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Research Paper

Effect of Cation Exchange Resin Treatment on Reduction of Non-Enzymatic Browning of Orange Juice and Semi-concentrates during Storage

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Abstract

Effect of removal of reaction substrate (amino acids) from sweet orange juice by using cation exchange resin was studied for reduction of browning of single strength juice and semi-concentrates of 15 and 30 ^{o}Brix. Cation exchange resin (CER), Dowex-50W was used for removal of amino acids. The treated and untreated juices were concentrated to 15 and 30^{o}Brix in a rotary vacuum evaporator. Single strength juice alongwith semi-concentrates of 15 and 30 ^{o}Brix were stored for a period of one month at refrigerated, ambient and accelerated temperature (37$\pm$2^{o}C, 65% RH). During 30 days storage, loss of amino acids was mere 3.07% in the concentrates prepared from cation exchange resin treated juice as compared to about 6.68% loss in the products prepared from untreated juice. Ascorbic acid content showed a net loss of 14.59% (dwb) after 30 days storage in products prepared from untreated juice as compared to far lesser losses of just 4.85% (dwb) in the products prepared from cation exchange resin treated juice under similar conditions of storage. Total sugar registered a net loss of 3.52% on dry weight basis. Treated juice showed higher amount of total sugars with mean value of 75.48% as compared to untreated juices with corresponding value of 71.01%. Cation exchange resin treatment of malta juice resulted into about three folds in non-enzymatic browning during storage.

Keywords: Sweet Orange, Malta, Non-enzymatic browning, Cation exchange resin, Juice, Semi-concentrates, Storage

Sweet orange, is the second most important citrus fruit cultivated in India after mandarin, mainly in Andhra Pradesh, Maharastra, Punjab, Haryana and Rajasthan. National Horticulture Board estimates sweet orange production in India to be 3.57 million tonnes from an area of 0.32 million ha area (NHB, 2009). Main varieties of sweet orange in India being cultivated on commercial scale are Blood Red, Mosambi and Satgudi, however, cv. 'Malta Common' is very popular in regions of Punjab, Haryana and Uttarakhand (Chadha, 2006). Juice is the most important product obtained from sweet orange which may be canned, frozen, converted into beverages or chemically preserved either as natural single strength juice or after concentration. But, browning of citrus juices and beverages during preparation as well as storage has been recognized as a big problem for the fruit processing industry (Sharma *et al.*, 2006).

Biochemically, browning predominantly results due to the reaction of sugars and amino acids leading to the formation of melanoidins (brown polymers). Browning reactions are supposed to slow down in the absence of any of these two substrates (Handwerk and Coleman, 1988; Sharma *et al.*, 2004). Several authors have reported that quality of fruit products is generally reduced during storage because of change in colour, aroma and taste of the products (Giese, 1992; Sharma *et al.*, 2006). Maintaining the product at low temperature is the common mean to avoid colour and flavour deterioration of processed citrus fruit juices, concentrates and dehydrated products in long term storage (Handwerk and Coleman, 1988). But when subjected to non-refrigerated storage, citrus juice rapidly develop objectionable colour and flavour making the product unacceptable. Sharma *et al.* (2006 and 2011) have reported about 96% reduction in amino acid (one of the browning reaction substrates) by use of Dowex-50W cation exchange resin (CER) and subsequent four-fold reduction in browning of lemon juice, concentrates and powder. But, the work on reduction of browning in sweet orange juice and concentrates during storage is scanty in literature.

Keeping the above facts in view, the present investigation was designed to evaluate the effect of cation exchange resin treatment for removal of amino acids (one of the browning reaction substrates) from sweet orange cv. Malta Common juice for subsequent reduction of colour and quality deterioration in single strength juice and semi concentrates during storage.

Materials and Methods

Fruits of sweet orange (*Citrus sinensis*) cv. Malta Common, devoid of blemishes, any visible signs of microbial infection, insect infestation and physical injury were procured from malta growing areas of district Tehri_Garhwal, Uttarakhand, India. Juice was extracted by using semi-automatic motor operated Screw type juice extractor (Bajaj Maschinen, Pvt. Ltd., New Delhi). The extracted juice was then strained through muslin cloth and pasteurized at 90°C for 10 sec followed by quick cooling to room temperature and preservation with 500 ppm SO_2 (Sharma *et al.*, 2004a and b) . The juice was clarified by using "Pectinase CCM" enzyme (Advanced Enzyme Technologies Ltd. Thane, Maharashtra, India) at 0.2% for 2 h at 50 $\pm$ 2^{o}C (Sharma *et al.*, 2001; Sharma, 2010).

For removal of amino acids (one of the browning reaction substrates), an acidic cation exchange resin, Dowex-50W, (Hi Media Laboratories Pvt. Ltd., Mumbai, India) was packed in a glass column (5 cm internal diameter) upto a height of 8 cm. About 120 ml of juice was allowed to pass through the glass column under gravity in one lot. The column, after use, was washed with 0.2 N HCl solution (3-4 times the volume of resin) and regenerated with 0.2 N NaOH solution (2-3 times the volume of resin) with EDTA (AOAC, 1995; Sharma *et al.*, 2004b). Excess of alkali was removed by washing the CER with 3-4 volumes of distilled water and dried under suction at room temperature. Properly washed and regenerated column was repeatedly used for separation of amino acids from malta juice without any practical loss in its activity (Fig 1). Samples of 120 ml juice were treated separately and the treated juice was collected and pooled.

Both types of juices i.e. one from which browning reaction substrates had been removed (treated) and untreated one were used for the preparation of semi-concentrates of 15,

Table 1 Effect of cation exchange resin treatment on amino-acid content (mg/100 g) of malta juice and semi-concentrates during storage

Storage conditions (S)	Concen-tration	Juice Type / CER treatment								Grand Mean	Mean (C)
		Treated				Untreated					
		Storage interval (Days)				Storage interval (Days)					
		Initial	15	30	Mean	Initial	15	30	Mean		
Refrigerated	Single Strength	4.26 (44.38)	4.25 (44.27)	4.22 (43.96)	4.24 (44.20)	256.30 (2288.39)	255.50 (2281.25)	255.00 (2276.79)	255.60 (2282.14)	129.92 (1163.17)	126.18 (1129.77)
	15^0Brix	7.00 (43.21)	6.96 (42.96)	6.90 (42.59)	6.95 (42.92)	361.70 (2127.65)	360.20 (2118.82)	358.80 (2110.59)	360.20 (2119.02)	183.58 (1080.97)	178.19 (1049.17)
	30^0Brix	13.70 (42.28)	13.6 (41.98)	13.50 (41.67)	13.60 (41.98)	707.30 (2080.29)	703.00 (2067.65)	698.80 (2055.29)	703.03 (2067.74)	358.32 (1054.86)	347.43 (1022.83)
	Mean	8.32 (43.29)	8.27 (43.07)	8.21 (42.74)	8.26 (43.03)	441.77 (2165.44)	439.57 (2155.90)	437.50 (2147.56)	439.61 (2156.30)	223.94 (1099.67)	
Ambient	Single Strength	4.26 (44.38)	4.22 (43.96)	4.20 (43.75)	4.23 (44.03)	256.30 (2288.39)	255.00 (2276.79)	253.90 (2266.96)	255.07 (2277.38)	129.65 (1160.71)	
	15^0Brix	7.00 (43.21)	6.93 (42.78)	6.88 (42.47)	6.94 (42.82)	361.70 (2127.65)	359.90 (2117.06)	358.10 (2106.47)	359.90 (2117.06)	183.42 (1079.87)	
	30^0Brix	13.70 (42.28)	13.50 (41.47)	13.30 (41.05)	13.50 (41.67)	707.30 (2080.29)	702.30 (2065.59)	697.40 (2051.18)	702.33 (2065.69)	357.92 (1053.68)	
	Mean	8.32 (43.29)	8.22 (42.80)	8.13 (42.42)	8.22 (42.84)	441.77 (2165.44)	439.07 (2153.15)	436.47 (2141.54)	439.10 (2153.38)	223.66 (1098.11)	
Accelerated	Single Strength	4.26 (44.38)	4.19 (43.65)	4.13 (43.02)	4.19 (43.68)	256.30 (2288.39)	233.00 (2080.36)	212.00 (1892.86)	233.77 (2087.20)	118.98 (1065.44)	
	15^0Brix	7.00 (43.21)	6.72 (41.48)	6.50 (40.12)	6.74 (41.60)	361.70 (2127.65)	327.30 (1925.29)	296.20 (1742.35)	328.40 (1931.76)	167.57 (986.68)	
	30^0Brix	13.7 (42.28)	13.20 (40.74)	12.65 (39.04)	13.18 (40.69)	707.30 (2080.29)	636.60 (1872.35)	572.90 (1685.00)	638.93 (1879.21)	326.06 (959.95)	
	Mean	8.32 (43.29)	8.04 (41.96)	7.76 (40.73)	8.04 (41.99)	441.77 (2165.44)	398.97 (1959.33)	360.37 (1773.40)	400.37 (1966.06)	204.21 (1004.03)	
	Grand Mean	8.32 (43.29)	8.18 (42.61)	8.03 (41.96)	8.17 (42.62)	441.77 (2165.44)	425.87 (2089.46)	411.45 (2020.83)	426.36 (2091.91)		
	Mean (D)	225.05 (1104.37)	217.03 (1066.04)	209.74 (1031.40)							

$CD_{0.05}$ CER Treatment (T) = 10.15, Concentration (C) = 12.43, Storage Condition (S) = 12.43, Storage Interval (D) = 12.43, T x C = 17.58, T x S = 17.58, T x D = 17.58, C x S = NS, C x D = NS , S x D = 21.52, T x C x S = NS, T x C x D = NS, T x S x D = 30.44, C x S x D = NS, T x C x S x D = NS

Table 2 Effect of cation exchange resin treatment on ascorbic acid content (mg/100 g) of malta juice and semi-concentrates during storage

Storage conditions (S)	Concen-tration	Juice Type / CER Treatment								Grand Mean	Mean (C)
		Treated				Untreated					
		Storage interval (Days)				Storage interval (Days)					
		Initial	15	30	Mean	Initial	15	30	Mean		
Refrigerated	Single Strength	70.23 (731.56)	70.10 (730.21)	70.00 (729.17)	70.11 (730.31)	74.00 (660.71)	73.95 (660.27)	73.56 (656.79)	73.84 (659.26)	71.98 (694.79)	69.18 (669.08)
	15^0Brix	116.00 (716.05)	115.5 (712.96)	115.00 (709.88)	115.50 (712.96)	102.00 (600.00)	100.90 (593.53)	99.90 (587.65)	100.93 (593.73)	108.22 (653.35)	103.57 (625.66)
	30^0Brix	225.00 (694.44)	224.30 (692.28)	223.50 (689.81)	224.27 (692.18)	196.30 (577.35)	193.30 (568.53)	190.40 (560.00)	193.33 (568.63)	208.80 (630.41)	198.97 (601.04)
	Mean	137.08 (714.02)	136.63 (711.82)	136.17 (709.62)	136.63 (711.82)	124.10 (612.69)	122.72 (607.44)	121.29 (601.48)	122.70 (607.21)	129.67 (659.52)	
Ambient	Single Strength	70.23 (731.56)	70.00 (729.17)	68.99 (718.65)	69.74 (726.46)	74.00 (660.71)	70.33 (627.95)	66.53 (594.02)	70.29 (627.56)	70.02 (677.01)	
	15^0Brix	116.00 (716.05)	115.00 (709.88)	114.00 (703.70)	115.00 (709.88)	102.00 (600.00)	94.96 (558.58)	88.40 (520.00)	95.12 (559.53)	105.06 (634.71)	
	30^0Brix	225.00 (694.44)	223.00 (688.27)	222.00 (685.19)	223.00 (689.30)	196.30 (577.35)	181.00 (532.35)	166.90 (490.88)	181.40 (533.53)	202.37 (611.42)	
	Mean	137.08 (714.02)	136.00 (709.11)	135.00 (702.51)	136.02 (708.55)	124.10 (612.69)	115.43 (572.96)	107.28 (534.97)	115.60 (573.54)	125.81 (641.05)	
Accelerated	Single Strength	70.23 (731.56)	67.44 (702.5)	64.74 (674.38)	67.47 (702.81)	74.00 (660.71)	63.18 (564.11)	53.70 (479.46)	63.63 (568.09)	65.55 (635.45)	
	15^0Brix	116.00 (716.05)	109.00 (672.84)	100.00 (617.28)	108.33 (668.72)	102.00 (600.00)	85.68 (504.00)	71.97 (423.35)	86.55 (509.12)	97.44 (588.92)	
	30^0Brix	225.00 (694.44)	205.00 (632.72)	190.00 (586.42)	206.67 (637.86)	196.30 (577.35)	162.90 (479.11)	135.20 (397.64)	164.80 (484.70)	185.74 (561.28)	
	Mean	137.08 (714.02)	127.15 (669.35)	118.25 (626.03)	127.49 (669.80)	124.10 (612.69)	103.92 (515.74)	86.96 (433.48)	104.99 (520.64)	116.24 (620.22)	
	Grand Mean	137.08 (714.02)	133.26 (696.76)	129.81 (679.39)	133.38 (696.72)	124.10 (612.69)	114.02 (565.38)	105.18 (523.31)	114.43 (567.13)		
	Mean (D)	130.59 (663.36)	123.64 (631.07)	117.50 (601.35)							

$CD_{0.05}$ CER Treatment (T) = 5.53, Concentration (C) = 6.77, Storage Condition (S) = 6.77, Storage Interval (D) = 6.77, T x C = 9.58, T x S = 9.58, T x D = 9.58, C x S = NS, C x D = NS , S x D = 11.73, T x C x S = NS, T x C x D = NS, T x S x D = 16.59, C x S x D = NS, T x C x S x D = NS

Table 3 Effect of cation exchange resin treatment on reducing sugars content (%) of malta juice and semi-concentrates during storage

Storage condition (S)	Concen-tration	Juice / CER Treatment								Grand Mean	Mean (C)
		Treated				Untreated					
		Storage interval (Days)				Storage interval (Days)					
		Initial	15	30	Mean	Initial	15	30	Mean		
Refrigerated	Single Strength	3.22 (33.54)	3.22 (33.54)	3.23 (33.65)	3.22 (33.58)	3.54 (31.61)	3.55 (31.70)	3.57 (31.88)	3.55 (31.73)	3.39 (32.66)	3.41 (32.73)
	15^0Brix	5.45 (33.64)	5.46 (33.70)	5.47 (33.77)	5.46 (33.70)	5.40 (31.76)	5.43 (31.94)	5.50 (32.35)	5.44 (32.01)	5.45 (32.86)	5.47 (32.93)
	30^0Brix	10.95 (33.79)	10.96 (33.83)	10.99 (33.92)	10.97 (33.85)	10.90 (32.06)	10.95 (32.21)	11.00 (32.35)	10.95 (32.21)	10.96 (33.03)	10.99 (33.11)
	Mean	6.54 (33.66)	6.55 (33.69)	6.56 (33.78)	6.55 (33.71)	6.61 (31.81)	6.64 (31.95)	6.68 (32.19)	6.65 (31.98)	6.60 (32.85)	
Ambient	Single Strength	3.22 (33.54)	3.23 (33.65)	3.25 (33.85)	3.23 (33.68)	3.54 (31.61)	3.58 (31.96)	3.60 (32.14)	3.57 (31.90)	3.40 (32.79)	
	15^0Brix	5.45 (33.64)	5.46 (33.70)	5.49 (33.89)	5.47 (33.74)	5.40 (31.76)	5.46 (32.12)	5.52 (32.47)	5.46 (32.12)	5.47 (32.93)	
	30^0Brix	10.95 (33.79)	10.99 (33.92)	11.10 (34.26)	11.01 (33.99)	10.90 (32.06)	10.98 (32.29)	11.12 (32.71)	11.00 (32.35)	11.00 (33.17)	
	Mean	6.54 (33.66)	6.56 (33.76)	6.61 (34.00)	6.57 (33.81)	6.61 (31.81)	6.67 (32.12)	6.75 (32.44)	6.68 (32.12)	6.63 (32.97)	
Accelerated	Single Strength	3.22 (33.54)	3.25 (33.85)	3.28 (34.17)	3.25 (33.85)	3.54 (31.61)	3.62 (32.32)	3.63 (32.41)	3.60 (32.11)	3.43 (32.98)	
	15^0Brix	5.45 (33.64)	5.47 (33.77)	5.50 (33.95)	5.47 (33.79)	5.40 (31.76)	5.49 (32.29)	5.55 (32.65)	5.48 (32.23)	5.48 (33.01)	
	30^0Brix	10.95 (33.79)	10.98 (33.89)	11.00 (33.95)	10.98 (33.88)	10.90 (32.06)	11.00 (32.35)	11.15 (32.79)	11.02 (32.40)	11.00 (33.14)	
	Mean	6.54 (33.66)	6.57 (33.84)	6.59 (34.02)	6.57 (33.84)	6.61 (31.81)	6.70 (32.32)	6.78 (32.62)	6.70 (32.25)	6.64 (33.05)	
	Grand Mean	6.54 (33.66)	6.56 (33.76)	6.59 (33.93)	6.56 (33.79)	6.61 (31.81)	6.67 (32.13)	6.74 (32.42)	6.68 (32.12)		
	Mean (D)	6.58 (32.74)	6.62 (32.95)	6.67 (33.18)							

$CD_{0.05}$ CER Treatment (T) = 5.40, Concentration (C) = NS, Storage Condition (S) = NS , Storage Interval (D) = NS, T x C = NS, T x S = NS , T x D = NS, C x S = NS, C x D = NS , S x D = NS, T x C x S = NS, T x C x D = NS, T x S x D = NS, C x S x D = NS, T x C x S x D = NS

Table 4 Effect of cation exchange resin treatment on total sugar content (%) of malta juice and semi-concentrates during storage

Storage condition (S)	Concen-tration	Juice / CER Treatment								Grand Mean	Mean (C)
		Treated				Untreated					
		Storage interval (Days)				Storage interval (Days)					
		Initial	15	30	Mean	Initial	15	30	Mean		
Refrigerated	Single Strength	7.35 (76.56)	7.34 (76.46)	7.32 (76.25)	7.34 (76.42)	8.51 (75.98)	8.49 (75.80)	8.46 (75.54)	8.49 (75.77)	7.92 (76.10)	7.88 (75.74)
	15^0Brix	12.35 (76.23)	12.33 (76.11)	12.30 (75.93)	12.33 (76.09)	12.20 (71.76)	12.08 (71.06)	11.90 (70.00)	12.06 (70.94)	12.20 (73.52)	12.03 (72.50)
	30^0Brix	24.50 (75.62)	24.00 (74.07)	23.99 (74.04)	24.16 (74.58)	24.00 (70.59)	23.89 (70.26)	23.60 (69.41)	23.83 (70.09)	24.00 (72.34)	23.71 (71.49)
	Mean	14.73 (76.14)	14.56 (75.55)	14.54 (75.41)	14.61 (75.70)	14.90 (72.78)	14.82 (72.37)	14.65 (71.65)	14.79 (72.27)	14.70 (73.99)	
Ambient	Single Strength	7.35 (76.56)	7.32 (76.25)	7.30 (76.04)	7.32 (76.28)	8.51 (75.98)	8.45 (75.45)	8.40 (75.00)	8.45 (75.48)	7.89 (75.88)	
	15^0Brix	12.35 (76.23)	12.25 (75.62)	12.10 (74.69)	12.23 (75.51)	12.20 (71.76)	12.00 (70.59)	11.82 (69.53)	12.01 (70.63)	12.12 (73.07)	
	30^0Brix	24.50 (75.62)	23.98 (74.01)	23.90 (73.77)	24.13 (74.47)	24.00 (70.59)	23.70 (69.71)	23.00 (67.65)	23.57 (69.32)	23.85 (71.90)	
	Mean	14.73 (76.14)	14.52 (75.29)	14.43 (74.83)	14.56 (75.42)	14.90 (72.78)	14.72 (71.92)	14.41 (70.73)	14.68 (71.81)	14.62 (73.62)	
Accelerated	Single Strength	7.35 (76.56)	7.31 (76.15)	7.24 (75.42)	7.30 (76.04)	8.51 (75.98)	8.35 (74.55)	8.16 (72.86)	8.34 (74.46)	7.82 (75.25)	
	15^0Brix	12.35 (76.23)	12.20 (75.31)	11.70 (72.22)	12.08 (74.59)	12.20 (71.76)	11.39 (67.00)	10.70 (62.94)	11.43 (67.23)	11.76 (70.91)	
	30^0Brix	24.50 (75.62)	24.40 (75.31)	24.30 (75.00)	24.40 (75.31)	24.00 (70.59)	22.00 (67.06)	19.62 (57.71)	22.14 (65.12)	23.27 (70.22)	
	Mean	14.73 (76.14)	14.64 (75.59)	14.41 (74.21)	14.59 (75.31)	14.90 (72.78)	14.18 (69.54)	12.83 (64.50)	13.97 (68.94)	14.28 (72.13)	
	Grand Mean	14.73 (76.14)	14.57 (75.48)	14.46 (74.82)	14.59 (75.48)	14.90 (72.78)	14.57 (71.28)	13.96 (68.96)	14.48 (71.01)		
	Mean (D)	14.37 (72.79)	14.12 (71.71)	13.76 (70.23)							

$CD_{0.05}$ CER Treatment (T) = 0.49, Concentration (C) = 0.60, Storage Condition (S) = 0.60, Storage Interval (D) = 0.60, T x C = 0.84, T x S = 0.84, T x D = 0.84, C x S = NS, C x D = 1.03 , S x D = 1.03, T x C x S = 1.46, T x C x D = NS, T x S x D = 1.46, C x S x D = NS, T x C x S x D = NS

Table 5 Effect of cation exchange resin treatment on non-enzymatic browning (OD 440_{nm}) of malta juice and semi concentrates during storage

Storage condition (S)	Concentration	Juice / CER Treatment								Grand Mean	Mean (C)
		Treated				Untreated					
		Storage interval (Days)				Storage interval (Days)					
		Initial	15	30	Mean	Initial	15	30	Mean		
Refrigerated	Single Strength	0.0103	0.0104	0.0105	0.0104	0.0235	0.0236	0.0238	0.0236	0.0170	0.0171
	150Brix	0.0139	0.0140	0.0142	0.0140	0.0348	0.0349	0.0350	0.0349	0.0245	0.0245
	300Brix	0.0178	0.0180	0.0181	0.0180	0.0528	0.0568	0.0572	0.0556	0.0368	0.0369
	Mean	0.0140	0.0141	0.0143	0.0141	0.0370	0.0384	0.0387	0.0380	0.0261	
Ambient	Single Strength	0.0103	0.0105	0.0108	0.0105	0.0235	0.0237	0.0242	0.0238	0.0172	
	150Brix	0.0139	0.0141	0.0143	0.0141	0.0348	0.0351	0.0354	0.0351	0.0246	
	300Brix	0.0178	0.0181	0.0184	0.0181	0.0528	0.0573	0.0578	0.0560	0.0371	
	Mean	0.0140	0.0142	0.0145	0.0142	0.0370	0.0387	0.0391	0.0383	0.0263	
	Grand Mean	0.0140	0.0483	0.0869	0.0142	0.0370	0.7841	1.4707	0.0382		
	Mean (D)	0.0255	0.0264	0.0266							

$CD_{0.05}$ CER Treatment (T) = 0.001, Concentration (C) = 0.001, Storage Condition (S) = NS, Storage Interval (D) = NS, T x C = .002, T x S = NS, T x D = NS, C x S = NS, C x D = NS, S x D = NS, T x C x S = NS, T x C x D = NS, T x S x D = NS, C x S x D = NS, T x C x S x D = NS

Accelerated	Single Strength	0.0103	0.0234	0.0605	0.0314	0.0235	1.2670	TDTR	-	-	
	15^0Brix	0.0139	0.0871	0.1545	0.0852	0.0348	TDTR	TDTR	-	-	
	30^0Brix	0.0178	0.2397	0.4804	0.2460	0.0528	TDTR	TDTR	-	-	
	Mean	0.0140	0.1167	0.2318	0.1209	0.0370	-	-	-	-	

TDTR: Too dark to read

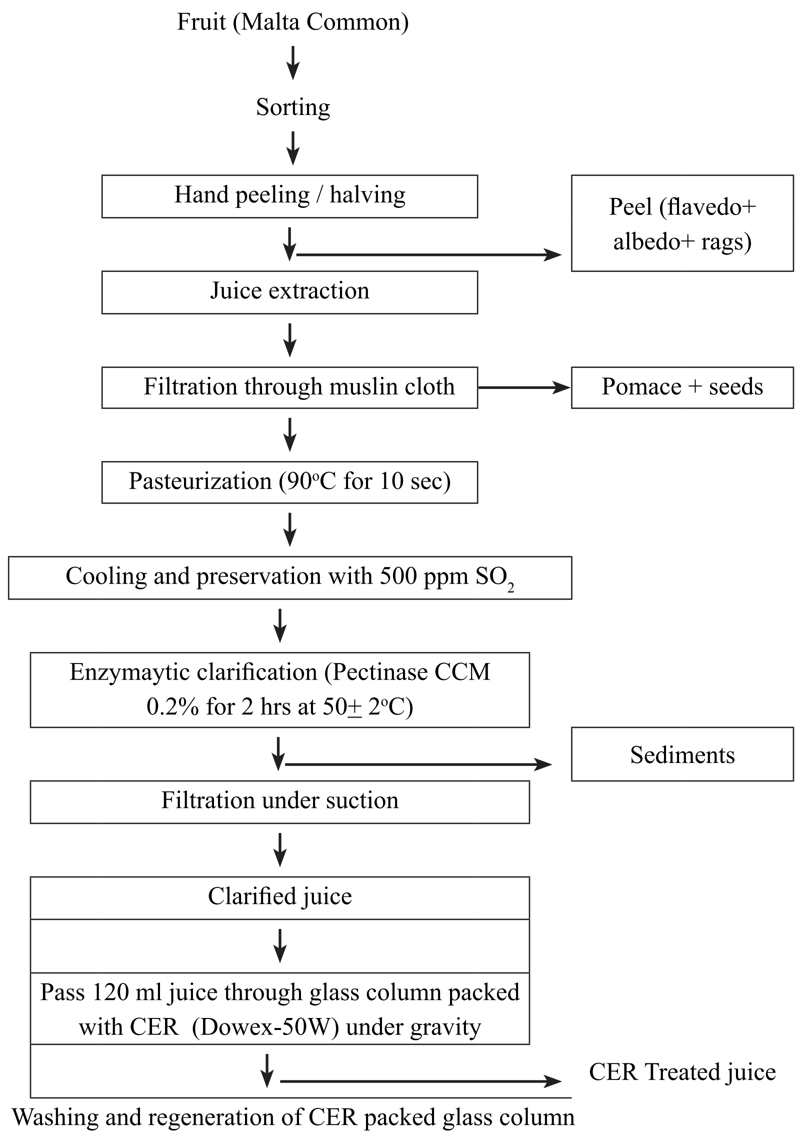
Table 6 Effect of cation exchange resin treatment on colour (OD 420_{nm}) of malta juice and semi-concentrates during storage

Storage condition (S)	Concentration	Juice / CER Treatment								Grand Mean	Mean (C)
		Treated				Untreated					
		Storage interval (Days)				Storage interval (Days)					
		Initial	15	30	Mean	Initial	15	30	Mean		
Refrigerated	Single Strength	0.0102	0.0102	0.0103	0.0102	0.0230	0.0230	0.0231	0.0230	0.0166	0.0167
	15^0Brix	0.0137	0.0137	0.0139	0.0138	0.0345	0.0345	0.0347	0.0346	0.0242	0.0242
	30^0Brix	0.0176	0.0176	0.0179	0.0177	0.0533	0.0533	0.0570	0.0545	0.0361	0.0365
	Mean	0.0138	0.0138	0.0140	0.0139	0.0369	0.0369	0.0383	0.0374	0.0256	
Ambient	Single Strength	0.0102	0.0103	0.0105	0.0103	0.0230	0.0232	0.0233	0.0232	0.0168	
	15^0Brix	0.0137	0.0138	0.0140	0.0138	0.0345	0.0348	0.0351	0.0348	0.0243	
	30^0Brix	0.0176	0.0178	0.0180	0.0178	0.0533	0.0571	0.0577	0.0560	0.0369	
	Mean	0.0138	0.0140	0.0142	0.0140	0.0369	0.0384	0.0387	0.0380	0.0260	
	Grand Mean	0.0138	0.0448	0.0757	0.0139	0.0369	0.7295	1.4011	0.0377		
	Mean (D)	0.0254	0.0258	0.0263							

$CD_{0.05}$ CER Treatment (T) = 0.0007, Concentration (C) = 0.0009, Storage Condition (S) = NS, Storage Interval (D) = NS, T x C = 0.001, T x S = NS, T x D = NS, C x S = NS, C x D = NS, S x D = NS, T x C x S = NS, T x C x D = NS, T x S x D = NS, C x S x D = NS, T x C x S x D = NS

Accelerated	Single Strength	0.0102	0.0285	0.0524	0.0304	0.0230	1.2130	TDTR	-	-	
	15^0Brix	0.0137	0.0832	0.1200	0.0723	0.0345	1.9170	TDTR	-	-	
	30^0Brix	0.0176	0.2081	0.4245	0.2167	0.0533	TDTR	TDTR	-	-	
	Mean	0.0138	0.1066	0.1990	0.1065	0.0369	-	-	-	-	

TDTR : Too dark to read



Step 1. Wash the column with distilled water (3-4 times vol. of resin)
Step 2. Wash the column with 0.2 N HCl solution (3-4 times vol. of resin)
Step 3. Regenerate with 0.2 N NaOH solution with EDTA (2-3 times vol. of resin)
Step 4. Wash the column with distilled water (3-4 times vol of resin) to remove excess of alkali
Step 5. Dry the column under suction at room temperature and reuse the column repeatedly

Fig. 1. Enzymatic clarification and cation exchange resin treatment of malta juice

30^o Brix using a rotary type vacuum evaporator (Rotating Evaporator RE 2000A, Associated Scientific Technologies, Delhi) at 50 ± 2^oC under 28 ± 2” Hg vacuum. In order to achieve fast condensation of vapours, the temperature of water circulating through the condenser was maintained between -15 and -5^oC by using a circulatory water bath. The freezing point of water was lowered by addition of 20% salt. All the semi-concentrates were packed in 200 ml glass bottles along with single strength juices (treated and untreated). Samples were stored under three storage conditions viz., refrigerated condition (6 ± 2^0C, 92 % RH), ambient condition (room temperature i.e. 20 ± 4 ^0C, 73% RH) and accelerated temperature (37 ± 2^0C, 65% RH) and were evaluated initially before storage (for one month) and at periodic intervals of 15 days for various qualities attributes.

Standard analytical procedures were followed for estimation of TSS, titratable acidity, colour and non-enzymatic browning of samples (Ranganna, 1997; Sharma and Nautiyal, 2009). Sugars were estimated by rapid colorimetric method of simultaneous determination of total reducing sugars and fructose (Ting, 1956), ascorbic acid by direct colorimetric method (Ranaganna, 1997), total amino acids were determined by ninhydrin colorimetric method (Ting and Deszyck, 1960). The data on chemical characteristics was analysed statistically by CRD (Cochran and Cox, 1963).

Results and Discussion

With the advancement of storage period, amino acid content decreased continuously, which was probably due to their involvement in browning reactions i.e. reaction with sugar, ascorbic acid and other juice constituents (Table 1). The reduction in amino acid content with advancement of storage period, have also been reported earlier (Sharma *et al*., 2004b, 2009 and 2011). However, the loss of amino acids was mere 3.07% in the concentrates prepared from cation exchange resin treated juice as compared to about 6.68% loss in the product prepared from untreated juice after 30 days of storage irrespective of folds of concentration, storage conditions and storage intervals. It might be due non-availability of sufficient amino acids to perform various non-enzymatic browning reactions, as cation exchange resin treatment removed 98.35 % of amino acids. Considerably lower contents of total amino acids in semi-concentrates prepared from treated juice may be attributed to their removal by cation exchange resin. The higher retention of amino acid under refrigerated condition might be attributed to slower reaction rate under refrigerated condition. On the contrary the retention of amino acid was significantly less at accelerated temperatures probably due to high rate of deteriorative reactions (Ajandouz and Puigserver, 1999).

Ascorbic acid degradation has been associated with browning of orange juice. It has been demonstrated that presence of amino acid accelerates ascorbic acid breakdown (Clegg, 1964) and in the presence of amine it is oxidized to dehydroascorbic acid that is the reaction intermediate in the pathway to brown pigment production (Hodge, 1953; Clegg, 1964). The mean value of ascorbic acid content was 1.23 times higher in treated juice as compared to untreated juice indicating better retention of vitamin C after cation exchange resin treatment (Table 2). Further, a net loss of 14.59 % (dwb) was recorded after 30 days storage in products prepared from untreated juice as compared to far lesser losses of just 4.85% (dwb) in the products prepared from cation exchange resin treated juice under similar conditions of storage, which might be due to non-availability of sufficient amino acids to react with ascorbic acid for its degradation. The semi-concentrates stored under refrigerated condition showed higher ascorbic content as compared to the semi-concentrate stored under accelerated temperature conditions.

A steady increase in reducing sugar during entire storage period irrespective of all other variables, as observed during the experiment has been presented in Table 3 probably due to hydrolysis or inversion of non-reducing sugar to reducing sugars. In similarity to the changes in amino acid and ascorbic acid contents, the reducing sugar contents also suffered lesser changes in the products prepared from cation exchange resin treated juice as compared to the untreated counterparts. There was a net increase of 1.92% in reducing sugars of products prepared from untreated juice as compared to only 0.80% increase in treated juice and semi-concentrates thereof after 30 days of storage, which might be due to non-availability of sufficient amino acids to react with sugars. Further, refrigeration of the products during storage was also effective in minimizing changes in sugar content.

The losses in total sugars may be attributed to their involvement in Maillard reactions leading to formation of brown pigments. Lesser losses in total sugar content of treated juice and its semi-concentrates, was probably due to non-availability of sufficient amino acids to react with sugars of treated juice (Table 4). These findings are in close conformity with earlier findings (Sharma *et al.*, 2004b & 2009). As far as the effect of cation exchange resin is concerned, treated juice showed higher amount of total sugars as compared to untreated juices on moisture free basis, which might be due to their corresponding higher folds of concentration. Higher retention of total sugar under refrigerated conditions was probably due to slower rates of browning reactions at lower temperature.

As depicted in (Table 5) there was a steady increase in non-enzymatic browning of malta juice and semi-concentrates during 30 days of storage, which might be due to the reaction of carbonyl and free amino groups that leads to formation of brown melanoidin pigments. Since, non-enzymatic browning of the products prepared from untreated juice at 15th and 30th day of storage at accelerated temperature were too dark to read their absorbance at 440 nm, the numerical data for the specific samples could not be generated. Therefore, statistical analysis the data pertaining for only refrigerated and ambient storage conditions were considered.

The cation exchange resin treatment of Malta juice had a significant effect on reduction of non-enzymatic browning, resulting into a mean reduction of about three folds in products prepared from treated juice as compared to untreated Malta juice, which might be due to the removal of browning reaction substrates (amino acids) from treated juice (Sharma *et al*., 2004, 2009 and 2014). As expected the non-enzymatic browning was more pronounced in concentrates of higher folds which were probably due to availability of more amount of browning substrate (amino acid). The increase in non-enzymatic browning with higher levels of concentration has also been reported by Sharma *et al*. (2006) in lemon juice concentrates.

Perusal of data in (Table 6) indicates that in similarity to the trends in non-enzymatic browning the measurement of colour (OD 420$_{nm}$) was also not possible in the products prepared from untreated juice during storage at accelerated temperature condition. Therefore, data from accelerated storage conditions was not considered for statistical analysis. The statistical analysis of rest of the data reveals that there was a continuous darkening in colour of samples with the advancement of storage periods, which might be attributed to non-enzymatic browning reactions as these reactions cause intense colour formation like yellow, orange or red. The colour measurement also reveals that the darkening of products was approximately three folds lesser in those prepared from treated juice as compared to counterparts prepared from untreated juice. The darkening of colour was slightly lesser during product storage at refrigerated condition than that at ambient condition. However, colour measurement also indicated product darkening with increase in concentration of sweet orange juice.

Conclusively, it emerges out that the use of cation exchange resin Dowex-50W is highly effective in removal of browning substrate (amino acids). Approximately 120 ml of sweet orange or Malta Common juice (pooled value of first six samples) when passed through a glass column (5 cm internal diameter) packed with cation exchange resin (Dowex-50W) upto a height of 8 cm, could remove about 98.35% of the amino acids from the juice with a slight loss in other juice constituents. The concentrates prepared from cation exchange resin treated juice underwent about 3 folds lesser darkening during storage alongwith better retention of nutritional and sensory quality attributes. The commercialization of this technology after pilot scale testing and validation may open new avenues for utilization of sweet orange and other citrus juices for preparation of juice concentrates and beverages.

References

Ajandouz, E.H. and Puigserver, A. 1999. Non-enzymatic browning reaction of essential amino acids: effect of pH on caramelization and Maillard reaction kinetics. *Journal of Agricultural and Food Chemistry* **47**(5): 1786-1793.

AOAC 1995.Official methods of analysis of AOAC international, 16th edn. Association of Official Analytical Chemists, Arlington, Virginia, USA, pp 66

Chadha, K.L. 2006. Handbook of Horticulture, Indian Council of Agricultural Research, New Delhi, pp 329.

Clegg, K.M. 1964. Non-enzymatic browning of lemon juice. *Journal of the Science of Food and Agriculture* **15**(12): 878-885.

Cochran, W.G. and Cox, G.W. 1963. Experimental Designs. John Wiley and Sons, Inc., New York, p 613.

Giese, J.H. 1992. Hitting the spot: beverages and beverage technology. *Food Technology* **46**(7): 70.

Handwerk, R.L. and Coleman, R.L. 1988. Approaches to the citrus browning problem – a review. *Journal of Agricultural and Food Chemistry* **36**: 231-236.

Hodge, J.E. 1953. Dehydrated foods - Chemistry of browning reactions in model systems. J Agri *Food Chemistry* **1**: 928.

NHB, 2009. Statewise area and production of sweet orange in India. www.hortibizindia.org website visited on 8th December 2009

Ranganna, S. 1997. Handbook of analysis and quality control for fruit and vegetable products, 2nd edn. Tata McGraw-Hill Pub Co Ltd, New Delhi, p1112

Sharma, P.C., Sharma, S.K. and Kaushal, B.B.L. (2001) Preparation and evaluation of some value added products from Hill lemon (*Citrus pseudolimon* Tan.) fruits. *Indian Journal of Agricultural Sciences* 71(11): 691-694.

Sharma, S.K., Sharma, P.C, and Kaushal, B.B.L. 2004a. Storage studies of foam mat dried Hill lemon (*Citrus pseudolimon* Tan.) juice powder *Journal of Food Science and Technology* 41(1): 9-13.

Sharma, S.K. 2010. Postharvest Management and Processing of Fruits and Vegetables – Instant Notes. New India Pub. Agency, New Delhi, 390p.

Sharma, S.K. and Nautiyal, M.C. 2009. Postharvest technology of horticultural crops - practical manual series – 2, New India Pub Agency, New Delhi, 229p

Sharma, S.K., Juyal, S., Rao, V.K., Yadav, V.K. and Dixit, A.K. 2014. Reduction of non-enzymatic browning in orange juice and semi-concentrates by removal of reaction substrate. *Journal of Food Science and Technology* 51(7):1302-1309.

Sharma, S.K., Kaushal, B.B.L. and Sharma, P.C. 2004b. Effect of temperature and removal of amino acids on non-enzymatic browning of lemon juice concentrates during storage. *Journal of Scientific and Industrial Research* **63**:444-451.

Sharma, S.K., Kaushal, B.B.L. and Sharma, P.C. 2006. Reduction of non-enzymatic browning of lemon juice concentrates by removal of reaction substrates. *Journal of Food Science and Technology* **43**(1): 83-88

Sharma, S.K., Kaushal, B.B.L. and Sharma, P.C. 2009. Effect of cation exchange resin treatment and addition of anti-caking agent on non-enzymatic browning of lemon juice powder during storage. *Journal of Scientific and Industrial Research* **68**: 611-616

Sharma, S.K., Kaushal, B.B.L. and Sharma, P.C. 2011. Effect of cation exchange resin treatment and addition of sugar as anti-caking agent on retention of nutritional and sensory quality of lemon juice powder during storage. *Journal of Food Science and Technology* 48(3): 296-304

Ting, S.V. 1956. Rapid colorimetric methods for simultaneous determination of total reducing sugars and fructose in citrus juices. *J Agril Food Chem* 4:263-266.

Ting, S.V. and Deszyck, E.J. 1960. Total amino acid content of chilled orange juice and frozen concentrate. *Proceedings of the Florida State Horticultural Society* **73**: 252-257.