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EFFECT OF THERMAL AND MACERATE ENZYMES PRETREATMENTS ON THE QUALITY OF COLD STORED PEACH JUICE.

Eissa, H.A.*; Wafaa H. Emam* and G. I. Wahab**
*** Food Technology Dept., National Res. Center, 12622 Cairo, Egypt.**
**** Dairy Department, National Research Center, 12622 Cairo, Egypt.**

ABSTRACT

Three indexes, namely colorimetric Hunter parameters (L, a, b and DE), β-carotene content, microbiology and sensory properties, were monitored to determine the quality loss of peach juice during cold storage at (5 °C). Total carotenoid (β-carotene) pigment content loss was not significant after thermal and enzymatic pretreatments. However, thermal effects on carotenoid pigment contents, especially β-carotene, were clearly observed and significant ($P < 0.05$). Water blanching of peach fruit reduced the content of β- carotene by 25% and steam blanching by 15%. Color parameters influenced by the presence and quantities of different carotenoids in juices analyzed and correlations between color and carotenoids have been observed. The β-carotene had a linear relationship ($p < 0.05$) with a* (positive) and L* (negative). Similarly, the β- carotene had a statistically significant ($p < 0.05$) linear relationship with a* (positive), b* (positive) and L* (negative). The results showed that Clostridium, *Staph. Aureus, E. coli*, Coliform group Counts and *Bacillus cereus* were not found in untreated and treated peach juice during cold storage. The results showed that the peach juices treated with enzyme + So_2 had also the highest reduction of yeast & molds and bacteria followed by treated withthose steamed and water blanched, but untreated samples were the lowest reduction of yeast & molds and bacteria for 28 days stored at 5 °C. It was clear that the refrigerated peach juice pretreated with enzymatic + So_2 gave the highest mean panel scores (7.78 - 9.22A) and were the most preferred in color, odor and appearance after 28 days of cold storage, but steam pretreatment was higher mean panel score (8.11^{A}) in taste characteristic.

Keywords: Peach juice, Color, β- carotene, Non-enzymatic browning; Clostridium, *Staph. Aureus, E. coli*, Coliform group Counts and *Bacillus cereus*, yeast & molds and bacteria.

INTRODUCTION

Peach is one of the most important commercial fruits of Egypt. The fruit can be consumed fresh or processed in various forms, peach juice is a popular product due to its very pleasant aroma and flavor. Thermal treatment is generally applied to extend shelf life of fruit products. However, heating processes can affect the quality of product which leads to consumer dissatisfaction. Non-enzymatic browning reactions and pigment destruction have been found to be major causes of such problems (Carabasa *et al.*, (1998), Buedo *et al.*, (2000) and Athanasios *et al.*, 2008).

Different methods can be used to determine the extent of color change. Color measurement is simple and faster than chemical analysis. The Hunter parameters (L, a, and b) have been proven to be useful for describing visual color change of various fruit products (Avila & Silva, 1999; Garza *et al.*, 1999; Andrea *et al.*, 2002; Ibarz *et al.*, 2005 and Murray *et al.*, 2007). The L

value represents the light–dark spectrum, a value is for the green–red spectrum and b value represents the blue–yellow spectrum (Ranganna, 1986). Other assays include the analysis of intermediates and final products of non-enzymatic browning reactions (Carabasa *et al.*, 1998 and Buedo *et al.*, 2000). The main objectives of this study were to investigate the effect of thermal (water and steam blanching) and commercially available pectinase and So_2 during cold storage at (5 °C) for 28 days on quality and color characteristics of peach juice, pathogenic microbiological, sensory evaluation and β- carotenoids content of peach juice.

MATERIAL AND METHODS

Fruit samples:

Peach (*Prunus persica* L. Batsch) fruit namely Sheikh Zowaied cultivars grown in the farms at North Sinai area, Egypt. It was purchased from The Ministry of Agriculture in season (2008) and kept at 3-5 °C until used in about 2-3 days for technological studies.

Technological Methods:

Extraction of peach juice samples:

One hour prior to use, fruits were removed from the refrigerator (5 °C) and equilibrated to room temperature. Peach fruits were rinsed with water, cut into halves, de-stoned and sectioned to longitudinal slices. Peach juice samples were extracted from individual Peach fruits with a juicerator and filtered by 3 layers of cheesecloth to obtain clear peach juice. Juice was collected in a beaker containing 5 mg ascorbic acid / 100ml juice with stirring. The amount of ascorbic acid used was not enough to prevent browning for more than 1hr. However, the ascorbic acid was used to prevent instantaneous browning, thereby providing a short lag time to allow test materials (natural extracts) to be added and mixed (Sapers and Douglas, 1987).

Pretreatments before processing:

Thermal pretreatments:

Thermal pretreatments were carried out by water and steam blanching for 3 min. of peach slices fruit, then extracted, analyzed and stored at refrigerator (5 °C) for 28 days (Genovese *et al.*, 1997).

Macerate Enzymes-So_2 pretreatments:

Peach fruits were hand peeled, and pulped in a stainless steel pulper, then pressed through three layers of cheesecloth to obtain clear peach juice. Whereas, Enzymatic pretreatment was carried out by 200 g of peach pulp and stirred with concentration 0.50% (W/w) pectinase. Pectinase enzyme (EC. 3.2.1.15) was 5,000 Units. This enzyme was derived from controlled fermentation's by selected strain of *Aspergillus niger* (SIGMA Chemical Co., St. Louis, MO, USA). Then, the enzyme treated pulp was incubated in a water bath at 50°C for 2 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 added So_2

(0.05%) and pressed through three layers of cheesecloth to obtain a clear juice, then tested, analyzed immediately and stored at refrigerator (5 °C) for 28 days. Both the pretreatments and the samples were repeated in duplicate (Eissa and Salama 2002).

Quality evaluation:

Physical and Chemical analyses:

The pH of juice samples was measured using a digital pH-meter (HANNA, HI 902 meter, Germany) at (25 °C) as described by A.O.A.C. (2000). The percent of Total Soluble Solids (TSS), expressed as °Brix (0-32), was determined with a Hand refractometer (ATAGO, Japan) according to A.O.A.C. (2000). Titratable acidity was determined according to the method described by Tung-Sun, *et al.*, (1995).

Non-enzymatic browning examination:

Non-enzymatic browning was measured spectrophotometrically by 4054 UV/Visible spectrophotometer, (LKB-Biochrom Comp., London, England), as absorbance at 420 nm using ethanol as blank according to the method of Birk *et al.*, (1998).

Microbiological Analysis:

1- Total viable bacterial count (TVBC):

Total viable bacterial count was determined in the juice on plate count agar. The plates were incubated at 35 °C for 48hrs (FDA, 1992).

2- *Staphylococcus aureus*:

Staphylococcus aureus cells were enumerated according to Baired-Parker (1962) using Baired-Parker's medium. Plates were incubated at 37 °C for 48 hrs.

3- Molds and Yeasts:

Molds and Yeasts were counted on Potato dextrose agar (Oxoid) acidified to pH 3.5 with sterile lactic acid solution (10%). Plates were incubated at 25 °C for 3-5 days (APHA, 1994).

4- Salmonella:

Salmonella was detected by surface plating technique on XLD (Xylose-Lysine Desoxycholates agar), Oxoid CM469, according to Doyle (2007). Plates were incubated at 37 °C for 24 hrs.

5- Coliforms:

Coliform bacteria were enumerated according to Harrigan and McCance (1996) using Violet Red Bile agar medium. The plates were incubated at 37 °C for 24 hrs.

6- *E.Coli*:

E.Coli was detected by surface plating technique on XLD (Xylose-Lysine Desoxycholates agar), Oxoid CM469, according to Doyle (2007). The plates were incubated at 37 °C for two days.

7- Spore Formers Count (SFC):

Spore formers count was determined by samples dilution heated to 85 °C for 15min. and counted with plate count agar (Difco), according to Doyle (2007). The plates were incubated for 48 hrs at 35 °C.

8- Clostridium:

Clostridium was counted on Reinforced Clostridial Medium (RCM) (Oxoid), according to Doyle (2007). The plates were incubated at 35 °C for 4 days.

Color characteristics and parameters determinations:

Hunter a*, b* and L* parameters were measured in the National Research Centre (Food Technology Dep.) with a color difference meter or the color of peach juice samples was measured using a spectro-colorimeter (Tristimulus Color Machine) with the lab color scale (Hunter, Lab Scan XE - Reston VA, USA) in the reflection mode. The instrument was standardized each time with white tile of Hunter Lab Color Standard (LX No.16379): X= 72.26, Y= 81.94 and Z= 88.14 (L*= 92.46; a*= -0.86; b*= -0.16) (Sapers and Douglas, 1987). Color difference, Delta E, was calculated from a*, b* and L* parameters, using Hunter-Scotfield's equation (Hunter, 1975) as follows.

Delta E = $(\text{delta } a^2 + \text{delta } b^2 + \text{delta } L^2)^{1/2}$

where : $a\text{-}a_o$, $b\text{-}b_o$ and $L\text{-}L_o$; subscript "$_o$" indicates color of control or untreated sample.

The Hue (H)*, Chroma (C)* and Browning Index (BI) was calculated according to the method of Palou *et al.*, (1999) as follows:

$H^* = \tan^{-1} [b^*/a^*]$.....(1)

C^* = square root of $[a^{2*} + b^{2*}]$.....(2)

BI = [100 (x-0.31)] 10.72.....(3)

Where:-

$X = (a^* + 1.75L^*) / (5.645L^* + a^* - 3.012b^*)$

β- carotene determinations:

β- **carotene** in untreated, thermal (water and steam blanching) and enzyme + So_2 pre-treated peach juice was determined by high performance liquid chromatography (HPLC) according to the method of Chen, *et al.*, (2007). Chromatographic analysis was carried out using HPLC Conditions as follows: The instrumentation consisted of as Agilent Series 1100 HPLC instrument (Agilent, Palo Alto, USA) equipped with a vacuum degasser, a quaternary pump, an auto sampler (50 µL injection volume), and a UV-vis diode array detector. For separation an Agilent Zorbax Eclipse XDB-C8 column, 150 x 4.6mm ID., particle size 5µm, was used. Detection was carried out at 360 nm. The optimized mobile phase consisted of water / methanol containing 10 mM oxalic acid. A gradient was run from 10% methanol to 60% methanol within 20 min. The flow rate was 1 $mLmin^{-1}$.

Sensory Evaluations

Sensory evaluation of the untreated and treated peach juices samples were evaluated by panelists of 10 selected judges utilizing a 10-point scale (where 10 = excellent and 1 = bad) for color, taste, odour and appearance (Crandall *et al.*, 1990).

Statistical analysis

All untreated and treated peach juices studied samples were conducted at least in duplicate and each sample was analyzed in duplicate. The results of sensory evaluation were analyzed statistically using analysis of variance, least significant difference (LSD) at 0.05 level and correlation coefficient (square root) as described by Richard and Gouri (1987).

RESULTS AND DISCUSSION

Effect of thermal and enzymatical pretreatments on the quality characteristics of peach juice during cold storage (28 days) at (5 °C):

Some properties of peach fresh juice and juice extracted after pretreatment by water and steam blanching and enzyme + So2 during cold storage at (5 °C) are given in Table (1). From data presented in Table (1) it could be noticed that pH, TSS and Titratable acidity did not change in samples of control and water blanching. pH of all pretreatment remained stable 4.93-4.99. Similar results have been noted by Barbanti *et al.*, (1992) and Ibarz *et al.*, (1999).

Table (1): Effect of thermal and enzymatical pretreatments on the quality of peach juice during cold storage (28 days) at (5 °C) .

Peach juice Samples	PH	TSS (Brix°)	% acidity*	TSS / acidity
Zero time				
C	4.99	12.8	1.17	10.93
T_1	4.93	11.8	1.12	10.51
T_2	4.99	12.4	0.96	12.92
T_3	5	13	1.25	10.42
7 days storage at 5 °C				
C	4.98	12	1.15	10.42
T_1	4.96	11.8	1.12	10.51
T_2	5	11.2	0.96	11.67
T_3	4.99	13	1.26	10.34
15 days storage at 5 °C				
C	5	12	1.15	10.42
T_1	4.98	12	1.13	10.60
T_2	4.93	12.6	0.96	13.13
T_3	4.9	13.2	1.25	10.58
28 days storage at 5 °C				
C	4.95	12.8	1.15	11.11
T_1	5	12	1.11	10.78
T_2	4.97	14	0.96	14.58
T_3	4.94	15	1.25	12.02

Control (C), Water blanching (T_1), Steam blanching (T_2) and Enzyme + So_2 (T_3)

It is clear in Table (1) that no different was observed for pH, TSS and acidity before and after 28 days storage of peach juice at refrigerator (5 °C). Peach juice contains predominantly citric acid. The % titratable acidity in untreated and water blanched treated of peach juices at zero time was about 1.12% to 1.25% (Table 1). With long-term storage at 5 °C, total acidity remained stable during 28 days storage of peach juices for every pretreatment was about 0.96-1.25. A similar result in acidity has been reported for peach with the results of Moller and Palmer, 1984. Ishida *et al.* (1979) found that three peach cultivars have 6.5-13% soluble solids, in the same time, green mature peach one represented 13.6-11.6% of ripened types (Nelson, 1983); It ranged between 12.2-13.5% for Indian peach (Moor *et al.*, 1984), and 8.4-16.3% for fleshed peach (Robertson *et al.*, 1990), meanwhile such values were very high being between 12.4-15.8%

(Dirlewanger *et al.*, 1999). This indicates that treated peach juice may be advisable for fresh use. However, it might be processed into acceptable quality juice.

Effect of thermal and enzymatical pretreatments on Color characteristics of peach juice during cold storage (28 days) at (5 °C):

Color is only part of the overall appearance, but is probably a major quality factor in peach juices. Color characteristics revealed that color did not change over storage time of enzymaticaly treated juice samples except in the samples steamed, water blanched and control (Table 2). In this case each of (L*-values) brightness, (a*-values) redness and (b*-values) yellowness decreased. The effects of steam treatment and enzymatically of peach juice in the inhibition of the browning reaction are listed in Tables (2). It is obvious that steam treatment of peach juice inhibited the development of red colour a*. For example, the a*-value of fresh juice at zero time was 14.07 compared to 15.06, 16.31 and 12.15 in case of juice treated with water blanched, steam blanched and enzymaticaly, respectively. The Hunter colour value of peach juice treated with enzymaticaly and So_2 chemical was lower than that of untreated juice. Similar results have been noted by Eissa and Salama (2002) who reported that the Hunter colour value of prickly peach juice was lower than that of untreated juice.

According to our results, the main color change in enzymatical treated peach juice was due to increase in chroma, hue angle and b*-value, which were in high correlation to browning measurement. Sapers and Douglas (1987) reported that decrease and increase in the L* value and a* value respectively correlated well with increases in apple browning. Hunter hue angle and saturation index (chroma) remained almost constant in all samples other than natural juices without treatments, which changed due to particle precipitation. Similar results have been noted by Andrea *et al.*, (2002), Ibarz *et al.*, (2005), Murray *et al.*, (2007).

Other color parameters such as Hue angle and chroma also indicated that heat (water and steam blanching) caused a slight color change. Similar results have been noted by Lee, (1997) who showed that the color parameters such as Hue angle and chroma also indicated that heat caused a slight color change of grapefruit juice. Tables (2), lists the values of H* and C* as well as BI for peach juice which have been treated with the steam, water blanching and enzymaticaly were shown. Results showed that the browning index (BI) results represented in Table (2). It is clear that enzymatically treatment markedly higher the BI. The samples enzymatically with So_2 treated had a BI equivalent to 542.48 compared to 469.66 in case of the fresh juice. But, water blanching lowered the BI to 432.11 compared to 457.36 in case of the steamed juice, as shown in Table 2. These results are in good agreement with those of Lee (1997), Palou *et al.*, (1999), Genovese *et al.*, (1997) and Marisa *et al.*, (2005). However, steam and enzymes treatment may be caused little browning during storage, but water blanching of peach juice caused clear browning as a Browning Index (BI) during storage at refrigerator (5 °C).

Delta E is the unit for computing the total color differences from the initial value. We found a good linear relation between Delta E and the storage time of untreated peach juice, as shown in Tables (2). This finding was consistent with many previous studies (Andrea *et al.*, 2002, Ibarz *et al.*, 2005, Murray *et al.*, 2007).

Previous studies of the color change during heat treatment showed similar results. Avila and Silva (1999) examined the color degradation of peach puree as affected by heat treatment. Peach puree became darker, corresponding to a decrease in L value and an increase in a value, with increasing temperature. Moreover, the loss of yellowness was also expressed by a decrease in the b* value. They concluded that the major causes of color change were due to carotenoid degradation and nonenzymatic browning (Maillard reaction).

The results also observed that enzymes and So2 had a positive controlling, or retarding color changes, when applied to natural peach juice without heat treatment. Steam treatment of peach juice was very effective in inactivating pectin methyl esterase (PME) enzymes as well as stabilizing cloudiness. McKenzie and Beveridge (1988) reported similar results during the steam blanching of apple juice. They attributed apple particulate stabilization to the formation of a protective colloid that prevented aggregation.

The surface colour of peach juice was measured with a colour difference meter, using the Hunter Lab colour scale. Under all tested conditions, enzymes and SO_2 showed much higher efficient values based on a*-values than non-enazymatic browning (NE-browning A420) measurements, whereas the other treatments behaved an opposite trend. Tested samples of pretreatments (enzymes and SO_2) showed an increase in the inhibition efficient. Such trend is in agreement with the previous studies of Buedo *et al.*, (2000) and Ozoglu and Bayindirh, (2002).The inhibitory effect of various enzymes macerate and chemical (anti-browning agents) pretreatments based on a measurements at their maximum concentrations as indicated in (table 2) for treated peach juice show of the following in a decrease order: enzyme + So_2 > steam blanching > water blanching. It is obvious that macerate enzymes and chemical pretreatments of peach fruits increased the development of red colour a* value as non-enzymatic browning. The Hunter colour values of treated samples in peach fruits juice were lower than those of untreated samples. Also, the Hunter colour value of enzymes and SO_2 pretreatment in peach juice was lower than that of water and steam pretreatments. These results indicated that the browning (redness) increased in untreated and water blanching treated samples than in enzymes and So2 treated samples for peach juice (Table 2). According to our results, the main colour change in untreated of peach fruits juice and pre-treated by blanching treatments was due to the increase in browning index (BI) and a*-value, which were in high correlation to browning measurement. Other colour parameters such as Hue angle and chroma also indicated that chemical pretreatments caused a slight colour change (Sapers and Douglas, 1987).

Table (2): Effect of thermal and enzymatical pretreatments on color characteristics in peach juice during cold storage (28 days) at (5 °C).

Peach juice Samples	L*	a*	b*	Delta E	C*	H*	BI	A_{420nm}**
Zero time								
C	40.22	14.07	42.57	66.82	44.84	71.72	469.66	1.95
T_1	45.32	15.06	46	66.35	48.40	71.87	432.11	2.59
T_2	46.3	16.31	48.32	67.22	51.00	71.35	457.36	2.52
T_3	53.7	12.15	61.5	71.36	62.69	78.82	542.48	2.5
7 days storage at 5 °C								
C	40.47	12.2	41.8	67.91	43.54	73.73	443.27	2.02
T_1	45.51	14.02	46.12	67.44	48.20	73.09	428.27	2.62
T_2	43.98	14.08	46.03	68.45	48.14	72.99	456.66	2.26
T_3	50.07	10.03	59.92	74.2	60.75	80.5	600.72	1.88
15 days storage at 5 °C								
C	38.54	12.25	40.28	68.7	42.10	73.08	455.19	1.74
T_1	42.94	13.64	42.91	67.24	45.03	72.37	418.34	2.47
T_2	42	13.79	42.45	67.68	44.63	72	428.32	2.25
T_3	51.36	10.65	59.82	73.63	60.76	79.91	562.77	2.12
28 days storage at 5 °C								
C	41.57	14.76	45.83	70.38	48.15	72.15	509.89	1.92
T_1	44.34	14.62	44.01	67.16	46.37	71.62	414.53	2.35
T_2	46.42	15.92	49.09	69.5	51.61	72.03	468.21	2.66
T_3	52.03	12.08	62.37	75.59	63.53	79.04	605.16	1.67

** Non-enzymatic browning (Absorption at 420nm)
H*= Hue angle, C*= Chromaticity and BI= Browning index.
Control (C), Water blanching (T_1), Steam blanching (T_2) and Enzyme + So_2 (T_3)

Effect of thermal and enzymatical pretreatments on β- carotene content of peach juice:

The major carotenoids, which are responsible for the colour of fresh, thermal and enzymatic chemical pretreatments of peach juices, were measured by reversed phase HPLC (Fig. 1). However, the major carotenoids in all juices were lutein, b-cryptoxanthin and zeaxanthin.

β- carotene or Total carotenoid content was 1.31± 0.44 mg/100ml juice (means ± standard deviation, n = 3) for fresh peach juices. In water and steam blanching juices, total pigment content decreased to 0.98 ± 0.44 and 1.11± 0.61 mg/100ml juice, which is about 25 and 15% loss respectively, and in enzymatic + So_2 juices, total content decreased to 1.16± 0.52 mg/100ml juice, which is about 11% loss. However, these losses were not significant. However, thermal pretreatments resulted in loss of β- carotene more than enzymatic-S02 pretreatments of peach fruit. Theses results were inagreement with the results of Athanasios *et al.*, 2008 who investigated the effect of short-term heat treatment on quality of fresh-cut peach. However, results also showed that the heat treatment increased the total carotenoids loss and reduced the chroma values of the peach slices.

The total carotenoids content of control was 1.31 mg/100ml juice, (Fig. 1), which is higher than those of all pretreatments of peach fruit juices. In addition to the enzymatic-S02 pretreatments of peach fruit resulted to produce peach juice very nearly to fresh juice. In addition, some researchers have shown increases in extractable carotenoids as a result of enzymatic

pretreatment of prickly peach juice (Eissa & Salama, 2002). The enhancing effect on extraction of carotenoids by enzymes macerating processing is attributed by affecting the membranes in fruit cells, whereas carotenoids are tightly bound to macromolecules, in particular to protein and membrane lipids, and high pressure processing is known to affect macromolecular structures such as proteins and polymer carbohydrates (Cassia *et al.*, 1995, and Butz & Tauscher, 2002).

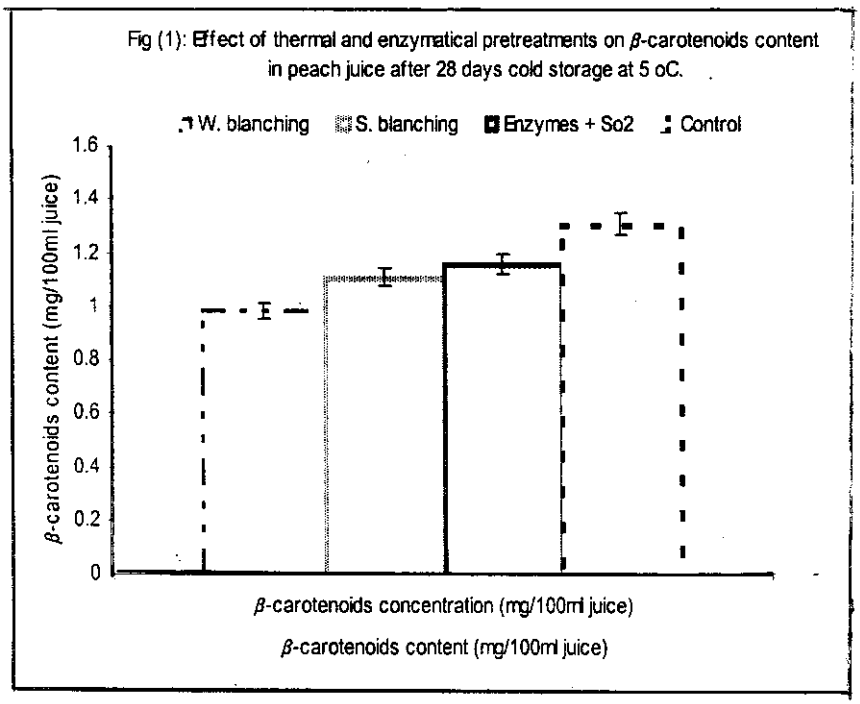

Fig (1): Effect of thermal and enzymatical pretreatments on β-carotenoids content in peach juice after 28 days cold storage at 5 oC.

Generally, total caroteniods increased with the addition of pectinase and cellulase enzymes. Whereas, these enzymes aid in release of pigments from plant cell. The increase in total caroteniods was observed as a result of pectinase enzyme, while cellulase addition had little effect on caroteniods release. The results showed that the average decrease in total caroteniod content of pectinase-So_2 treated peach fruit juice was about 11% and 15-25 loss respectively as compared to the water and steam blanching of fruit juice, as seen in fig. 1. Theses results agreed with the results of Cassia *et al.*, (1995), Tung-Sun *et al.*, (1995) and Eissa and Salama (2002).

Color parameters are influenced by the presence and quantities of different carotenoids in juices analyzed and correlations between color and carotenoids have been observed. The β- carotene had a linear relationship ($p < 0.05$) with a* (positive) and L* (negative). Similarly, the β- carotene had a statistically significant ($p < 0.05$) linear relationship with a* (positive), b* (positive) and L* (negative), as shown in fig.1 and table 2.

Effect of thermal and enzymatical pretreatments on microbial counts of peach juice during cold storage (28 days) (5 °C):

Effects of thermal and enzymatical pretreatments processing on the microbiological properties of peach juices are shown in Table (3). The control proved to be naturally contaminated by the microorganisms normally occurring in peach juice, at the same levels as those found in this product. The thermal pretreatment processing and enzymatical pretreatments processing both resulted in most efficient inactivation of all the microorganisms to a level below the detection limit in this study. These two pretreatments products appeared stable during storage at 5 °C for at least 28 days.

However, the enzymatical pretreatments could efficiently inactivate yeasts and molds, the both microorganisms still germinated or grew after storage. Some researchers have proved that enzymatical pretreatments already yielded a microbially stable peach juice product regardless of pH values of 4.0–5.0. Enzymatical pretreatments resulted in inactivation of natural flora to a level below the detection limit in peach juice even after storage at 5 °C for 4 weeks, however, that up to water and steam blanching decreased of natural peach juice (Hsu Kuo-Chiang *et al.*, 2008). We suggested that the different effects of thermal and enzymatical pretreatments processing processing on inactivation of microorganisms were mainly attributed by the various peach juice products. In this study, thermal and enzymatical pretreatments processing was sufficient to produce microbially stable peach juices for refrigerated storage at least for 28 days.

The microbial stability of peach juice treated with water, steam and enzymatic SO_2 during storage at refrigerator temperature (5 °C) for 28 days was investigated. The ability of various pretreatments to control the endogenous micro flora in peach juice can be shown in Table (3).

The results showed that the Clostridium, *Staph. Aureus, E. coli*, Coliform group Counts and *Bacillus cereus* (B.C) were not found in untreated and treated peach juice during storage at refrigerator temperature (5 °C). These results are partially confirmed by those of (FAO / WHO 2003; Janisiewicz 2006; Doyle, 2007). Enzyme + So_2 was effective on the bacteria, yeast and molds populations whilst water blanching and steam blanching pre-treated caused weaker activity in inhibition of microbial counts. Results obtained showed that the water and steam blanching produced a peach juice product with unacceptable taste, while the Enzyme + So_2 produced a peach juice with acceptable taste.

The reduction in bacteria, yeast and molds counts was high by using of enzyme + So_2 pre-treated. For the purpose of maintaining an acceptable degree of freshness or sensory value and reduction in microbial counts enzyme + So_2 pre-treated were used.

Total microbial counts of different peach juices treatments with water, steam, enzyme + So_2 and of untreated peach juices were followed up through 28 days at 5 °C. The effect of treating peach juices with the studied various pretreatments and stored at 5 °C for 28 days on inhibiting the microbial counts are shown in Tables (3). It can be observed that the peach juices treated with enzyme + So_2 have the highest inhibition of yeast & molds and

other bacteria followed by those treated with water and steam blanching after 28 days at 5 °C. Results show that the untreated peach juices were $90x10^2$ CFU/ml of bacteria compared to 3x10 CFU/ml in case of those yeast & molds. Whereas, the peach juices treated with enzyme + So_2 were 0 CFU/ml of yeast & molds and 2x10 CFU/ml of bacteria.

The results from Table 3 showed that the peach juices treated with enzyme + So_2 have also the highest reduction of yeast & molds and bacteria followed by steam and water blanching, but untreated samples showed the lowest reduction of yeast & molds and bacteria for 28 days stored at 5 °C. These results are partially confirmed by those of (Kniel *et al.*, 2003; Patricia *et al.*, 2004; Janisiewicz 2006).

Table (3): Effect of thermal and enzymatical pretreatments on pathogenic and total viable bacteria count (TVBC) in peach juice during cold storage (28 days) at (5 °C).

Peach juice Samples	Y&M	SFC	TVBC	*Clostridium*	B.C	*Staph. aureus*	*E. Coli*	Coli form group Count
Zero time								
C	3x10	$60x10^2$	$90x10^2$	Nil	Nil	Nil	Negative	Nil
T_1	Nil	$40x10^2$	$50x10^3$	Nil	Nil	Nil	Negative	Nil
T_2	Nil	$40x10^2$	$10x10^4$	Nil	Nil	Nil	Negative	Nil
T_3	Nil	Nil	2x10	Nil	Nil	Nil	Negative	Nil
7 days storage at 5 ° C								
C	6x10	$50x10^2$	$50x10^4$	Nil	Nil	Nil	Negative	Nil
T_1	Nil	$80x10^2$	$60x10^4$	Nil	Nil	Nil	Negative	Nil
T_2	Nil	$30x10^2$	$12x10^5$	Nil	Nil	Nil	Negative	Nil
T_3	Nil	Nil	3x10	Nil	Nil	Nil	Negative	Nil
15 days storage at 5 ° C								
C	9x10	5x10	$15x10^2$	Nil	Nil	Nil	Negative	Nil
T_1	Nil	$50x10^2$	$30x10^4$	Nil	Nil	Nil	Negative	Nil
T_2	Nil	5x10	$9x10^2$	Nil	Nil	Nil	Negative	Nil
T_3	Nil	Nil	3x10	Nil	Nil	Nil	Negative	Nil
24 days storage at 5 ° C								
C	5x10	$5x10^2$	$10x10^2$	Nil	Nil	Nil	Negative	Nil
T_1	Nil	$10x10^2$	$20x10^4$	Nil	Nil	Nil	Negative	Nil
T_2	Nil	4x10	30x10	Nil	Nil	Nil	Negative	Nil
T_3	Nil	Nil	10	Nil	Nil	Nil	Negative	Nil
28 days storage at 5 ° C								
C	10	$3x10^2$	$10x10^2$	Nil	Nil	Nil	Negative	Nil
T_1	Nil	1x10	$11x10^3$	Nil	Nil	Nil	Negative	Nil
T_2	Nil	1x10	$5x10^2$	Nil	Nil	Nil	Negative	Nil
T_3	Nil	Nil	Nil	Nil	Nil	Nil	Negative	Nil

Control (C), Water blanching (T_1), Steam blanching (T_2) and Enzyme + So_2 (T_3)

On the other hand molds and yeasts were not counted after 28 days of storage period at refrigerator temperature (5 °C) with steam, water and enzyme + So_2 pretreatments of peach juice. Also, results from Tables (3) show that refrigeration temperature 5 °C of peach juice could enhance the inhibitory effect of enzyme + So_2 pretreatments.

These results nearly in consistent with results given by Ting and Deibel, 1992 who appeared that refrigeration temperature (5 °C) could enhance the inhibitory effect of pretreatments. These results are partially

confirmed by those of Levi *et al.*, (1988); Barbanti *et al.*, (1992); Stratford *et al.*, (2000). Results in table (3) show that the peach juices treated with enzyme + So_2 had also the highest reduction of spore formers count (SFC) followed by steam and water blanching, but untreated samples were the lowest reduction of SFS for 28 days stored at 5 °C. For example, results show that the untreated peach juice was $60x10^2$ CFU/ml of SFC compared to $40x10^2$ CFU/ml in case of that water and steam blanching. Whereas, the peach juices treated with enzyme + So_2 was 0 CFU/ml of SFC, as shown in table (3). These results are partially confirmed by those of Parish (2000).

In general, the refrigeration of peach juices effects increased the inhibition of microorganism's count. Also, the results showed that the peach juices treated with enzyme + So_2 were non-browning and with the lowest microorganisms count during storage at 5 °C for 28 days.

Effect of thermal and enzymatical pretreatments on the sensory evaluations of peach juice during cold storage (28 days) (5 °C).

The results of sensory evaluation of the refrigerated peach juice based on color, taste, odor and appearance are shown in Table (4). It is clear that the refrigerated peach juice pretreated with enzymatical treatment gave higher mean panel scores (7.78 - 9.22A) than the other refrigerated samples of peach juice or were the most preferred in all the studied characteristics (color, odor and appearance) after 28 days storage at 5 °C, but steam pretreatment was higher mean panel score (8.11^{A}) in taste characteristic.

Table (4): Effect of thermal and enzymatical pretreatments on sensory evaluation of peach juice after 28 days storage at (5 °C).

Juice samples	Color	Taste	Odour	Appeachance
Peach juice control	5.00^{C}	7.00^{AB}	7.00^{A}	5.67^{C}
Water blanching	5.78^{BC}	6.11^{B}	6.78^{A}	6.22^{BC}
Steam blanching	7.00^{B}	8.11^{A}	6.89^{A}	7.44^{AB}
Enzyme + So_2	9.22^{A}	7.56^{AB}	7.78^{A}	8.44^{A}
LSD (P>0.05)	1.55	1.63	1.70	1.57

Least Significant Differences (P>0.05)
Mean values in each column having different superscript (A.B.C.AB.BC) are significantly different at P > 0.05 level.

After 28 days storage, there was a significant difference (P<0.05) between these samples. There were no odor or flavor changes after 28 days storage at 5 °C. These results are confirmed by those results of Crandall *et al.*, (1986); Levi *et al.*, (1988); Barbanti *et al.*, (1992).

On the contrary, the water blanched and control samples gave a lower panel score than the steam and enzymatical samples after 28 days storage at 5 °C. These results are confirmed by those results of Monsalve *et al.*, (1995). They reported that the apple juice preserved by combined methods and refrigeration (5 °C) was, in essence, similar to minimally processed apple slices products. Hence, organoleptic characteristics such as flavour and color were similar to the fresh counterparts without compromising the wholesome characteristics e.g. microbial safety of the apple juice.

Results obtained showed that the water and steam blanching produced a peach juice product with unacceptable taste, while the Enzyme + So_2

produced a peach juice acceptable taste and caused a marked (Siegmund and Pollinger-Zierler 2006).

CONCLUSION

In conclusion, among the two commercial enzymes pectinase and cellulase investigated improved overall quality of peach juice, pectinase with So_2 addition resulted in a more stable color, higher caroteniods contents, better flavor, and sediment free, clear juice compared to thermal (water and steam blanching) pre- treatments. Losses in carotenoids due to thermal pretreatments were more than enzymatic + $S0_2$ pretreatments of peach fruit. The results showed that the Clostridium, *Staph. Aureus, E. coli*, Coliform group Counts and *Bacillus cereus* (B.C) were not found in untreated and treated peach juice during storage at refrigerator temperature (5 °C). In general, the results showed that enzyme + So_2 with refrigeration at 5 °C are considered to be a potentially useful tool for extending the shelf life of fresh juice, preserving its fresh taste and appearance than other methods, in controlling both inactivation of enzymatic browning in peach juice and microorganisms, respectively.

It is concluded that the technique used produced good results, the product showed good stability during processing with respect to microbiological, physical, chemical and sensory properties. The values found in our work were higher because we saponified samples and quantified using standard curves. The results obtained were interesting because the losses of carotenoids with important functions for human health were not significant.

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تأثير المعاملات الأولية الحرارية و انزيمات النقع المحللة على جودة عصير الخوخ المبرد

هشام أمين على عيسى* ، وفاء حسين امام* و جمال ابراهيم وهاب**

*** قسم الصناعات الغذائية – المركز القومى للبحوث ١٢٦٢٢ – القاهرة – مصر**

**** قسم الألبان – المركز القومى للبحوث ١٢٦٢٢– القاهرة – مصر**

فى هذا البحث تم فحص الخصائص اللونية L*, a*, b*, DE و محتوى صبغة البيتا كاروتين و الأحياء الدقيقة و الخصائص الحسية لتقدير جودة عصير الخوخ المعامل بالمعاملات الأولية الحرارية و انزيمات النقع المحللة اثناء التخزين بالثلاجة على درجة حرارة (٥°م).

نتجة التحليل الاحصائى للنتائج المتحصل عليها أظهرت ان تأثير تأثير المعاملات الحرارية و الانزيمية على فقد محتوى صبغة البيتا كاروتين كان واضحا ومعنويا و خاصة المعاملات الحرارية. فالسلق بالماء لثمار الخوخ خفض محتوى صبغة البيتا كاروتين الى ٢٥% و الى ١٥% بالسلق بالبخار.

و كذلك الخصائص اللونية L*, a*, b*, DE تأثرت بوجود و كمية محتوى صبغة البيتا كاروتين فى عصير الخوخ و حيث وجدت علاقة قوية بين اللون و محتوى صبغة البيتا كاروتين.

حيث اتضح أن محتوى صبغة البيتا كاروتين له علاقة ايجابية واضحة مع قيم a اللونية فى حين له علاقة اخرى سالبية مع قيم L اللونية.

كما اتضح أن بكتريا

Clostridium, *Staph. Aureus, E. coli*, Coliform group Counts and *Bacillusc cereus*

كانت غير موجودة فى عصير الخوخ الغير معامل و المعامل أثناء التخزين و أن عصير الخوخ المبرد و المعامل بالمعاملة الأولية الانزيمية كان الأعلى انخفاضا للعد البكتيرى و الخميرة و الفطر تلاه المعاملة الأولية بالسلق بالبخار و بالماء وان عينات العصير الغير معامل (الكونترول) كانت أقل انخفاضا للعد البكتيرى و الخميرة و الفطر أثناء ال ٢٨ يوم تخزين بالثلاجة.

كما أوضحت النتائج أن عصير الخوخ المبرد و المعامل بالمعاملة الأولية الانزيمية أعطت أعلى قيمة تقييم حسي (A9.22 - 7.78) عن العينات المبردة و المعاملات الأخرى و أن عصير الخوخ المبرد و المعامل بالمعاملة الأولية الانزيمية أعطى أفضل خصائص حسية (اللون و الرائحة و الشكل العام) لكن المعاملة الأولية بالبخار كانت أعلى قيمة تقييم حسي (A 8.11) فى خاصية الطعم.