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Effect of enzymatic treatment on carrot cell wall for increased juice yield and effect on physicochemical parameters

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Combination of crude pectolytic and cellulolytic enzymes (both prepared from *Aspergillus foetidus*) was used as pretreatment for extraction of carrot juice since pectinases and cellulases contribute to the breakdown of pectin and cellulose, respectively, hence enhance the juice yield and clarity. Juice yield was increased by 11.38% due to enzyme action. Juice samples along with control were stored at room (30 ± 5°C) and refrigeration temperature (5 ± 1°C) for 3 months. Evaluation of physicochemical and sensory changes was done on 0, 15, 30, 45, 60, 90th day of storage. Initially, due to enzymatic treatment, acidity, total soluble solids and β-carotene content was significantly higher as compared to control, whereas pH, viscosity and pectin content for enzymatically treated juice samples was lower than that of the control samples. β-Carotene, pectin content and total acidity decreased, whereas pH and reducing sugar content increased during storage. Colour parameters L- and a- decreased while b- increased slightly which resulted in increased browning index (BI) and total colour difference. Zero- and first-order kinetic models was used to describe the L-, a-, b-value, BI and β- carotene at both temperatures. Sensory analysis revealed that enzymatically treated juice was more acceptable at refrigeration temperature (5 ± 1°C) for 3 months.

Key words: Carrot juice, enzyme pretreatment, storage, physicochemical properties, sensory evaluation.

INTRODUCTION

Carrot (*Daucus carota* Sativus) is one of the most important seasonal root vegetable of *Apiaceae (Umbelliferae)* family, grown extensively in India during winter season. It is an excellent source of β-carotene, a precursor of vitamin A, which protects cells from free radicals which may damage the basic cell structure of healthy cells (Demir et al., 2004; Yoon et al., 2005). Carrot is rich in antioxidants like α-carotene, β-carotene, phytochemicals, glutathion, calcium, phosphorus and consumed as versatile vegetable with excellent source of calcium pectate, an extraordinary pectin fiber that has the cholesterol lowering properties (Kaur et al., 2012). Carrot juice has the therapeutic property which improves the boosting of immunity, helps to heal minor wounds, injuries, reduce the risk of heart disease and blood pressure. It cleans the liver by excreting fats and bile, helps to fight anaemia, improves eye health, reduces the risk of high blood pressure, stroke, heart disease and some types of cancer (Wrolstad, 2004; Bahkru, 1993). Because of all these properties, it is considered as health drink. Several researchers have worked on the extraction of carrot juice and the storage stability (Sharma et al.,

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2009; Chadha et al., 2003).

Enzymatic treatment alone or in combination with others, is one of the potential pretreatment, which results in increased yield with better juice quality, colour and acceptability. Khandare et al. (2011) reported that enzyme-assisted black carrot juice processing significantly ($p < 0.05$) improved the antioxidant composition of black carrot juice. According to this study, there was an overall increase of 33% in juice yield, 27% in total phenolics and 46% in total flavonoids. Bahrmian et al. (2011) used pectinase and cellulose enzymes for sugar extraction process from date fruits. About 18% increase in the amount of extracted sugars was reported in the case of pretreatment of fruits by each of these two enzymes equally, while using a combination of two enzymes in proper ration and a suitable condition resulted in a further increase of sugar to about 46%, in relation to untreated samples. Kaur et al. (2009) optimized the conditions for clarification of guava fruit juice using commercial pectinase enzyme. Optimum conditions reported were: incubation temperature 45.35°C, incubation time 7.23 h and enzyme concentration of 0.70 mg/100 g guava pulp with ascorbic acid 77.71 mg/100 g, clarity 34.54% transmittance, viscosity 1.24 cps and color (L value) 23.33. Sharma et al. (2005) optimized the enzymatic process parameters for increased juice yield from carrot. In this study, it was reported that enzymatic treatment resulted in increase in juice yield by 13.95% and decrease in viscosity by 0.45 cP. Singh et al. (2013) reported that bael fruit juice yield, viscosity, and clarity are functions of enzymatic hydrolysis conditions. The usage of either crude or commercial enzymes significantly enhanced juice yield and clarity as compared to the control. Several researchers have reported enhanced juice yield by use of enzymes (Khandare et al., 2011; Wang et al., 2009; Sun and Tang, 2007).

Colour is an important attribute in food products, since it is perceived immediately by the consumer. Wavelength coming from the surface of the object falls on the retina of the eyes which determines the colour of the object (Tijskens et al., 2001). Therefore, angle of view, light, source of light etc are the important factors affecting the colour of object (Giese, 2000). Carrot juice is rich in color due to the pigments. The change in colour of juice during storage may be due to degradation of β-carotene which may be due to presence of air in the head space, because β-carotene reacts with air present in head space resulting in loss of carotene and color of the juice during storage (Demir et al., 2004). Other factors affecting colour of carrot juice during storage are pH, acidity, processing temperature, duration and fruit cultivar.

Kinetic modeling is necessary to derive basic information for a system in order to describe the reaction rate as a function of experimental variables and to predict changes in a particular food during processing and storage (Mohammadi et al., 2008). Studies related to the enzymic extraction of carrot juice have been reported in literature (Sharma et al., 2005; Chadha et al., 2003) but work relating to the effect of enzymic treatment on physicochemical, microbial and sensory quality of carrot juice and kinetic study related to storage of enzymatically treated carrot juice has not been studied so far. Therefore, the present study was undertaken to study the effect of enzymic treatment on physicochemical parameters (β-carotene, reducing sugar, pectin, pH, acidity, specific gravity, color, total solids, total soluble solids andviscosity) and sensory parameters (color, flavour and overall acceptability) of carrot juice and to study the kinetics of degradation of β-carotene and colour during storage.

MATERIALS AND METHODS

Fresh carrots (variety: *'Local red'*) were procured from the local market, Longowal, Punjab (India). The fungal strains *Aspergillus foetidus* (MTCC-151) and *Trichoderma reesei* (MTCC-*164) were obtained from Institute of Microbial Technology (IMTech), Chandigarh. All the chemicals used in the study were of AR grade.

Preparation of enzymes and activity assay

Foundation cultures of *A. foetidus* (MTCC 151) and *T. ressei* (MTCC *164) were multiplied in recommended growth medium, that is, Czapek Yeast Extract Agar-CYA medium and Streptomyces medium, respectively. Then, the working culture was prepared in Potato Dextrose Agar (PDA) medium in slants. It was incubated at 30 and 25°C, for *A. foetidus* and *T. ressei*, respectively, for 3 days for further multiplication of the source microorganism (IMTech, 2000).

The pectin rich medium was used to prepare the crude pectolytic enzyme. An inoculum of *A. foetidus* was added to the medium and incubated in shake flask at 45°C, 110 rpm for 7 to 8 days. Crude enzyme was obtained as supernatant after filtration and centrifugation (Chadha et al., 2003). For preparation of cellulolytic enzyme, the Yeast Phosphate Soluble Starch (YPSS) medium was inoculated with *T. resesi* picked from a well-grown working culture in slants. Incubation was done at 45°C at 110 rpm for 3 days. Finally, for the preparation of enzyme from the source microorganism, the three days old culture grown on YPSS was inoculated onto wheat bran medium. Incubation was done at 45°C and 110 rpm in an incubator shaker for 7 days. Then, filtration was done followed by centrifugation at 10000 rpm, 10°C for 10 min. Supernatant was obtained as crude cellulolytic enzyme (Chadha et al., 2003).

Protein content (mg/mL) was determined with Folin-phenol reagent. The phenolic group of tyrosine and trytophan residues (amino acid) in a protein produces a blue purple color complex with Folin-Ciocalteau reagent which consists of sodium tungstate molybdate and phosphate. Thus, the intensity of color depends on the amount of these aromatic amino acids present and thus varies for different proteins. Bovine Serum Albumin (BSA) was used as a standard protein following the method of Lowry et al. (1951). Cellulase activity was assayed as per the method given by Mandels et al. (1976) and pectinase activity was determined using pectin as substrate (Phutela et al., 2005).

Enzymatic juice extraction

The carrots were washed under running tap water, trimmed using

stainless steel vegetable knife and peeled by using peeler to remove dirty skin and undesirable hair. Then grating was done (thickness ~2 mm and width ~3 to 4 mm) using a hand grater. The grated carrots were immediately blanched. Enzymatic extraction of carrot juice using juice mixer grinder was carried out as per the process given by Sarkar and Sharma (2010) under the optimum conditions: enzyme concentration, 210.7 mg/kg of grated carrot; pectolytic and cellulolytic enzyme ratio, 3.84:6.16; incubation time, 130 min and incubation temperature 47°C (Sharma et al., 2005). For control samples, all the steps followed were the same, except enzymatic treatment. Juice samples were filtered followed by addition of sugar (3%) and preservative (200 ppm). Then, it was filled into bottles and immediately pasteurized at 80°C for 25 min. Processed juice samples were stored at room temperature (30 ± 5°C) and at refrigeration temperature (5 ± 1°C) and microbial changes were evaluated on 0 and 90th day of storage, whereas physicochemical and sensory changes were evaluated on 0, 15, 30, 45, 60, 90th day of storage. Samples in triplicates were used for each treatment.

Physicochemical and sensory parameters

Juice was subjected to various physicochemical parameters such as acidity, total solids, total soluble solids and color by standard methods (Ranganna, 1991). Acidity was expressed in terms of citric acid and determined by titration of a known amount of sample with 0.1 N NaOH using phenolphthalein as indicator. Total solids content was determined by hot air oven drying method, and total soluble solids content was determined by hand refractometer. Colour was measured by color spectrophotometer (Gretag Macbeth model i5 Regensdorf Switzerland) and expressed in terms of L, a and b. Pectin was estimated as calcium pectate by the standard method based on the saponification and precipitation by the addition of calcium chloride (Ranganna, 1991). pH (Digital pH meter, Thermo-Orion 720) and specific gravity (using specific gravity bottle) were evaluated by AOAC (1985) methods. β-Carotene content was estimated colorimetrically using β-carotene as standard (Edward and Lee, 1986) and reducing sugar was determined using DNSA by Miller's method (1959). Viscosity was measured using Ostwald viscometer (Pandey et al., 2004). Hedonic rating (Ranganna, 1991) was used to study the sensory characteristics of samples which relates to pleasurable or unpleasurable experiences. The tests were performed using 9-point hedonic scale, where 9 was like extremely and 1 was dislike extremely.

Colour values

The color values were expressed as L (whiteness/darkness), a (redness/greenness) and b (yellowness/blueness), respectively. And also, the total colour difference (Equation 1) and browning index (BI) were calculated from the Hunter L, a, b values and used to describe the colour change during storage (Maskan, 2001):

$$\Delta E = \sqrt{(L_0 - L)^2 + (a_0 - a)^2 + (b_0 - b)^2} \quad (1)$$

Where subscript "0" refers to the colour reading of juice initially, which was used as the reference and larger ΔE value denotes greater colour change from the reference material.

Statistical analysis

Correlation coefficient (Pearson's r) was calculated using MS Excel, 2007, and level of significance was determined at 99 and 95% level (Mendenhall et al., 1989). ANOVA (Data Analysis function, MS Excel 2007) was used to determine the effect of enzyme treatment on parameters of carrot juice at 99 and 95% level. Coefficient of determination (R^2) was used to represent the percent of the data that is closest to the line of best fit.

Zero order and first order reaction kinetic models were used for this study (Equations 2 and 3) as majority of workers reported changes in food by zero order and first order degradation kinetics (Pua et al., 2008; Maskan, 2001).

$$C = C_0 \pm k_0 t \quad (2)$$

$$C = C_0 \exp(\pm k_1 t) \quad (3)$$

Where, k_0 and k_1 are the reaction rate constants for the zero order and first order model respectively. C is the measured value of a parameter at time 't', whereas C_0 is the initial value of parameter.

RESULTS AND DISCUSSION

Carrot juice yield for enzyme assisted samples was found to be 71.26% whereas for control samples it was 59.88%. The juice yield increased by 11.38% due to enzyme action which is due to the action of pectolytic and callulolytic enzyme on carrot cell (containing juice) wall. Results are in agreement with the findings of Chadha et al. (2003).

β-Carotene and kinetics

Carrot gets its characteristic and bright orange colour from β-carotene, which is a precursor of vitamin A. Enzyme assisted carrot juice extraction helps to release phytochemicals thus helping in greater recovery of phenols and anthocyanins (Sowbhagya and Chitra, 2010). In the present study, the β-carotene content of enzymatically treated samples was found to be higher than that of control samples. β-Carotene content of enzyme treated samples was found to be 3.93 mg/100 mL, which initially decreased to 3.31 and 3.64 mg/100 mL at room and refrigeration temperature, respectively, whereas β-carotene content of control samples was found to be 3.88 mg/100 mL which initially decreased to 2.98 and 3.55 mg/100 mL at room and refrigeration temperature, respectively (Table 1). This may be due to action of enzymes which facilitate the extraction of β-carotene to a greater extent. Results are in agreement with the findings of Sims et al. (1993) and several other researchers. Khandare et al. (2011) also reported that enzymatic treatment of black carrot resulted in 30% increase in antioxidant activity, 27% increase in total phenolics, 46% in total flavonoids and 33% in overall juice yield. Sun and Tang (2007) worked on asparagus juice and reported a significant increase in the free radical scavenging activity of juice extracted with viscozyme. Results are also in agreement with the findings of Landbo and Meyer (2004). They worked on enzyme assisted processing of black currants and reported increase in antioxidant activity. Total antioxidant

Table 1. Changes in physicochemical parameters of enzyme treated carrot juice and control samples with storage time.

Quality parameter	Sample condition	Storage time (days)					
		0	15	30	45	60	90
β-Carotene (mg/100 mL)	ET (RT)	3.93±0.15	3.86±0.12	3.75±0.20	3.64±0.17	3.53±0.04	3.31±0.24
	C (RT)	3.88±0.08	3.71±0.07	3.62±0.18	3.57±0.03	3.31±0.15	2.98±0.27
	ET (Ref. T)	3.93±0.15	3.87±0.16	3.82±0.26	3.79±0.13	3.73±0.17	3.64±0.21
	C (Ref. T)	3.88±0.08	3.82±0.11	3.79±0.09	3.72±0.06	3.64±0.13	3.55±0.23
Pectin content (%)	ET (RT)	0.97±0.13	0.96±0.06	0.96±0.06	0.95±0.08	0.95±0.14	0.94±0.07
	C (RT)	1.01±0.09	0.99±0.05	0.99±0.03	0.98±0.10	0.98±0.08	0.98±0.11
	ET (Ref. T)	0.97±0.13	0.97±0.12	0.96±0.07	0.96±0.11	0.95±0.06	0.95±0.09
	C (Ref. T)	1.01±0.09	1.01±0.05	1.00±0.10	0.99±0.07	0.99±0.09	0.99±0.05
Reducing sugar (%)	ET (RT)	2.47±0.08	2.49±0.06	2.51±0.11	2.51±0.07	2.54±0.08	2.58±0.10
	C (RT)	2.43±0.05	2.50±0.09	2.50±0.11	2.54±0.13	2.56±0.08	2.62±0.07
	ET (Ref. T)	2.47±0.08	2.48±0.07	2.48±0.05	2.50±0.13	2.50±0.09	2.56±0.08
	C (Ref. T)	2.43±0.05	2.43±0.12	2.45±0.07	2.51±0.09	2.53±0.11	2.60±0.04
Total acidity (%, expressed as citric acid)	ET (RT)	0.32±0.00	0.30±0.00	0.29±0.00	0.29±0.00	0.28±0.02	0.28±0.00
	C (RT)	0.29±0.00	0.28±0.00	0.27±0.00	0.26±0.00	0.26±0.02	0.24±0.00
	ET (Ref. T)	0.32±0.00	0.32±0.00	0.31±0.00	0.31±0.00	0.3±0.001	0.30±0.00
	C (Ref. T)	0.29±0.00	0.28±0.00	0.27±0.01	0.26±0.00	0.25±0.00	0.25±0.00
pH	ET (RT)	4.47±0.00	4.47±0.05	4.49±0.01	4.52±0.00	4.52±0.00	4.55±0.01
	C (RT)	4.48±0.00	4.51±0.00	4.52±0.01	4.56±0.02	4.56±0.00	4.58±0.00
	ET (Ref. T)	4.47±0.00	4.47±0.00	4.49±0.02	4.49±0.00	4.50±0.01	4.53±0.01
	C (Ref.T)	4.48±0.00	4.49±0.00	4.52±0.00	4.52±0.01	4.53±0.00	4.54±0.00
Viscosity (cP)	ET (RT)	1.45±0.03	1.44±0.02	1.44±0.04	1.44±0.01	1.44±0.01	1.44±0.01
	C (RT)	1.77±0.01	1.77±0.06	1.75±0.01	1.75±0.04	1.75±0.01	1.75±0.02
	ET (Ref. T)	1.45±0.03	1.45±0.04	1.45±0.01	1.45±0.01	1.45±0.03	1.45±0.01
	C (Ref. T)	1.77±0.01	1.76±0.04	1.76±0.01	1.76±0.01	1.76±0.02	1.76±0.02

ET= Enzyme treatment; C = control; RT = room temperature; Ref. T= refrigeration temperature; room temperature = 30 ± 50°C; refrigeration temperature = 5±10°C.

activity of black carrot juice was found to be exceptionally higher than the corresponding values found in black cherry, red grapes, prune, pineapple and apple juice (Seeram et al., 2008).

The effect of enzymic treatment on β-carotene content was significant at 95 and 99% ($P<0.01$) level at room and refrigeration temperature, respectively. Effect of storage time on this parameter was found to be statistically significant ($P<0.01$) at both temperatures. The reduction in β-carotene content may be due to presence of air in the head space. β-Carotene reacts with air present in head space resulting in loss of carotene and hence color of the sample during storage. Demir et al. (2004) also reported similar results in the case of carrot juice produced with lactofermentation and acidification.

The results of regression analysis of β-carotene content from zero-order and first-order reaction kinetics for enzyme treated and control samples are presented in Table 2. This analysis indicated that both reaction kinetics models can be used adequately. The coefficient of determination (R^2) value varied from 0.96 to 0.99 for zero order and from 0.95 to 0.99 for first order kinetics (Table 2). Therefore, both models fitted well with the data of β-carotene at both temperatures. Figure 1a and b represents the correlation between the model predicted values and experimental values. Reaction rate constants for the zero and first order reactions were found to be lesser for the enzymatic treated and control samples at refrigeration temperature, indicating that the rate of degradation of β-carotene was slower at refrigeration temperature as compared to room temperature (Table 2).

Colour parameters and kinetics

Colour is an important parameter as it is associated with

Table 2. Non-linear regression analysis results of colour parameters from zero- and first-order reaction kinetics for enzyme treated carrot juice and control samples.

Sample condition	Parameter	Zero order model			First order model		
		K_0	C_o	R^2	K_1	C_0	R^2
ET (RT)	β-carotene	0.0070	3.95	0.99	0.0019	3.95	0.99
	L	0.0050	1.69	0.98	0.0034	1.70	0.99
	a	0.0144	4.97	0.93	0.0034	5.02	0.91
	b	0.0028	2.08	0.97	0.0013	2.08	0.96
	ΔE	0.0155	-0.06	0.96	0.0256	0.15	0.96
	BI	3.9132	351.42	0.97	0.0074	368.33	0.99
ET (Ref. T)	β-carotene	0.0032	3.92	0.99	0.0008	3.92	0.99
	L	0.0028	1.72	0.97	0.0018	1.72	0.97
	a	0.0036	4.85	0.95	0.0008	4.85	0.95
	b	0.0020	2.06	0.97	0.0009	2.06	0.97
	ΔE	0.0050	0.01	0.97	0.0217	0.07	0.82
	BI	1.4627	366.82	0.98	0.0034	369.25	0.98
C (RT)	β-carotene	0.0097	3.90	0.96	0.0029	3.92	0.95
	L	0.0050	1.65	0.99	0.0035	1.66	0.99
	a	0.0176	4.94	0.93	0.0045	5.01	0.90
	b	0.0031	2.14	0.91	0.0014	2.14	0.90
	ΔE	0.0186	-0.13	0.95	0.0296	0.13	0.96
	BI	5.6190	349.23	0.93	0.0092	382.52	0.98
C (Ref. T)	β-carotene	0.0038	3.88	0.98	0.0010	3.88	0.98
	L	0.0022	1.65	0.98	0.0014	1.65	0.99
	a	0.0040	4.77	0.98	0.0009	4.77	0.98
	b	0.0021	2.11	0.98	0.0009	2.11	0.98
	ΔE	0.0050	0.00	0.98	0.0214	0.07	0.90
	BI	1.4963	394.76	0.99	0.0032	397.38	0.99

ET = Enzyme treated; C = control; RT = room temperature; Ref. T = refrigeration temperature; L, a, b = colour parameters; ΔE = total colour difference; BI = browning index; β-carotene: mg/100 mL; room temperature: 30 ± 5°C; refrigeration temperature: 5±1°C.

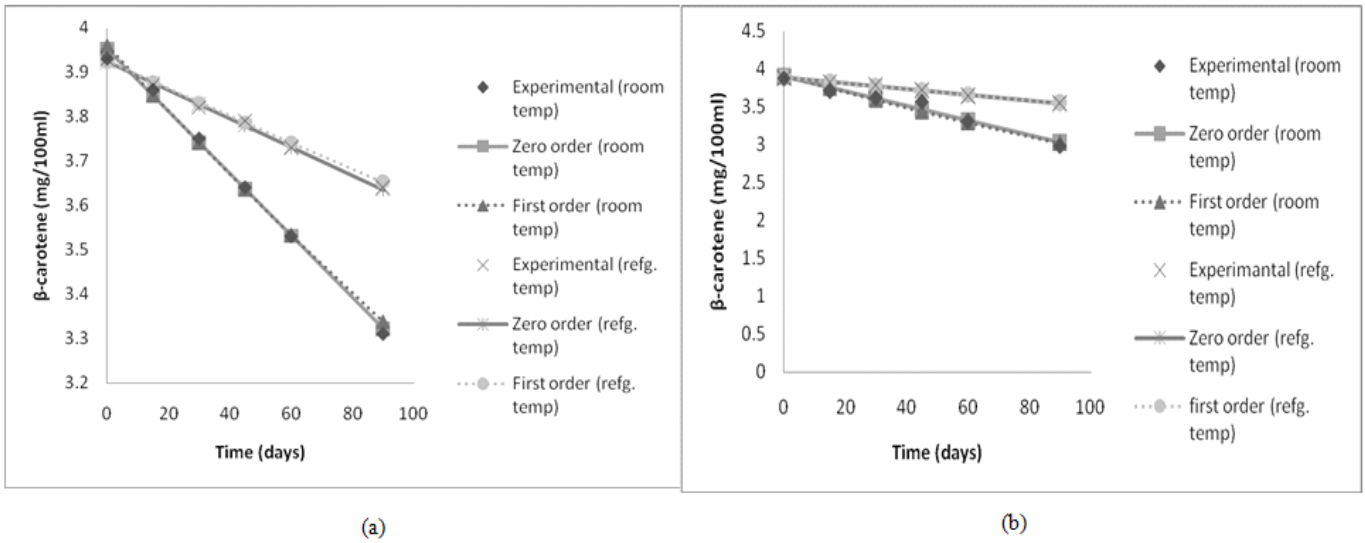

Figure 1. Kinetics of degradation of β-carotene as a function of time during storage for (a) enzymatically treated carrot juice samples and (b) control samples. refg temp: refrigeration temperature; room temp: room temperature.

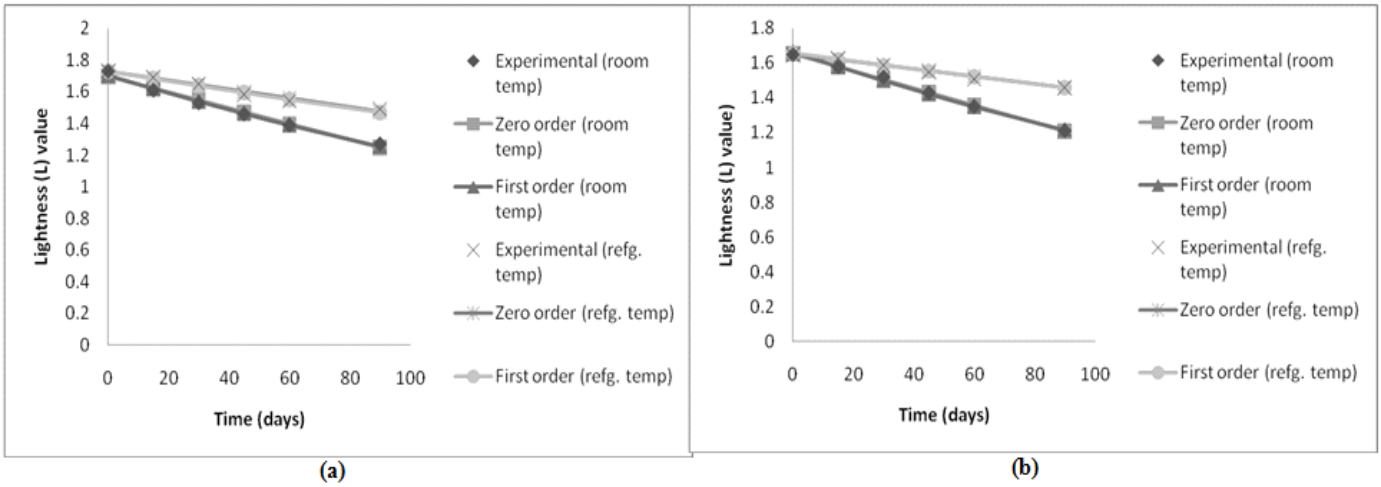

Figure 2. Kinetic changes in L- value as a function of time during storage for (a) Enzymatically treated carrot juice samples and (b) control samples. refg temp: refrigeration temperature; room temp: room temperature.

the β-carotene content of carrot juice. Colour measurement by standardized instrumental method corresponds to a visual assessment of the colour and is important for determination of conformity of food product quality. Colour measurement is also an indication of the changes taking place in a food product. Colour parameters were expressed as L-, a-, b-, BI and ΔE. L- indicates the lightness, and a- and b- are the chromaticity coordinates, BI (browning index) and ΔE (color change) were calculated from a- and b- values. The lightness value "L" of enzyme assisted carrot juice and control samples at refrigeration and room temperature is presented in Figure 2a and b. Initially, the lightness (L-) value of enzymatically treated samples was 1.73 which decreased to 1.27 at room temperature and 1.49 at refrigeration temperature at the end of storage period, whereas the L-value for control sample was 1.65 which decreased to 1.21 at room temperature and 1.46 at refrigeration temperature. The decrease in the lightness of juice samples can be taken as the colour degradation. Since it is a measure of the colour in the light-dark axis, these falling values indicated that the samples were turning darker. This may be because of the phenolic polymerization due to auto oxidation which is responsible for color loss in processed carrot products (Talcott et al., 1999). Chen et al. (1995) also reported that processing of carrot juice at higher temperature causes colour loss. ANOVA revealed the significant difference ($P<0.05$) in the L-value of enzymatically treated samples and control samples at both temperatures. Sims et al. (1993) also reported that enzymatic extraction improves the color of carrot juice.

The a-value of enzyme assisted as well as control samples decreased with storage time, which denoted decrease in redness value of juice samples during storage. This may also be correlated to β-carotene content, because β-carotene imparts red colour on carrots. Initially, the a-value of enzyme assisted carrot juice was 4.88 which decreased to 3.52 at room temperature and 4.57 at refrigeration temperature, whereas a-value of control sample was 4.79 initially, which decreased to 3.17 at room temperature and 4.44 at refrigeration temperature (Figure 3a and b). The b-value of enzymatically treated samples was found to be 2.07 initially, which increased to 2.32 at room temperature and 2.24 at refrigeration temperature, whereas it was 2.11 for control samples which increased to 2.38 at room temperature and 2.29 at refrigeration temperature (Figure 4a and b). The reduction in a-value and slight increase in b-value may be due to degradation of β-carotene and presence of air in the head space, because β-carotene reacts with air present in head space resulting in loss of carotene and hence red color of the sample during storage. These results are in agreement with the findings of Demir et al. (2004) in the case of carrot juice produced with lactofermentation and acidification. It has been reported that the color change of carrot juice during processing can be correlated with carotenoid content and the formation of *cis* isomers. Moreover, all-*trans*-*β*-carotene in carrot juice plays a more important role in color performance than *cis* isomers. During manufacture of carrot juice, processes such as peeling, blanching, pasteurization and storage, all-*trans*-*β*-carotene is partly converted into its *cis* isomers, because of the light or thermal treatment (Qin et al., 2005). Chen et al. (1996) also reported that *cis* isomers increase during processing leading to increase in yellowness of juice. Initially, the colour of enzymatically extracted juice was better than control. This may be due to action of enzymes which facilitate the extraction of β-carotene to a greater extent (Sims et al., 1993). Analysis of variance revealed that the effect of enzymatic treatment on a- and b-value was

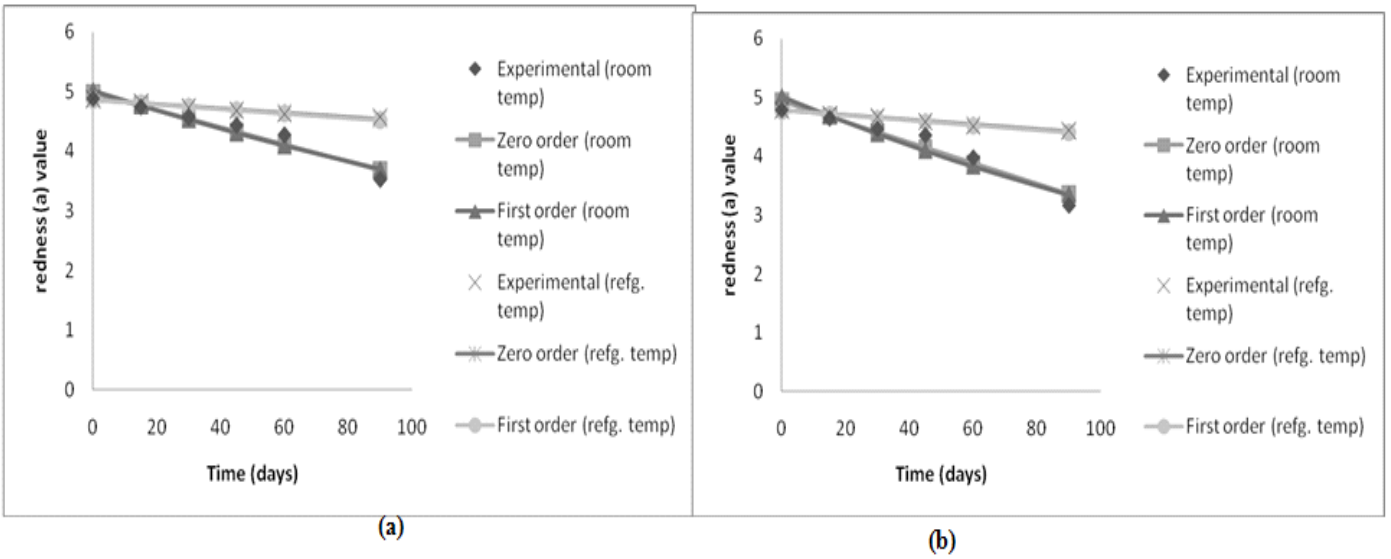

Figure 3. Kinetic changes in a- value as a function of time during storage for (a) Enzymatically treated carrot juice samples and (b) control samples. refg temp: refrigeration temperature; room temp: room temperature.

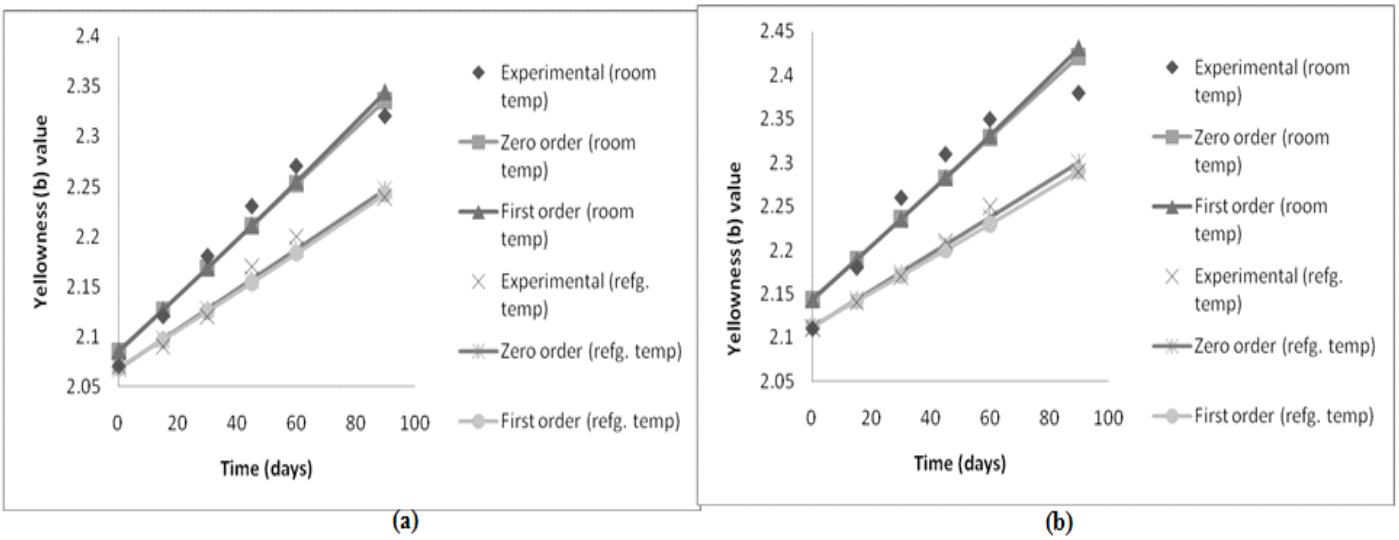

Figure 4. Kinetic changes in b- value as a function of time during storage for (a) Enzymatically treated carrot juice samples and (b) control samples. refg temp: refrigeration temperature; room temp: room temperature.

significant ($P<0.05$) at both temperatures.

Experimental data for change in parameters L-, a-, b-values, ΔE and BI were fitted to different kinetic models. Non linear regression analysis was applied for the kinetic equations of zero (Equation 2) and first-order (Equation 3). Table 2 shows the kinetic parameters obtained for these fittings. The results revealed that regardless of the enzyme treatment, both the zero-order and first-order reaction kinetic models can be used adequately for L-, a-, b- value, and BI values with R^2 values from 0.90 to 0.99 (Table 2). Figures 2 to 4 represents the correlation between the model predicted values and experimental values for L-, a- and b-values. However, the first order kinetic model has been reported better for L and b values of tomato paste (Barreiro et al., 1997), peach puree (Garza et al., 1999) and pear puree (Ibarz et al., 1999).

The total colour difference (ΔE) was calculated using Equation 1. It is a colorimetric parameter extensively used to characterize the variation of colours during processing. It is a combination of parameters L-, a- and b-values. An increase in ΔE for both samples at both temperatures was observed. For enzymatically treated

samples, it increased to 1.45 at room temperature and 0.42 at refrigeration temperature, whereas for control samples, it increased to 1.7 at room temperature and 0.43 at refrigeration temperature. Zero-order model fitted well with the data of ΔE for enzymatically treated sample at refrigeration temperature (R^2: 0.97) whereas both reaction kinetics models fitted well with the data of ΔE of other samples (R^2: 0.90, 0.98) as shown in Table 2. Similar zero-order reaction has been reported by Rahim et al. (1989) in the study of kinetics of colour change in grape juice.

BI represents the purity of brown colour and reported as an important parameter in processes where enzymatic and non-enzymatic browning takes place (Palou et al., 1999). The browning index for enzyme assisted carrot juice samples was found to be 370.66 initially, which increased to 730.28 at room temperature and 494.89 at refrigeration temperature, whereas it was 400.46 for control samples initially and increased to 915.95 at room temperature and 529.01 at refrigeration temperature.

The kinetic rate constants for the colour change were observed to have different values for enzymically treated and control samples at both temperatures (Table 2). It is clear from Table 2 that rate of degradation of colour parameters L-, a- and b- was slower at refrigeration temperature as compared to room temperature for enzymatically treated as well as control samples. Zero-order kinetic rate constants for variation of L-value for enzyme treated and control samples were found to be 0.0050 at room temperature and 0.0028 at refrigeration temperature, respectively.

For first-order reaction kinetics model, the kinetic rate constants for L-value were found to be 0.0034 and 0.0018 for enzyme treated sample and 0.0035 and 0.0014 for control at room temperature and refrigeration temperature, respectively. Similarly, as shown in Table 2, it is clear that the rate of degradation of L- and a- values at refrigeration temperature was slower than at room temperature and also, the rate of increase of total color difference and browning index was slower at refrigeration temperature as compared to room temperature for both models. This may be due to the fact that with increase in temperature, rate of conversion of *trans-β*-carotene into its *cis* isomers is higher (Qin et al., 2005). Chen et al. (1996) also reported that processing of carrot juice at higher temperature causes colour loss.

Physicochemical and sensory parameters

The pectin content of enzymatically treated samples was lower than that of control samples (Table 1) and effect of enzyme treatment was found to be significant (P<0.01) at both temperatures during storage. This may be due to breakdown of pectin by action of pectinase enzyme (Torres et al., 2005). Effect of time was also found statistically significant (P<0.01) at both temperatures.

Tapre and Jain (2012) studied the effect of pectinase on banana for clarification of banana pulp. They reported that the use of enzyme resulted in 49.28% juice yield and 62.31% clarity, which is mainly due to breakdown of pectin by pectinase enzyme. The decrease in pectin content with storage time has also been reported by Wang et al. (1995).

Initially, the reducing sugar content in the case of enzymatically treated samples was slightly more than that of the control samples (Table 1). This may be due to action of cellulolytic enzyme which causes conversion of cellulose to glucose (Mandels et al., 1976). But effect of enzymatic treatment was found to be statistically non significant (P>0.05) during storage period. Reducing sugar content increased all the samples at both temperature conditions during storage (Table 1). Reducing sugar content was found to be positively correlated with storage time at 99% level (α = 0.01, n = 6) for all the samples. Variation was attributed to the hydrolysis of sucrose leading to the formation of reducing sugar. Bawa and Saini (1987) also reported similar behaviour in the case of carrot juice (without enzymatic treatment).

Acidity of enzyme assisted carrot juice was higher than that of the control samples, whereas pH was lower than that of the control samples (Table 1). This may be due to the action of pectolytic enzyme on carrot cell wall constituents consisting of protopectin and pectin and thereby increasing acidity of the product and decreasing the pH (Sharma et al., 2005; Chadha et al., 2003). Effect of enzyme treatment on acidity and pH was found to be statistically significant (P<0.01) at both temperature conditions. Acidity decreased with storage time and effect of storage time was found to be significant at 99 (P<0.01) and 95% (P<0.05) level for samples stored at room and refrigeration temperature, respectively. The correlation between pH values and storage time was statistically significant at 99% (P<0.01) level (α=0.01, n=6) for all the samples. Bawa and Saini (1987) also reported significant decrease in acids with storage time of untreated carrot juice.

Viscosity of enzymatically treated sample was slightly lesser than that of the the control sample initially (Table 1). This may be due to action of pectolytic enzyme on carrot cell (containing juice) wall consisting of protopectin and pectin (Sharma et al., 2005). Effect of enzymatic treatment on viscosity was found to be statistically significant (P<0.01) and effect of storage time was found to be non significant (P>0.05) at both temperature conditions. The correlation coefficient between viscosity and storage time was also found to be statistically non significant ($r_{cal}<r_{crit}$, α = 0.05, n = 6). Grewal and Jain (1987) also reported that there was no change in viscosity during storage of skim milk carrot juice beverage at room temperature.

Specific gravity of enzyme assisted carrot juice and control sample was 1.03 and 1.04, respectively, and total

Table 3. Changes in sensory properties of enzyme treated carrot juice and control samples with storage time.

Quality parameter	Sample condition	Storage time (days)					
		0	15	30	45	60	90
VC	ET (RT)	8.30±0.48	8.20±0.42	7.90±0.31	7.80±0.42	7.60±0.51	7.50±0.52
	C (RT)	8.10±0.31	8.00±0.00	7.80±0.42	7.70±0.48	7.50±0.52	7.40±0.51
	ET (Ref. T)	8.30±0.48	8.30±0.48	8.20±0.42	7.90±0.31	7.90±0.31	7.80±0.42
	C (Ref. T)	8.10±0.31	8.10±0.31	7.90±0.31	7.70±0.48	7.70±0.48	7.70±0.48
F	ET (RT)	8.00±0.00	7.90±0.31	7.70±0.48	7.60±0.51	7.40±0.51	7.20±0.42
	C (RT)	7.90±0.31	7.70±0.48	7.50±0.52	7.40±0.51	7.20±0.42	7.10±0.31
	ET (Ref. T)	8.00±0.00	7.90±0.31	7.90±0.31	7.80±0.42	7.70±0.48	7.60±0.51
	C (Ref. T)	7.90±0.31	7.80±0.42	7.60±0.51	7.60±0.51	7.50±0.52	7.40±0.51
OA	ET (RT)	8.10±0.31	7.90±0.31	7.70±0.48	7.70±0.48	7.50±0.52	7.30±0.48
	C (RT)	7.90±0.31	7.70±0.48	7.60±0.51	7.40±0.51	7.30±0.48	7.10±0.31
	ET (Ref. T)	8.10±0.31	8.10±0.31	7.90±0.31	7.80±0.42	7.80±0.42	7.70±0.48
	C (REF. T)	7.90±0.31	7.80±0.42	7.70±0.48	7.70±0.48	7.50±0.52	7.40±0.51

VC = Visual colour; F = flavour; O A= overal acceptability; ET = enzyme treated; C = control; RT = room temperature; Ref. T= refrigeration temperature; room temperature = 30±50°C; refrigeration temperature = 5±10°C.

solids content of enzyme assisted and control samples was 9.79 and 9.78, respectively. These values were almost constant during storage. ANOVA revealed that effect of enzymatic treatment as well as storage time on specific gravity and total solids was non significant ($P>0.05$) at both temperatures. Grewal and Jain (1987) reported similar observations in the case of skim milk carrot juice beverage with storage time. Similar results have been reported by Dhaliwal and Hira (2004) in the case of carrot spinach and carrot-pineapple juices. Total soluble solids content of enzymically treated sample (9.00) was more than that of the control sample (8.50) initially and no changes were observed during storage. This may due to the enzymatic action thereby increase in TSS and decrease in viscosity (Sharma et al., 2005). Effect of enzymatic treatment was found significant ($P<0.01$) at both temperatures whereas effect of storage time was non-significant.

Correlation coefficient between total soluble solids and storage time was also statistically non significant ($r_{cal}<r_{crit}$, $\alpha = 0.05$, $n = 6$). Results are in agreement with findings of Bawa and Saini (1987) in the case of untreated carrot juice.

The values for sensory parameters decreased slightly with storage time for all the samples (Table 3). Effect of enzymatic treatment was found statistically significant ($P<0.01$) at both temperature conditions. This may be due to extraction of colour and flavour compounds to a greater extent by enzymatic treatment (Sims et al., 1993) which probably caused enhanced overall acceptability. Effect of storage time was found significant ($P<0.01$); nevertheless sensory scores of colour, flavour and overall acceptability were good after storage of 3 months. There was negative correlation between hedonic scale values and storage time which was found to be significant at 99% significance level (Table 3).

Conclusions

The present study revealed that enzymatic treatment caused significant increase in yield of juice and there was significant increase in acidity, TSS and β-carotene content initially as compared to control samples whereas significant decrease was observed in pH, viscosity and pectin content for enzymatically treated juice samples. The color values, L- and a- decreased while b- increased slightly during storage which resulted in increased browning index and total colour difference. Zero- and first-order kinetic models were found to describe the L-, a-, b-value, BI and β- carotene at both temperatures. The effect of enzymatic treatment on sensory quality of juice was found to be statistically significant.

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