59 mg/100 g) and malic acid was the third one (0.01 g/100 g). According to Redgwell and Turner[13], citric and malic acid levels of pepino fruit were 0.124 and 0.010 g/100 g FW, respectively.

Pepino fruit is a rich source of ascorbic acid, which is an important antioxidant. In our study, concentration of ascorbic acid in pepino fruit was 31-59 mg/100 g (Table 2). It was found that the ascorbic acid content of the pepino (cultivar Miski) analyzed in the present study was very similar to that of El Camino (48-68 mg/100 g) [13]. Rodriguez-Burruezo *et al.* [14] reported a range of ascorbic acid concentrations considered as medium or high for *S. muricatum* between 25 and 50 mg/100 g. Ascorbic acid content of different pepino varieties analyzed in previous studies was found to be ranging from 20 to 70 mg/100 g [12-14, 31].

Conclusions

In this study, for the first time, organic acids, sugars and other quality characteristics of the pepino fruit and juice obtained from the cv. Miski were examined. The results indicated that the total sugar content of pepino was lower than in other fruits. The major organic acid and sugar were determined quantitatively as citric acid and fructose, respectively, in the ripe pepino fruits.

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