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Effect of Incubation, Enzymes and Thermal Pre-treatments on the Quality of Pumpkin Juice

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Abstract

Incubating fresh pumpkin *(Cucurbita pepo L.)* vegetable slices treated with thermal pre-treatments and a commercial exogenous enzyme preparation cellulase and α-amylase produced a pumpkin juice. HPLC was also carried out to fractionate the major sugar component found in fresh and treated pumpkin juice. Sucrose was the major sugar of the pumpkin juice which hydrolysis during water and steam blanching and cellulase enzyme pre-treatments to glucose and fructose. Results concluded that the incubated pumpkin have the lowest WIS and water content compared to the fresh pumpkin juice without treatment. No pectin-methyl-esterase (PME) activity was found in water, steam and enzymes pre-treatments of pumpkin juice compared with the fresh pumpkin juice without treatment. It was found that the yield was increased in water blanching, followed by cellulase addition and then steam blanching. The inhibitory effect of various thermal and enzymes pre-treatments based on a*-values measurements at their high time and maximum concentrations for pumpkin juice treated in the following decrease order steam blanching > microwave > water blanching > α-amylase enzyme > cellulase enzyme. This research proved that pumpkin can be used as a pumpkin juice with thermal and enzymes pre-treatments after incubated fresh pumpkin vegetable slices at 55°C for 3 hours.

Keywords: Pumpkin; Juice; Sucrose; Glucose; Fructose; Color; WIS; Cellulase; α-amylase; PME; Water; Steam; Microwave; Incubation

Introduction

In the last ten years, there has been a great increase in the international consumption of tropical vegetable juices, including pumpkin, according to FAO (Food and Drug Administration. 1989). The chemical composition of the pumpkin (fruit, seeds, flowers and leaves) and medical properties were summarized by Agnieszka et al., and Vucetic et al., [1] with indication of the beneficial constituents. Also, pumpkin fruits are a source of nutrients and vitamins [2] and are eaten fresh or preserved in jams, syrups or candies [3].

Therefore, in certain countries the contribution of calories from the consumption of fresh sugar may be substantial. A modification of sugar composition of the juice may be useful if the total calories could be reduced to a lower value and functional ingredients produced. Few commercial enzyme preparations that are normally used for juice processing have potentials for the modification of sugar composition [4]. It would be possible to use the enzyme preparation for modification of sugar composition of pumpkin juice [5,6]. Conservation of pumpkin vegetables by enzymatic pre-treatment aiming to increase and improvement of pumpkin juice yield and quality without changes in the physical and chemical characteristics, leading to improve in the sensorial and nutritional characteristics [7-9]. Reports concerning use of exogenous enzymes in tropical fruit processing are scarce. Enzymatic liquefaction of guava, papaya, plum, mango and Prickly pear has been reported in several studies [10-12]. In general, we can think of carbohydrates as either simple or complex. Simple carbohydrates include the following: fructose (which is the sugar in fruits), galactose, lactose, maltose, glucose and sucrose (common table sugar). Major free sugars in the pumpkin were fructose, glucose and sucrose. In the flesh, fructose and glucose were the major free sugars, corresponding to 87% of total free sugars. Total sugar content in the flesh was 3 times higher than that in the funicular attachments of the seeds, fibre and skin [13,14], whereas, conservation of vegetables by thermal treatment aiming to increase the life, controlling the action of enzymes and microorganism activity may affect the physical and chemical characteristics of the juice, leading to variations in the sensorial and nutritional characteristics. Alterations in this sense are inevitable but the optimization of the process would decrease the problem considerably. Understanding the reasons leading the juice to modify its characteristics during thermal treatment and processing, along with the extension of these variations, may be used to limit or inhibit these processes and may be a determining factor for the success of a product on the market [15,16]. In some cases, cell wall polysaccharide-degrading enzymes are used as aids to improve the yield of fruit juices (liquefaction) or to transform the pulp into nectar or juice (maceration). This is an important mechanism which affects the edible quality of fruit as well as its processing in juices or nectars. This textural change depends on the cellular anatomy of the tissue, the water content of the cells and the composition of the cell walls [17]. However, Ella Missang et al., [18] and Massiot et al., [19] found that the enzymatic treatment of the apple tissues was more effective the longer the storage; yields correlated well with the enzyme hydrolysis of WIS). Also they found that the water insoluble solids (WIS) preparation was preferred to alcohol insoluble solid (AIS) preparation because (1) the endogenous enzymes should act as they did in the crushed apple pulp and (2) the cell wall polysaccharides were more accessible to exogenous enzymes in the WIS than in the AIS material. However, WIS considered indicating the cellulose hydrolysis and the breaking of cells improving access to the pectins and quality of pumpkin juices In the present investigation, we will examine whether the different characteristics changes (Yield, WIS and water content) during pumpkin juice process

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as a natural juice are capable of modifying the hydrolysis of cell wall polysaccharides with conventional thermal and exogenous enzymes pre-treatments for modification of sugar composition of pumpkin juice , thus improving the quality of pumpkin juice.

Materials and Methods

Materials

Pumpkin *(Cucurbita pepo L.)* grown in the Damietta Governorate, Egypt were purchased from the Ministry of Agriculture in season 2007 at the commercial maturity and stored at 3-4°C until used in about 3 days for chemical and technological studies.

Methods

Juice extraction: Pumpkin samples were prepared according to the method of Hanson [20] with minor modification. They were cut into pieces which were handily peeled and the seeds as well as undesirable materials were discarded. The peeled pumpkin were extracted and screened by using the pulpier machine and the obtained tissues were pressed through three layers of cheesecloth to extract clear pumpkin juice, then tested and analyzed immediately.

Technological process

Incubation pre-treatments: Untreated pumpkin slices were incubated at different temperatures 40, 50 and 55°C for 3, 6, 9 and 24 hours, then the following analyzed immediately were carried out water content and water insoluble solids.

Thermal pre-treatments: Thermal pre-treatments were carried out by water and steam blanching for 1, 2 and 5 min. and microwave blanching (Power 10) for 10, 20, 30, 40, 50 and 60 seconds of pumpkin fruit slices and incubated in an incubator at 55 oC for 3 hours, then extracted to juice and analyzed immediately.

Enzymes pre-treatments: Enzymatic pre-treatment were carried out by α-amylase enzyme 5,000 Units (EC. 3.2.1.1) and cellulase enzyme 5,000 Units (EC. 3.2.1.4). These enzymes were derived from controlled fermentation's by selected strain of *Aspergillus niger* (SIGMA Chemical Co., St. Louis, MO, USA). Whereas, 200 g of pumpkin pulp was stirred with different concentrations 5, 10 and 50 unit of α-amylase enzyme and 10, 50 and 100 unit of cellulase enzyme respectively. Then, the enzyme treated pulp was incubated in an incubator at 55°C for 3 hours. The enzyme treated pulp was then placed in a boiling water bath for 5 minutes to inactivate the enzyme. The enzyme treated pulp was then rapidly cooled by cold water to 25°C. Following the enzyme treatment, the enzyme treated pulp was pressed through three layers of cheesecloth to obtain or extract clear pumpkin juice, then tested and analyzed immediately. The incubated samples at 55°C for 3 hours were considered as a blank or untreated sample. The following analyses were carried out in pretreated pumpkin juice: water content, water insoluble solids, acidity, pH, TSS, Yield, PME enzyme activity, colour characteristics and sugar compositions as a sugar profile.

The technological scheme plan of pumpkin juice processing by incubating, thermal and enzymatic pre-treatments is shown in Figure 1. Both the pre-treatments and the samples were repeated at least in duplicate.

Analytical methods

Water content determination: The water content of pumpkin samples was determined gravimetrically according to the methods recommended by the AOAC (2000) by drying at 70°C under reduced pressure to constant weight.

Water insoluble solids (WIS) determination: The water insoluble solids (WIS) were determined according by the method of Massiot et al. [19]. However, the pumpkin slices were immersed in distilled water and homogenizing at 4°C in a Waring blender for 30 second. The mixture was filtered through a glass filter and filter paper Whatman No. 1. The WIS was washed with distilled cold water until the filtrate showed a negative reaction in the phenol-sulphuric acid test (Dubois et al 1956).

Quality evaluation

Chemical properties: The pH of juice samples was measured using a digital pH-meter (HANNA, HI 902 meter, Germany). The percent Total Soluble Solids (TSS), expressed as °Brix (0-32) or g/Kg, was determined with a Hand refract meter (ATAGO, Japan). Treatable acidity was determined according to the method reported by Tung-Sun, et al, [21]. Juice yield was determined in a duplicate as g juice/100 g pumpkin pulps.

Color characteristics determination: Hunter a*, b* and L* values of the investigated samples were measured using a spectro-colourimeter (Tristimulus Colour Machine) with the CIE lab colour scale (Hunter, Lab Scan XE-Reston VA, USA) in the reflection mode. The instrument was standardized each time with white tile of Hunter Lab Colour Standard (LX No.16379): X=72.26, Y=81.94 and Z=88.14 (L*=92.46; a*=-0.86; b*= -0.16) [22]. The Hue (H)*, Chroma (C)* and Browning Index (BI) were calculated according to the method of Palou et al. [23].

Assay of pectinmethylesterase (PME) enzyme activity: Pectinmethylesterase enzyme (EC 3.1.1.11) activity was assayed according to the method of Arreola et al., [24] using a 0.1N NaOH solution.

HPLC reducing and non-reducing sugar determinations (sugar profile): Sugars (Reducing sugars (glucose and fructose) and non-reducing (sucrose)) in untreated, incubated, thermal and enzyme pre-treated pumpkin juice were determined by high performance liquid chromatography (HPLC) according to the method of Delcio, et al., [16] with minor modification. The samples were prepared diluting at the proportion of 1:10 with distilled water and centrifuged twice, at 5,000 × g for 10 minutes, and at 15,000 × g for 30 minutes. The samples were then frozen and kept at -18°C until analysis. After thawing, the samples were filtered in a polyethylene HV Millex syringe with a Durapore membrane (0.45 μm) (Waters-Milipore, Bedford, MA) and analyzed immediately.

Chromatographic analysis was carried out using a HP- Hewlett Packard HPLC 1050 Series with an automatic injector. Hewlett Packard absorbance/UV detector 1050 series was set at 214 nm. The apparatus was controlled automatically by Hewlett Packard computer, which also could calculate the obtained data and curved the chromatogram by the inset Chemo station Program. Also, Hewlett Packard HPLC Column was used as following characteristics:

- Apparatus - Hewlett Packard HPLC 1050 Series with an automatic injector – 1995
- Column - Restek (Pinnacle II Amino 3 μ 105 × 4.6 mm
- Column length - 50 mm
- Mobile phase - Acetonitrile 80% and water 20%

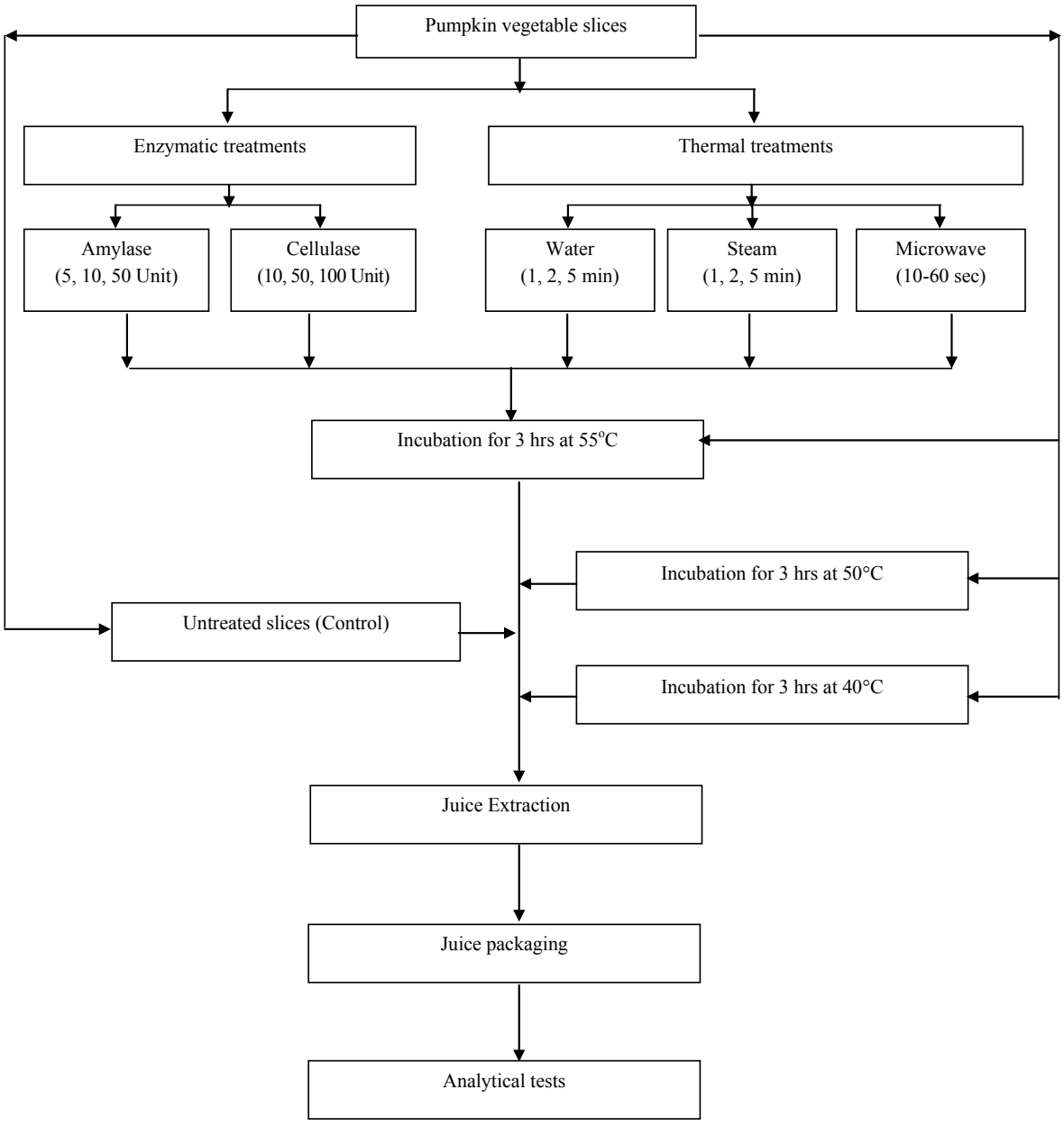

Figure 1: Technological Scheme plan of pumpkin juice processing.

- Flow rate - Flow rate
- Sample size - 10 ml
- Detection - Shodex Ri – 71
- Detector - GC1
- Range - Bipolar, 1250 mV, 10-Samp. Per Sec.
- Peak Width - 0.300 min
- Temperature - 30°C
- Pressure - 85 Bar

Peaks were quantified by area measurement and identified by retention time of standards (Merck, Darmstadt, Germany). A 50 mL loop was used in the injection.

Statistical analysis: For each pre-treatment and sample, three replicates were used. The mean values as well as the standard deviation of the three replicates of each sample were computed.

Results and Discussion

Effect of different incubation temperatures on water content and water-insoluble solids (WIS) of pumpkin vegetable slices during storage for 24 hours: The water and Water-insoluble solids (WIS) contents of pumpkin vegetables can be seen in Table 1. The water content of the pumpkin tissue decreased with a losses 3% in different incubation temperatures during the 24 hours incubation period. These

Incubation Times (hrs)	Different temperatures (°C)	Water content (g/100 g)	Water content reduction (%)	WIS (g/100 g)	WIS reduction (%)
0	40	89.97 ± 0.12	100	1.03 ± 0.06	100
0	50	89.87 ± 0.09	100	1.13 ± 0.08	100
0	55	89.54 ± 0.013	100	1.46 ± 0.11	100
3	40	89.03 ± 0.04	98.96	0.93 ± 0.15	90.6
3	50	88.89 ± 0.06	98.91	0.99 ± 0.09	87.12
3	55	88.45 ± 0.07	98.78	1.10 ± 0.05	75.05
6	40	88.14 ± 0.11	97.97	0.89 ± 0.11	86.50
6	50	88.00 ± 0.08	97.92	0.88 ± 0.04	77.60
6	55	87.48 ± 0.12	97.70	0.97 ± 0.09	66.43
9	40	87.44 ± 0.11	97.19	0.70 ± 0.16	67.90
9	50	87.23 ± 0.04	97.06	0.77 ± 0.06	67.45
9	55	86.72 ± 0.09	96.85	0.76 ± 0.11	52.13
24	40	86.93 ± 0.05	96.62	0.52 ± 0.14	50.00
24	50	86.60 ± 0.07	96.36	0.63 ± 0.07	55.68
24	55	86.04 ± 0.08	96.09	0.68 ± 0.13	46.46

± Standard Deviation (SD) with n=3

Table 1: Effect of different temperatures incubation on water content and water-insoluble solids of pumpkin fruit during storage for 24 hours.

results agreed quite well with that reported earlier of Massiot et al., [17] and corresponded in all probability to evaporation of water during storage of apple tissue. Over 24 hours incubation, the water content decreased in pumpkin fruit by 96%, in 40, 50 and 55°C, respectively, as seen in Table 1. The WIS content was 1.03, 1.13, and 1.46 g/100 g of pumpkin flesh in 40, 50 and 55°C incubation temperatures, respectively. These amounts of cell wall material as a WIS and water content were in agreement with previous reported results on apple cell walls as a WIS and water content [17,18]. Over 24 hours incubation, the WIS content decreased by 46.5, 55.7 and 50%, in 40, 50 and 55°C, respectively, as seen in Table 1. Also, Fischer and Amado, 1994 found similar decreasing of cell wall material during storage of apple. Such decreasing may be attributed to the catabolic activity of the endogenous pumpkin enzymes such as pectin-methyl-esterase and poly-galacturonase. A water insoluble solids (WIS) preparation of pumpkin samples were preferred than alcohol insoluble solid (AIS) preparation as described by Ella Missang et al, [19] and Massiot et al., [17]. From Table 1 results could be concluded that the incubated pumpkin vegetable slices for 3 hours at 55°C decreased WIS and water content 75.1 and 98.8%, respectively compared to the un-incubated sample and other incubated temperature of pumpkin samples.

The water and Water-insoluble solids (WIS) contents of pumpkin vegetables were evaluated during incubations and storage which are capable of modifying the hydrolysis of cell wall polysaccharides with both (thermal and enzymes) pre-treatments of pumpkin fruit slices, thus improving the quality of pumpkin juice.

We report on thermal and enzymes pre-treatments to hydrolysis of the cell wall polysaccharides from pumpkin fruits stored for 24 hours at three incubation temperatures.

However, Massiot et al., [17] reported that the sum of the WIS and water content, the polysaccharide content decreased by 10% during incubation and storage and about 20% of the total polysaccharides became water soluble after storage.

The degree of maceration of cell wall material in the tissue increased with the length of incubation and storage time whereas the solubilisation of pectins from the WIS with the pectinmethylesterase was similar (Tables 1-3). In contrast, there was a good correlation between the degree of liquefaction of cell wall material in the tissue and the solubilisation limits of pectins from the WIS (Table 1), indicating the cellulose hydrolysis and the breaking of cells improving access to the pectins and quality of pumpkin juices. Hence, under liquefying and incubation conditions, as pumpkin fruit slices storage time lengthened, the pectic polysaccharides became more accessible to the thermal and enzymes pre-treatments [17]. The thermal and enzymes pre-treatments of the pumpkin tissues was more effective the longer the storage; yields correlated well with the enzyme hydrolysis of WIS.

Effect of pre-treatments (thermal and enzymes) and incubation on physico-chemical properties of pumpkin juice (PJ): Chemical and physical properties of the fresh, microwave (with different seconds), water and steam blanching (1, 2 and 5 min.) and enzymes pre-treatments (with different concentrations) of pumpkin juice are given in Tables 2 and 3. It could be noticed that all thermal pre-treatments did not change TSS, while enzymes pre-treatments increased TSS with range about 11-22%. Whereas, addition of cellulose and α-amylase enzyme increased total soluble solids and decreased insoluble solids of pumpkin juice. Pilnik and Voragen [20] found similar increased in soluble solids content in studies involving commercial enzymes which release polysaccharides from apple cell wall, in addition to degrading pectic material, thus increasing soluble solids content.

The pumpkin juice pre-treated with α-amylase enzyme had a higher total acidity and lower ratio of TSS/Total acid it than others pre-treatments and control juice, which may be due to enzymatic desertification and degradation of pectin resulting in an increase of total acid (Tables 2 and 3). This result confirmed with the results of Tung-Sun et al., [21]. It is presumed the TSS/acid ratio has the major analytical measurement for quality in juices. Also, the pumpkin juice pre-treated with water blanching had a higher total acidity and lower ratio of TSS/Total acid than steam and microwave pre-treatments or control (Table 1). pH values of pumpkin juice were lightly affected by thermal and enzymes pre-treatments (Tables 2 and 3), which ranged from 5.77-6.72. These findings are in principal agreement with the results of Tung-Sun et al. [21].

No pectin-methyl-esterase (PME) enzyme activity was found in water and steam pre-treatments for 5 minutes and in cellulase and high concentration of α-amylase enzymes pre-treatments of pumpkin juice, while it was very low in other thermal or low concentration of α-amylase enzyme pre-treatments compared with untreated pumpkin juice, as seen in Tables 2 and 3. The PME inactivation obtained was

Blanching methods	Time (sec)	PH	TSS	Total acidity*	TSS / Total Acidity	PME	WIS	Water content (g/100 g)	Yeilds (%)
Microwave	Fresh	6.36 ± 0.11	9	0.096 ± 0.12	93.75	2.66 ± 0.07	0.58	90.42 ± 0.1	47
Blanching	10	6.58 ± 0.08	9	0.096 ± 0.08	93.75	1.66 ± 0.04	0.23	89.98 ± 0.04	54.5
	20	6.53 ± 0.09	9	0.096 ± 0.09	93.75	1.0 ± 0.1	0.48	89.74 ± 0.08	54
	30	6.52 ± 0.12	9	0.096 ± 0.13	93.75	0.66 ± 0.12	0.44	88.98 ± 0.12	55
	40	6.54 ± 0.1	9	0.096 ± 0.11	93.75	0.53 ± 0.08	0.76	87.89 ± 0.14	58
	50	6.32 ± 0.09	9	0.115 ± 0.12	78.26	0.33 ± 0.05	0.72	87.17 ± 0.07	58
	60	6.48 ± 0.06	9	0.096 ± 0.07	93.75	0.13 ± 0.07	1.09	86.73 ± 0.05	59
Steam blanching	Fresh	6.36 ± 0.08	9	0.096 ± 0.06	93.75	2.66 ± 0.08	7.78	83.22 ± 0.08	56
	60	6.38 ± 0.13	9	0.096 ± 0.1	93.75	0.46 ± 0.15	3.52	80.73 ± 0.13	53.5
	120	6.35 ± 0.16	9	0.096 ± 0.09	93.75	0.26 ± 0.07	4.37	77.20 ± 0.07	65
	300	5.84 ± 0.1	9	0.115 ± 0.06	78.26	0.00 ± 0.08	5.89	71.32 ± 0.04	77.5
Water blanching	Fresh	6.72 ± 0.09	9	0.125 ± 0.11	72	2.66 ± 0.05	1.09	89.91 ± 0.11	57.5
	60	6.14 ± 0.06	9	0.125 ± 0.09	72	0.66 ± 0.01	2.72	86.32 ± 0.09	62.5
	120	6.10 ± 0.12	9	0.115 ± 0.12	78.26	0.33 ± 0.11	2.88	83.44 ± 0.1	80
	300	5.77 ± 0.07	9	0.125 ± 0.14	72	0.00 ± 0.1	4.03	79.42 ± 0.14	82.5

*Total acidity expressed as citric acid (%), ± Standard Deviation (SD) with n=3

Table 2: Effect of incubation and thermal pre-treatments on physico-chemical properties of pumpkin juice.

Enzyme treatments	Conc. (unit)	PH	TSS	Total acidity*	TSS / Total Acidity	PME	WIS	Water content (g/100 g)	Yeilds (%)
Cellulase	Fresh	6.15 ± 0.09	9	0.144 ± 0.11	62.5	2.66 ± 0.06	0.66	90.34 ± 0.08	50.5
enzyme	10	6.36 ± 0.12	10	0.115 ± 0.13	86.96	0.00 ± 0.15	0.63	89.39 ± 0.1	68.5
	50	6.36 ± 0.07	11	0.125 ± 0.09	88	0.00 ± 0.11	0.72	88.67 ± 0.14	79.5
	100	6.38 ± 0.13	11	0.125 ± 0.14	88	0.00 ± 0.08	0.72	87.95 ± 0.09	81
α-amylase enzyme	Fresh	6.15 ± 0.08	9	0.125 ± 0.06	72	2.66 ± 0.06	0.78	90.22 ± 0.07	50
	5	5.95 ± 0.06	10	0.490 ± 0.07	20.41	0.26 ± 0.07	0.82	89.36 ± 0.14	57
	10	5.99 ± 0.11	11	0.538 ± 0.06	20.45	0.2 ± 0.09	0.41	89.11 ± 0.12	60
	50	5.96 ± 0.08	11	0.461 ± 0.13	23.86	0.00 ± 0.00	0.25	88.70 ± 0.07	62.5

*Total acidity expressed as citric acid (mg kg^{-1}), ± Standard Deviation (SD) with n=3

Table 3: Effect of enzymes pre-treatments and incubation on physico-chemical properties of pumpkin juice.

similar to that reported by Villamiel et al. [22], who heated orange juice in pasteurization under similar condition. Highest yield of pumpkin juice was obtained in pumpkin slices treated with water blanching after 5 min. (82.5%), followed by 100 unit cellulase enzyme additions (81%) and then 5 min. steam blanching (77.5%). While the average yield of untreated juices was about 47-57% (Tables 2 and 3). The cellulase enzyme pre-treatment enhanced liquefaction of pumpkin pulp resulting in higher juice yields, and should be considered as an important economic value. This coincides with research by the results of Tung-Sun et al., [22].

The water content in all thermal and enzymes pre-treatments of pumpkin slices was decreased by increasing of times and concentrations respectively compared with untreated juice. Tables 2 and 3 include the water insoluble solids (WIS) and water content of all thermal and enzymes pre-treatments of pumpkin slices. The water insoluble solids (WIS) content was increased in thermal pre-treatments by increasing of time and in cellulase enzyme pre-treatments by increasing of concentration, whereas decreased in α-amylase enzyme pre-treatments by increasing of concentration. These result similar to the findings of Hang and Woodams, [6,23].

Effect of pre-treatments (thermal and enzymes) and incubation on different parameters of color characteristics and non-enzymatic browning (A420 nm) of pumpkin juice (PJ): The surface colour of pumpkin juice was measured with a colour difference meter, using the Hunter Lab colour scale (Tables 4 and 5). Under all tested conditions, steam and microwave blanching showed much higher efficient values based on a-values than A420 measurements, whereas the other pre-treatments behaved an opposite trend. For all tested samples the increase in the time of the thermal pre-treatments revealed increase in the inhibition efficient. Such trend is in agreement with previous studies of Janovitz-Klapp [24] and Ozoglu and Bayindirh [25]. The inhibitory effect of various thermal and enzymes pre-treatments based on a*-values measurements at their high time and maximum concentrations on Tables 4 and 5 for pumpkin juice treated in the following decrease order steam blanching > microwave > water blanching > α-amylase enzyme > cellulase enzyme. It is obvious that α-amylase and cellulase enzymes pre-treatments of pumpkin juice increased the development of red colour a* value as non-enzymatic browning. The Hunter colour values of steam blanching samples in pumpkin juice were lower than that of water blanching and microwave samples. Also, the Hunter colour value of α-amylase enzyme pre-treatment in pumpkin juice was lower than that of cellulose enzyme pre-treatment. These results indicated that the browning (redness) increased in enzymes pre-treatments samples than in thermal pre-treatments samples for pumpkin juice, respectively, seen in Tables 4 and 5. According to our results, the main colour change in untreated of pumpkin juice and pre-treated by thermal and enzymes pre-treatments was due to increase in browning index (BI) and a*-value, which were in high correlation to browning measurement. However, Beta-carotene partially lost its red color after heat treatment, probably because it changed to the *cis* form [26-28]. Other colour parameters such as Hue angle and chroma also

Blanching methods	Time (sec)	b*	a*	L*	C*	H*	BI	A 420 nm
Microwave	Fresh	62.4	25.8	48.6	67.5	67.51	726.8	0.132
Blanching	10	36.6	2.55	35.6	36.7	86.01	413.8	0.11
	20	38.3	4.46	37.2	38.5	83.36	420.5	0.112
	30	35.4	1.79	35.2	35.4	87.1	391.9	0.116
	40	34.4	2.24	34	34.4	86.27	398.6	0.122
	50	34.1	2.33	34.7	34.2	86.09	375.5	0.122
	60	36.1	-0.44	30	36.1	89.3	592.4	0.116
Steam blanching	Fresh	62.4	25.8	48.6	67.5	67.51	726.8	0.132
	60	34.5	1.52	33.7	34.6	87.48	407.8	0.113
	120	21.8	-1.7	27.5	21.9	85.55	234.3	0.111
	300	19.3	-2.39	26.2	19.4	82.92	200.5	0.114
Water blanching	Fresh	62.4	25.8	48.6	67.5	67.51	726.8	0.132
	60	60.6	21.6	49.6	64.4	70.39	645.3	0.11
	120	59.6	21.4	49.2	63.3	70.22	632.1	0.112
	300	60.5	23.2	49.4	64.8	69.04	649.6	0.116

Table 4: Effect of thermal pre-treatments and incubation on different parameters of color characteristics and non-enzymatic browning (A 420 nm) of pumpkin juice.

Untreated and Treated, Pumpkin Juice Samples	Sucrose	Glucose	Fructose	Maltose	Lactose
Fresh PJ – Control	46.27	104.24	55.54	57.68	25.53
Incubated PJ at 55°C for 3 hr	41.57	67.77	90.92	94.91	125.04
Water 2 min. PJ	39.85	70.85	99.84	115.92	129.05
Steam 2 min. PJ	36.46	98.66	88.73	30.72	48.24
Microwave P10 – 60 sec. PJ	46.39	101.87	46.81	58.68	56.49
Cellulase 50 Unit PJ	35.47	103.49	101.39	32.8	-
A-amylase 10 Unit PJ	45.59	70.35	61.05	-	-

Table 5: Effect of enzymes pre-treatments and incubation on different parameters of color characteristics and non-enzymatic browning (A 420 nm) of pumpkin juice.

indicated that enzymes pre-treatments caused a slight colour change. The samples of water blanching had a BI higher than in case of the steam blanching and microwave samples. But, BI values in α-amylase enzyme pre-treatment samples was lower than in case of the cellulose enzyme treated samples, as seen in Tables 4 and 5. These results are in good agreement with those of Janovitz-Klapp et al. [24]; Genovese et al. [29]; Palou, et al., [30]; Biesiada et al., [31]; Agnieszka and Hayta [32] and Ozoglu and Bayindirh [25]. However, cellulase and α-amylase enzyme samples had the higher increase in colour as optical density (A420 nm) compared to the untreated and thermal treated pumpkin juice samples, as seen in Tables 4 and 5. Increasing of α-amylase and cellulase enzyme concentration did not influence chroma (saturation index) and hue angle of treated pumpkin juice. This indicates that how much a color differs from gray.

Hunter Color results indicate that pumpkin juice was darker and redder after α-amylase enzyme and cellulose enzyme treatment. These results are in accordance with the same results of Tung-Sun, et al, 1995 who found that the Hunter Color results indicate that plum juice was darker and purpler after enzyme treatment. The results showed that the browning (O.D 420) was decreased from 0.165 and 0.207 in untreated pumpkin juice to 0.152 and 0.186 in low concentration of α-amylase and cellulase treated pumpkin juice respectively, as seen in Table 5. This indicates that the α-amylase and cellulase enzyme pre- treatments prevented browning in pumpkin juice compared to untreated pumpkin juice [33,34].

With time, the increase in the intensity of the dark colors, such as the red one decreased the yellow intensity, contributing negatively to the maintenance of the characteristic color of the juice. Looking at these results, it was possible to conclude that the pumpkin juice pre-treated with thermal and enzymes treatments and incubated at 55°C/3 hrs had the smaller variation compared to the control, for the Hunter dimensions.

From the above mentioned results it could be concluded that the pretreated pumpkin juice with microwave, steam blanching and α-amylase enzyme have the best colour values (a* and BI) and lower non-enzymatic browning compared to the other pre-treatments, as seen in Tables 4 and 5.

Effect of pre-treatments (thermal and enzymes) and incubation on Sugar compositions of pumpkin juice (sugar profile): Sucrose, glucose and fructose were the major soluble neutral sugars and predominant sugar found in the incubated, untreated and treated pumpkin juice samples, Furthermore, Bian et al., [35] found similar results in peach puree. Glucose contents were relatively higher than sucrose and fructose contents in fresh pumpkin juice samples (Table 6). The incubation of pumpkin slices at 55°C for 3 hours increased fructose, maltose and lactose but decreased glucose and sucrose content in PJ compared with untreated sample, as seen in Table 6. This conversion of sucrose to invert sugars maltose and lactose has been successfully used as a functional ingredient in various health food products [5]. Also, this association of high invert sugar with incubation of pumpkin vegetable juice agreed with that reported for fruit juice [6,23].

Steam, microwave and cellulase enzyme pre-treatments increased the glucose content than the other sugar contents, while water blanching pre-treatment increased fructose, maltose and lactose content than sucrose in PJ, as seen in Table 6.

The aforementioned results could be interpreted by the findings of Bian et al., [35], which they found that the total sugar level and soluble sugars of peach puree did not change with either pasteurized or sodium benzoate treatments. However, thermal pre-treatments showed a

Enzyme treatments	Conc. (unit)	b*	a*	L*	C*	H*	BI	A 420 nm
Cellulase	Fresh	57.5	22.4	45.9	61.7	68.8	686.1	0.214
Enzyme	10	56.1	19	48.2	59.3	71.3	574.7	0.186
	50	56.9	19.5	48.2	60.1	71	592.7	0.205
	100	55.9	18.9	48.6	59	71.3	560	0.208
α-amylase enzyme	Fresh	62.4	25.8	48.6	67.5	67.5	726.8	0.184
	5	54.8	18	46.4	57.7	71.8	592.7	0.152
	10	51.8	17.2	46.6	54.6	71.6	519	0.166
	50	57	19.8	48.5	60.4	70.8	587.4	0.162

Table 6: Effect of pre-treatments (thermal and enzymes) and incubation on sugar compositions of pumpkin juice (sugar profile).

decrease in sucrose with a concurrent increase of glucose and fructose.

Sucrose was more stable in untreated and incubated juices, while juices treated with water and steam blanching showed more hydrolysis of sucrose. These results were similar to those found by Dalal and Salunkhe [36], Fang et al. [26], Ewaidah [37] and Park et al. [15] where the concentration of reducing sugars (fructose and glucose) increased over heating time while that of sucrose decreased.

Results showed that the microwave pretreated juice decrease sucrose hydrolysis because the effect of microwave treatment on contents of sugar prevented sucrose degradation. The same results was found by Fujiwara et al. [38-42] when analysis of the effects of the microwave treatment on contents of sugars in the grape pulp indicated that microwave heating was necessary in order to prevent sucrose degradation during extraction for sugar analysis. It would be possible to use the enzyme preparation or thermal operation after incubation for modification of sugar composition of pumpkin juice used as a natural juice. So, water and steam blanching and cellulase enzyme pre-treatments might cause hydrolysis of sucrose to glucose and fructose [43-47].

Conclusion

Thermal (water, steam and microwave) and enzymes (amylase and cellulase) pretreatments after incubating pre-treatment could be used to modify sugar composition and improve quality of pumpkin juice. Even though the change in sugar composition may affect sweetness attribute of the pumpkin juice, this pre-treatments could also be considered beneficial for production of pumpkin juice from pumpkin vegetable slices.

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