Zagazig Journal of Agricultural Research
www.zu.edu.eg/agr/journals

ANTIOXIDANT ACTIVITY OF ORANGE FRUIT PECTIC SUBSTANCE

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ABSTRACT

The oxidation of ascorbic acid and the consequent browning reactions is considered as a serious quality defect in orange juice. Therefore, the control of these reactions is of prime importance to preserve the nutritional value and the quality of orange juice. The aim of this study was to evaluate the effect of pectin fractions isolated from orange fruit as retardation of ascorbic acid oxidation and browning reaction of orange juice. The results indicated that the orange fruits were rich in total soluble solids (12.9%) total titratable acidity as citric acid 1.59% and ascorbic acid (58.93mg/100 g). Alcohol insoluble solids (AIS) of orange fruits contained anhydrogalacturonic acid; 35.34%, degree of esterification (DE); 75%, protein; 6.52% and ash; 3.75% as well as pectic substances such as water soluble pectin; 19.87%, hot water soluble pectin; 25.18% and HCl-soluble pectin, 15.26%. Water soluble pectin and hot water soluble pectin inhibited ascorbic acid oxidakion and colour deterioration as browning index at 420 nm of orange juice, while HCl-soluble pectin showed lowest effect. On the other hand, pectin fractions led to improve quality charteristics of packaged orange juice during storage at room temperature for 3 months. This work colud be recommended that the hot water soluble pectin is considered a very effective natural antioxidant and antibrowning agent for orange juice.

Key words: Pectin, fractions, ascorbic acid, oxidation, browning reaction, orange juice.

INTRODUCTION

Apart from its pleasant colour, aroma and taste orange juice occupies an important position in human diet due to its content of vitamins (mainly ascorbic acid), minerals, organic acids and sugars (Li-Ying *et al.*, 2008). Moreover, it is a rich source of important dietary fibers and biologically active compounds such as phenolic compounds, flavanones (mainly hesperidin and narirutin) and caroteoides (DeMajo *et al.*, 2005).

These compounds possess health promoting properties and have been suggested as one of the possible cancer preventing agents (Peterson *et al.*, 2006 and Roussos, 2012).

Thermal processing and storage of orange juice have negative impact on the quality of its fresh flavour, degradation of ascorbic acid and discolouration (Sandhu and Minhas, 2006). Omar *et al.* (2006) found that the processing of navel orange compote caused higher losses in ascorbic acid compared to losses resulted from storage at 5°C for 180 days. Aerobic and anaerobic degradation of ascorbic acid is one of the possible mechanism responsible for the non-enzymatic browning in orange juice, leading to the formation of undesirable end products (Nagy *et al.*, 1989 and Johnson *et al.*, 1995). This is mainly correlated with dissolved oxygen in the juice. Therefore, control of the oxidation of ascorbic acid by natural antioxidants is of prime importance to preserve the quality of orange juice.

Pectin is one of the major polysaccharides of the cell walls of fruit and vegetable tissues. It is probably the most complex macromolecule in nature as it can be composed out of as many as 17 different monosaccharaides containing more than 20 different linkages (O'Neill *et al.*, 2004). The amount and composition of pectin in fruits and vegetables determine to a large extent the quality parameters of fresh and processed food

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products (Voragen *et al.*, 2009). Pectic substances are polygalacturonides with non-uronide carbohydrates covalently bound to an unbranched chain of (1 - 4) α-D-galacturonic acid units (Siliha, 1985). The carboxyl groups of the galacturonic acids are partially esterified with methanol. Some hydroxyl groups on C2 and C3 may be acetylated. The non-uronide carbohydrates are in the form of neutral sugars consisting of predominantly of rhamnose, arabinose, galactose, glucose, xylose and mannose, in addition to other sugars present in minor concentration. Rhamnose is an integral part of the main chain, while other neutral sugars are present as side chains. Ferulic acid groups are ester-linked mainly on C2 of the arabinose units and on C6 of the galactose units in sugar beet pectins (Saulnier and Thibault, 1999). Pectins from plant cell walls can be fractionated according to its solubility into water-soluble pectin, ammonium oxalate soluble pectin, acid-soluble pectin and alkali-soluble pectirn (Carle *et al.*, 2001).

Although, pectin is used widely in food industry as thickening and gelling agent, its antioxidant activity has gained an increasing interest during the last 10 years. Motomura (2002) and Motomura and Yoshida (2003) showed that hot- water soluble pectin fraction extracted from alcohol insoluble solids (AIS) of apples and lemons acted as antioxidant. Its activity in linoleic acid solution and free radical scavenging activity on 2-2- Diphenyl-1-picryl-Hydrazyl (DPPH) was higher than cold -water soluble pectin fraction, while HCI-soluble pectin fraction showed no effect. Pectins with low degree of esterification had higher activity than those with higher degree of esterification. On the other hand. Renard *et al.* (1999), found that pectins extracted by hot HCI from AIS of quinoa leaves were rich in ferulic acid. It has been found that low esterified pectin normalizes the level of malonic dialdehyde and the activity of glutathione peroxidase in the liver (Khasina *et al.*, 2003). Strong antioxidant capacity was displayed by hot water soluble polysaccharides isolated from AIS of *Manhifera Pajong* (Al-Sheraji *et al.*, 2012).

This investigation was undertaken to study the antioxidant activity of pectin fractions isolated from orange fruits (*Citrus sinensis*).

MATERIALS AND METHODS

Materials

Rip orange fruits var. Balady (*Citrus sinensis*) were obtained from Horticultural Research, Institute Agric. Res. Center. The chemicals of pure grade were obtained from the El-Gomhoria Medicine and Chemical company, Egypt.

Methods

Preparation of alcohol insoluble solids (AIS)

After removing flavedo layer, orange fruits were cut, heated in 95% ethanol for 30 min at 70°C, filtered through whatiman filter papper number 1. The pellet was suspended in 80% ethanol for 1h and filtered. The pellet was washed with 70% ethanol and the residue was suspended in acetone, dried at room temperature Shewfelt (1965).

Isolation of pectin fractions

Alcohol insoluble solids were fractionated into water soluble pectin using distilled water at room temperature for 1h, hot water soluble pectin using distilled water at 70℃ for 1 h Motomura and Yoshida (2003), and HCl-soluble pectin using 0.5N HCl at room temperature for 1h Motomura (2002), then filtered. The soluble pectin was recipitated in 70% ethanol, filtered. The residue was washed with 70% ethanol, acetone and dried at room temperature.

Preparation of orange juice

The fruits were sorted, washed, cut into several pieces and pressed manually. The juice was filtered using two layers of cheese cloth. After that, different concentrations of 0.5%, 1%, 1.5% and 2% from water soluble pectin (WSP), hot water soluble pectin (HWSP) and HCL-soluble pectin (HCSP) were added separately to juice, mixed well and left at room temperature for 30 min with continuous stirring to study and choose the best treatment from the previous pectin fractions as retardation of ascorbic acid and browning reaction. The fresh juice was used as a comparable sample. All samples were treated with 0.05% sodium benzoate, packaged in glass bottles (200ml), pasteurized at 85°C for

15 min, sealed well and directly cooled. Finally, glass bottle was stored at room temperature for 3 months and subjected to the analytical procedures.

Analytical methods

Moisture content, total soluble solids, total titratable acidity (as citric acid), pH value, total, reducing and non-reducing sugars, ash, protein, β-carotene, ascorbic acid using (2.6 dichlorophenol indophenol) and colour index as browning at 420 nm were determined according to the methods described in the AOAC, (2005).

Physical properties of orange fruits

The amount of juice yield, peel flavedo, peel albedo, pulp and seeds were measured. Anhydrogalacturonic acid content of alcohol insoluble and pectin fractions was determined according to the method described by Ahmed and Labavitch, (1977). Degree of esterification (DE) was determined by saponification of pectin and titration with 0.1 N NaOH (Ranganna, 1979).

Antioxidant activity of pectin fractions against ascorbic acid oxidation of orange juice were detrmined according to the method described by Tebourbi *et al.* (2006) and Alexeev (1980). By Knowing the amount of intial iodine (I_2) and determining the amount of I_2 reacted with ascorbic acid, and the amount of untreated I_2 that were determined by titration with $Na_2 S_2 O_3$ solution. Oxided ascorbic acid % was determined.

RESULTS AND DISCUSSION

Some Chemical and Physical Charactirstics of Orange Fruits and Juice

From the result given in Table 1, it could be observed that Balady orange juice possessed high total soluble solids (12.9%), total titratable acidity as citric acid (1.59%), total sugars (9.79%), reducing sugars (4.25%) and non-reducing sugars (5.59%), β-Carotene (0.251 mg/100 g) and ascorbic acid 58.93mg/100g. Omar *et al.* (2006) found that Navel orange juice contained 10.98%, 1.22%, 7.74%, 3.98%, 3.76%, 0.013mg/100gm and 25.67mg/100g respectively. These results agreed with the finding of El-Ashwah *et al.* (1975) and Mohamed *et al.* (2002). Physical analysis show that the balady orange fruits contained 52% juice, 9.5% peel flaved, 27.5% peel albedo, 4% pulp and 7% seed (Table 2).

Table 1. Chemical composition of raw Balady orange juice

Parameters	Value
Moisture content %	85.32
Total soluble solids %	12.9
Total titratable acidity as citric acid%	1.59
pH value	3.63
Total sugars %	9.79
Reducing sugars %	4.25
Non-reducing sugars %	5.59
Ascorbic acid mg/100 g	58.93
β.Carotene (mg/100 g)	0.251

Table 2. Physical properties of raw Balady orange fruits

Parameters	Value %
Juice %	52
Peel flavedo %	9.5
Peel Albedo %	27.5
Pulp %	4
Seeds %	7

Chemical Composition of Alcohol Insoluble Solids (AIS) of Orange Fruits

Data presented in Table 3 show that the alcohol insoluble solids of orange fruits contained 5.30% moisture, 6.52% protein, 35.34 anhydrogalacturonic acid, 75% dergree of esterification (DE) and 3.75% ash. Siliha (1993) found that the fiber pectin of lime peel had 9.44% moisture contents 2.67% ash, 37.60% anhydrogalacturonic acid and 80.72% dergree of esterification. Table 3 also shows that the amount of pectin fractions isolated from alcohol insoluble solids were as follows:

Water soluble pectin 19.87% hot water soluble pectin 25.18% and HCl-soluble pectin 15.26% as anhydrogalacturonic acid % of alcohol insoluble solids. These data were relatively higher compared to that mentioned by Omer (1993) of water soluble pectin (16.30%) for lime fruit.

Effect of Different Concentration of Pectin Fractions on Ascorbic Acid Oxidation of Orange Juice

Fig. 1 shows that the hot water soluble pectin inhibited the oxidation of ascorbic acid followed by using water soluble pectin, whereas HCl soluble pectin showed little effect. The inhibion of the oxidation of ascorbic acid of orange juice was increased as the concentration of fraction was increased. The control sample showed that 35% of ascorbic acid was oxidized by O_2., while 1.5% pectin fractions showed that 20%, 3% and 33% ascorbic acid oxidation in case of water soluble pectin, hot water soluble pectin and HCL-soluble pectin respectively. Although, several researchers indicated that pectin polysaccharides possess antioxidant activity, the explaination of this effect was not thoroughly discussed (Motomura, 2002). The fact that pectin in cell wall contain phenolic acids such as ferulic acid of beet (Saulnier and Thibault, 1999) and coumaric acid of fruit (Fry, 1983). Linked on the C2 of the arabinose and/or C6 of the galactose residues, suggests that the antioxidant activity is probably due to the antioxidant activity of phenolic acid. This assumption is confirmed by the recent data obtained by (Kostalova *et al.*, 2013). They fractionted the pectin polysaccharides of pumpkin biomass into hot water, ethylen diamin tetra acetic acid (EDTA) and HCl-soluble pectin. Hot water soluble pectin fraction exhibited the highest free radical scavenging activity on DPPH. Phenolic compounds and protein occurred mainly in the hot water soluble pectin fraction. The authors concluded that the antioxidant activity correlated with the total phenolic content.

Effect of Different Concentrations of Pectin Fractions on Browning of Orange Juice

Colour is an important quality factor in the marketing of citrus juice. Browning of citrus products has been a problem throughout processing and storage. Fig. 2 shows that the browning was lower in samples treated with hot water soluble pectin than that of the control. This may be due to the presence of water soluble pectin. Tong *et al.* (1995) mentioned that commercial pectin inhibited browning of fresh apples juice may be due to the oxalic acid of pectin and this may chelate the critical coper prosthetic group of polyphenol oxidase (PPO).

Effect of Addition of Different Pectin Fractions on some Quality Parameters of Orange Juice during Storage

Total soluble solids, total titratable acidity, pH value of the control sample were 12.88%, 1.80% and 3.67, respectively, whereas the respective value of orange juice treated with 1.5% pectin fractions were 12.92%, 1.86% and 3.63 in water soluble pectin; 12.94%, 1.87% and 3.63 in hot water soluble pectin and 12.88%, 1.81% and 3.63 in HCL-soluble pectin, respectively (Tables 4-6), hot water soluble pectin had the highest content which means the best effect. Total, reducing and non-reducing sugers showed the highest content compared to control. HCl-soluble pectin indicated no effect.

The addition of water soluble pectin to orange juice may be lead to reduce the chemical reaction during storage at room temperature for 3 month as an antioxidant agent. Tables (4-6) also show that the orange juice treated with 1.5% water soluble pectin were rich in ascorbic acid and low browning. Cornell *et al.*, 1987 stated that the greatly accelerated loss of ascorbic acid of orange drink is due to the presence of oxygen content with an increase of the formation of browning pigment during storage at 24°C. In conclusion, this work indicate that the hot water soluble pectin is considered a very effective natural antioxidant and antibrowning agent for orange juice.

Table 3. Chemical composition of extracted alcohol insoluble solids (AIS) from Balady orange fruits and pectin fractions

Parameters	Value
Moisture content %	5.30
Protein %	6.52
Anhydrogalacturic acid %	35.34
Degree of estrification (DE) %	75
Ash %	3.75
Water soluble pectin %	19.87
Hot water soluble pectin %	25.18
HCl-soluble pectin %	15.26

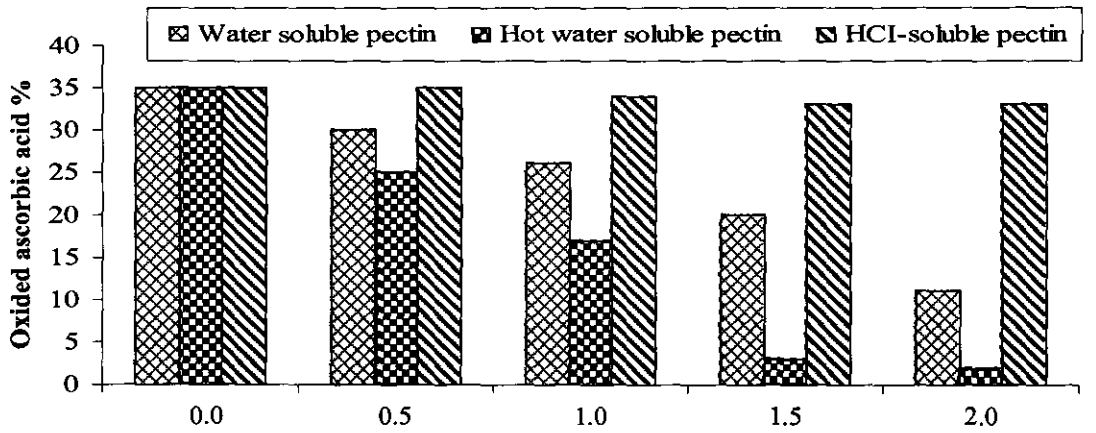

Fig. 1. Oxidated ascorbic acid % of orange juice as affected by pectin fractions

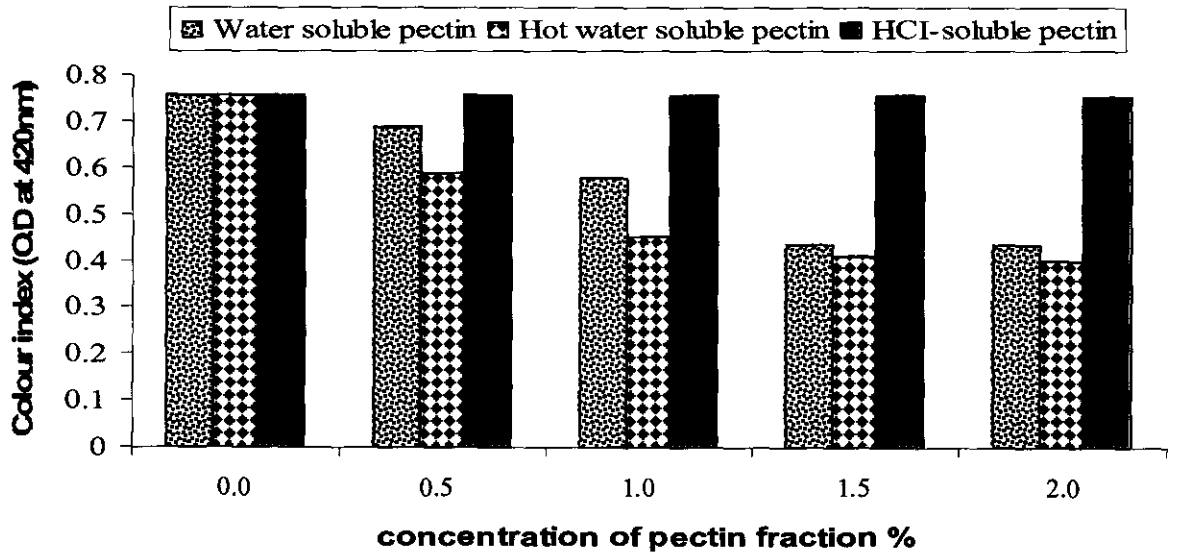

Fig. 2. Colour index of raw orange juice as affected by pectin fractions

Table 4. Chemical composition of packaged Balady orange juice during storage at room temperature for 3 months as affected by adding 1.5% WSP

Parameters	Control	1.5%WSP
Total soluble solids (%)	12.88	12.92
Total titratable acidity as citric acid (%)	1.80	1.86
pH value	3.67	3.63
Total sugars (%)	9.52	9.71
Reducing sugars (%)	4.07	4.29
Non-reducing sugars (%)	5.45	5.42
Ascoric acid mg/100 ml	50.78	56.92
B-carotene mg/100 ml	0.228	0.236
Colour index at 420 n m (Optical density) (OD)	0.587	0.415

WSP: Water soluble pectin.

Table 5. Chemical composition of packaged Balady orange juice during storage at room temperature for 3 months as affected by adding 1.5%HWSP

Parameters	Control	1.5%HWSP
Total soluble solids (%)	12.88	12.94
Total titratable acidity as citric acid (%)	1.80	1.87
pH value	3.67	3.63
Total sugars (%)	9.52	9.77
Reducing sugars (%)	4.07	4.33
Non-reducing sugars (%)	5.45	5.44
Ascoric acid mg/100 ml	50.78	57.12
B-carotene mg/100 ml	0.228	0.236
Colour index at 420 nm (Optical density) (OD)	0.587	0.410

HWSP: Hot water soluble pectin.

Table 6. Chemical composition of packaged Balady orange juice during storage at room temperature for 3 months as affected by adding 1.5% HCSP

Parameters	Control	1.5%HCSP
Total soluble solids (%)	12.88	12.88
Total titratable acidity as citric acid (%)	1.80	1.81
pH value	3.67	3.63
Total sugars (%)	9.52	9.56
Reducing sugars (%)	4.07	4.10
Non-reducing sugars (%)	5.45	5.46
Ascoric acid mg/100 ml	50.78	55.92
B-carotene mg/100 ml	0.228	0.228
Colour index at 420 nm (Optical density) (OD)	0.587	0.567

HCSP: HCL. Slouble pectin.

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النشـــاط المضـــاد للأكســـدة للمـــواد البكتينيـــة في البرتقـــال

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يعتبر أكسدة حمض الاسكوربيك وما يتبعه من تفاعلات التلون البني من العوامل التي تؤثر علي جودة عصير البرتقال. يهدف هذا البحث إلي دراسة تأثير إضافة المواد البكتينية المفصولة من ثمار البرتقال علي إمكانية التحكم في التلون البني والحفاظ علي ثبات حمض الأسكوربيك في عصير البرتقال. أظهرت النتائج إن ثمار البرتقال البلدي غنيه بالمواد الصلبة الذائبة حيث كانت ١٢,٩% والحموضة الكلية مقدرة كحمض ستريك كانت ١,٥٩% وحمض الاسكوربيك ٥٨,٩٣% مليجرام/١٠٠ جرام. كما أظهرت النتائج أن المواد الصلبة الغير ذائبة في الكحول تحتوي علي كلاً من حمض جلاكتونيك ٣٥,٣٤% ودرجة الأستر ٧٥% والبروتين ٦,٢٥ والرماد ٣,٧٥% و إن المواد البكتينية المستخلصة من المواد الصلبة الغير ذائبة في الكحول وهي البكتين الذائب في الماء علي درجة حرارة الغرفة لمدة ساعة ١٩,٨٧% والبكتين الذائب في الماء الدافئ لمدة ساعة ٢٥,١٨% والبكتين الذائب في حمض الهيدروكلوريك لمدة ساعة ١٥,٢٦%. وأظهرت النتائج إن إضافة البكتين الذائب في الماء علي درجة حرارة الغرفة أو المستخلص علي الساخن يحافظ علي ثبات حمض الاسكوربيك ويحسن لون العصير وزيادة تركيز المواد البكتنية المضافة يحسن خواص عصير البرتقال البلدي علاوة على تحسن خصائص الجودة في العصير المعبأ في زجاجات والمخزن علي درجة حرارة الغرفة لمدة ٣ شهور. هذا العمل يوصي بأن المواد البكتينية الذائبة في الماء الساخن تعتبر مضاد طبيعي للأكسدة والتلون البني في عصير البرتقال.

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