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PRODUCTION OF CLOUDY APPLE DRINKS USING PECTINASES

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ABSTRACT

Apple fruits (*Malus spp*., Anna cultivar)which collected from Racheed (El-Behara governorate) in Egypt were processed to produce cloudy apple juices using commercial pectinases. Cellulase treatments were considered as comparable treatments. Apple pulp was blanched in microwave for 4 min for browning inhibition. Enzymes were applied to microwave-blanched apple mashes at different concentrations for 30 min at 45°C. Cloudy apple drinks stored at refrigerator temperature (4°C) for six months. Drinks were evaluated periodically for TSS, acidity % (as citric acid), pH, cloud stability (absorbance at 660nm), colour (L, a and b value), reducing sugars and total sugars % . The data showed that, addition of pectinases and cellulase increased TSS and juice yield on the expenses of clould stability. Conducted concentrations of the enzymes showed good balance between yield, TSS and cloud stability. All treatments including the control were highly accepted by the panelists and showed not less than "satisficatory" by the end of the storage period for any quality aspects. Also drinks remained good microbiological quality up to the end of the storage period.

Keywords: Anna apple, cloudy apple juices, pectinases, chemical and physical characteristics and sensory assessment.

INTRODUCTION

Cloudy apple juices are complex systems containing fine pulp particles dispersed in a serum constituted by macro molecules (pectins, protiens, etc.) colloidally dissolved in a true solution of low-molecular weight components, sugars, organic acids, etc.(Wucherpfennig *et al*.1987). Cloudy or unclarified apple juice contains significant quantities of suspended pulp and is perceived as a natural food product that supplies dietary fiber and important nutrients (Quoc *et al*. 2006).

A variety of sparkiling clear juices and cloudy juices production depends on pectic enzyme treatment (Whitaker, 1984 and Voragen and Pilnik, 1998).Enzyme treatment can increase the efficiency of pressing and juice extraction and even enhance flavour and colour release (Downing, 1988; Voragen and Pilnik, 1998).

Mostafa (2004) produced pulpy apple juice from Anna apple by macerated microwaved –blanched apple pulp with polygalacturonase (PG); pectin lyase (PL); pectinesterase (PE) and commercial mixture of pectinases. Mechanical treatment without enzyme addition was also prepared. Mostafa (2004) found that, apple juices from macerated pulp with combination action of PG and PL for 15 min/ 45 °C showed the highest cloud stability value and better colour

and acceptability than those prepared with mechanical process treatment during storage period for three months at 8°C.

Will *et al*. (2008) manufactured pulp-enriched cloudy apple juices from two apple varieties by blending of apple puree with natural cloudy apple juice in order to enrich bioactive secondary plant substances.To reach this aim they manufactured cloudy apple juices and purees from table and cider apples and adjusted them to an optimal drinking consistency. Treatment of the mash with a maceration enzyme (Rohament PL, 150 ml/t, ABenzymes, Darmstadt, Germany) and a pectinase-free amylase (Fructamyl HT, 80 ml/t, Erbslo¨h, Geisenheim, Germany) for 1 h / 50 °C to produce apple puree blended with cloudy apple juice to produce blended pulp-enriched cloudy apple juices. The maceration enzyme used in the mash for puree production degraded insoluble protopectin of the middle lamella of the cells in soluble hydropectin. This separated apple cells from each other. As a result purees with certain thickness and viscosity were obtained.

One of the main problems with cloudy apple juice production is the assurance of colour stability. Control of enzymatic browning during processing and storage is important to preserve the original appearance and nutrition of cloudy apple juices (Gui *et al*. 2005). Enzymatic browning in apple products is caused by the action of polyphenol oxidases, which catalyzes oxidation of phenolic compounds containing two o- dihydroxy groups to the corresponding o- quinone (Nicolas *et al*. 1995). Several methods have been used to attain the inhibition of polyphenol-oxidase (PPO) activity in apple juice, including microwave heating (Oszmian´ ski *et al.* 2008).

The aim of this work is to produce cloudy apple juices from Anna apple cultivated in Egypt, using commercial pectinases. Cloudy apple drinks were evaluated chemically, physically, sensorial and microbiologically within six months at refrigerator temperature (4°C). The effect of pectinases on cloud stability and other juice characteristics was pointed out.

MATERIALS AND METHODS

Materials

Anna apple fruits were collected at mature stage, from Racheed (El-Behara governorate), then stored at refrigerator temperature untill use, within two weeks.

Commercial pectinolytic enzymes; Pectinase 444L and Macer8^{TM}FJ and also cellulase (Cellulase 13L, for comparison purpose) were obtained from Biocatalysts Limited, Wales, UK.

Apple esseence (green apple, 0.017 %, R9410G) were obtained from Delta Aromatic Company, El-Geeza, Egypt.

Methods

Browning inhibition of apple pulp.

Slices of apple pulp were heated in the microwave (Moulinex, 2450 MHz, 1300 Watt) for 1, 2 , 3, 4, 5, 6 and 7 minutes, 350 g each patch. Activities of polyphenol oxidase (PPO) were determined in fresh pulp and in microwave-

blanched pulps. The least heating time capable of polyphenol oxidase inhibition was applied in processing of cloudy apple drinks.

Processing of cloudy apple juice drinks.

For preparing cloudy apple juices; mature apple fruits were, washed, peeled and cored. Sliced apple pulp was heated in the microwave for 4 min and cooled in ice water for 2 min, then mashed using moulinex mincer and homogenized (10000 rpm/min, Jank & Kunkel, Ika-labortechnik, GMBH). Apple mashes were treated with Pectinase 444L and Macer8^{TM}FJ at concentrations of 10; 25 and 50 ppm, and Cellulase 13L at concentrations of 0.1; 0.05 and 0.025 % (V/W) for 30 min at 45°C which referred to by P10; P25; P50; M10; M25; M50; C0.1; C0.05 and C0.025, respectively besides the control treatment (con). Enzyme treatments were optimized upon repeated experiments of different enzyme concentrations and holding times. Mashes were heated in a water bath to 90°C/1 min for enzyme inactivation then cooled. Juice squeezed through double layer opaque cloth. For drinks preparation, juices were adjusted to 14 °Brix with sugar then diluted to 1:1 (v/v) with 14 °Brix sugar solution, bottled, pasteurized (90°C/ 3min) then cooled and stored at refrigerator temperature for six months.

Drinks were analyzed for TSS, acidity % (as citric acid), pH, cloud stability (absorbance at 660nm), colour (L, a and b value), ascorbic acid content, reducing sugars % and total sugars % at zero-time and at one month intervals for the total period of the cold storage. Sensory and microbiological evaluations were carried out at zero-time and at one month and half intervals for the total storage period. Also 30 ppm of flavouring agent was added to the highest enzyme treatment to serve as flavoured treatment then evaluation was carried out.

Chemical analysis

Total titratable acidity and reducing and total sugars were determined as described in the AOAC (1990). Value of pH was measured using a pH-meter, Jenway Ltd., UK, at 25°C. Ascorbic acid content (mg/100g) was determined according to Mazumdar and Majumder (2003)a and activity of polyphenol oxidase was determined according to Mazumdar and Majumder (2003)b.

Physical anslysis

Total soluble solides (° Brix) were measured according to the AOAC (1990) by Abbe refractometer at 20°C. Colour attributes (L, a and b) were measured according to Gui *et al.* (2005) using a Minolta color reader CR-10, Minolta Co. Ltd., Japan. Cloud stability was determined according to Staelehamatschek (1989) with little modifications (Mostafa, 2004). Apple juice centrifuged at 4000 rpm for 25 min. The turbidity of the supernatant was measured by a spectrophotometer at 660 nm, against blank.

Sensory anslysis

Cloudy juices were sensorial evaluated for colour, taste, aroma, appearance and mouth-feel. Sensory assessments were carried out by ten semi-trained panelists on a 7-point hedonic scale from excellent, very good, good, satisfactory, poor, very poor and extremely poor, which for analysis were scored from 7 to 1 (McBride and Richardson, 1983).

Microbiological anslysis

Microbiological qualities of cloudy apple juices were periodically examined for bacteria; mold and yeast. For total count of bacteria, trypton glucose extract agar was used (Marsall, 1992)a. Counts of molds and yeasts were carried out by antibiotic agar method using standard methods agar, according to Marsall (1992)b. Antibacterial buffer (2%) was added to sterile media befor use, for inhibition of bacterial growth. Dilutions up to 1: 10^3 of tested treatments were cultivated.Counts of plates were taken after incubation of 24 hrs/37°C and 72 hrs/25°C for bacteria and molds and yeasts respectively.

Statistical anslysis

The analysis of variance (ANOVA) and LSD were performed by CoStat 6.311, Copyright(c) 1998-2005, CoHort Software, at probability ≥ 0.05 (Snedecor and Cochran, 1967). Statistical analysis of sensory evaluations depended on ten replicates of degrees by the ten panelists.

RESULTS AND DISCUSSION

Preliminary experiments were carried out to select the best enzyme treatments that showed the best cloud stabilities in addition of higher TSS and juice yield. Some data are presented in Table (1) which showed that enzyme treatment increased juice yield significantly in comparison with the control.

Table (1): Percent of juice yield and TSS of crude juice and cloud stability of cloudy drinks, extracted from apple mashs treated with different enzyme concentrations for half an hour at 45°C.

Characteristics / Treatment	Yield %	TSS	Cloud stability
Cellulase at 0.3%	63.6a	11.5a	0.176g
Cellulase at 0.15%	59.5a	11.0b	0.189g
Cellulase at 0.1%	57.3a	10.8b	0.213f
Cellulase at 0.05%	55.0a	10.5c	0.289bc
Pectinase at 50 ppm	68.4a	11.0b	0.261cd
Pectinase at 25 ppm	65.5a	10.5c	0.311b
Pectinase at 10 ppm	60.0a	10.0d	0.317b
Macer at 50 ppm	69.7a	11.5a	0.230de
Macer at 25 ppm	66.9a	11.0b	0.252d
Macer at 10 ppm	65.8a	10.5c	0.297b
Control	51.0b	10.0d	0.390a

Mean in the same column with different superscripts are significantly different (p< 0.05). Abbreviations: Cellulase : Cellulase 13L; Pectinase: Pectinase 444L and Macer: Macer8^{TM}FJ. Control: cloudy apple juice/drink extracted without enzyme additiont.

The highest enzyme treatments; cellulase at 0.3%, pectinase at 50 ppm and macer at 50 ppm had juice yields of 63.6, 68.4 and 66.9%, respectively. Also TSS increased significantly in proportional with increasing of enzyme concentration while cloud stability decreased with the increase of enzyme concentration. So enzyme concentrations for ongoing experiments were

selected to provide good yield, high TSS and suitable cloud stability. According to Beveridge (2002), cloud stability is one of the visual quality attributes decisive for consumer acceptance of cloudy apple juices. Also Will *et al*. (2008), mentioned that, the most important economical parameters for the assessment of an apple juice are soluble solids (°Brix) and total titratable acidity.

Table (2): Activity of polyphenol oxidase for microwave-blanched apple pulp at different durations of microwave heating.

Duration (minute)	Activity of polyphenol oxidase	Reached temperature of apple pulp (°C)
0	1.37	25
1	1.04	74
2	0.70	84
3	0.54	89
4	-	91

Activity of polyphenol oxidase =changing of absorbance at 495 nm/min/g

From the results in Table (2), it was shown that, heating the apple pulp in the microwave for 4 min was enough to inactivate the PPO. Mostafa (2004) found that, apple slices required 4.5 min for blanching by microwave energy to inactivate PPO. Similar results were reported by Yemenicioglu *et al.* (2000). Whereas Gui et al. (2005) conducted the apple cloudy juice to 95°C for 90 seconds to inactivate PPO completely.

Trying to control of phenol oxidase activity in apple slices, Nahed (1993) found that the optimum temperature for inactivation of the enzyme was between 60-70 °C for 15 min.

Physical and chemical analysis

Since cloudy apple drinks were modified to 14°Brix, the TSS of all treatments was equal within the first two months. The mean of TSS of all treatments increased significantly ($p < 0.05$) to 14.3°Brix at the fourth month and to 14.88°Brix at the sixth month. The treatments interacted significantly with the storage time and showed different significant means over the storage period (Table 3). Increasing of °Brix could be due to the disintigration of pulp particles which were suspended in aqueous media for six months.

Table (3): TSS at one month intervals of cloudy apple drinks produced by different concentratons of Cellulase 13L ,Pectinase 444L and Macer8^{TM}FJ, during storage at refrigerator temperature (4°C).

Treatment	Storage (month)							Mean
	0	1	2	3	4	5	6	
Con	14.0	14.0	14.0	14.5	14.5	15.0	15.0	14.43 a
C0.1	14.0	14.0	14.0	14.0	14.5	15.0	15.0	14.31 ab
C0.05	14.0	14.0	14.0	14.5	14.5	14.7	15.0	14.38 ab
C0.025	14.0	14.0	14.0	14.5	14.5	14.7	15.0	14.38 ab
P50	14.0	14.0	14.0	14.5	14.5	14.8	15.0	14.41 ab
P25	14.0	14.0	14.0	14.5	14.5	14.8	15.0	14.41 ab
P10	14.0	14.0	14.0	14.0	14.0	14.2	14.7	14.12 cd
M50	14.0	14.0	14.0	14.0	14.0	14.0	14.5	14.07 d
M25	14.0	14.0	14.0	14.0	14.0	14.7	14.7	14.14 cd
M10	14.0	14.0	14.0	14.0	14.3	14.8	15.0	14.24 bc
mean	14.00d	14.00d	14.00d	14.25 c	14.30 c	14.58b	14.88a	

Mean in the same column with different superscripts are significantly different (p< 0.05).

Enzymatic treatments produced drinks with significantly different cloud stabilities. According to their stability means over the storage period, treatments were arranged descendeningly in this order; P10, C0.025, M10, P25, M0.05, M25, P50, C0.1 and M50 which showed means of 0.250, 0.250, 0.233, 0.231, 0.212, 0.209, 0.209, 0.198 and 0.182, respectively, in comparison with control treatment which had significantly the best stability mean of 0.273.The mean of treatments stability had significantly decrement at each month (Table 4), from 0.311 at zero-time to 0.139 at the sixth month. Beveridge (1997), explained that it is very difficult to produce a cloudy or unclarified apple juice with good opalescence stability due to the presence of pectin methylesterases (PME). The pectin molecules present in suspension are degraded by the PME enzymes resulting in a loss of opalescence stability. In addition, concerning pulp-enriched apple juice, Will *et al*. (2008) indicated that a long-term cloud stability can not be expected from this kind of products, and after a two weeks storage of the samples in upright standing bottles a distinct sedimentation of the solids could be observed. Mostafa (2004) increased cloud stability of pulpy apple juice by maceration apple pulp with combination action of pectin lyase and polygalacturonase for 0.5 and 0.25 hr, which gave absorbance of 0.310 and 0.324 at 660nm, respectively, in comparison with the control (0.152). Treatments retained their turbidity than control after 3 months of storage at 8°C, as absorbance were 0.05, 0.26 and 0.29 for control, PG PL/ 0.5hr and PG PL/ 0.25hr, respectively. Results were in agreement with those of Zhao *et al*. (2008) who found that the turbidity of cloudy apple juice decreased during its storage for 120 days at 4, 22 and 40 ˚C.

Table (4): Cloud Stability at one month intervals of cloudy apple drinks produced by different concentratons of Cellulase 13L , Pectinase 444L and Macer8^{TM}FJ, during storage at refrigerator Temperature (4°C).

Treatment	Storage (month)							Mean
	0	1	2	3	4	5	6	
Con	0.373	0.308	0.290	0.263	0.248	0.232	0.198	0.273 a
C0.1	0.271	0.240	0.232	0.228	0.167	0.137	0.115	0.198 e
C0.05	0.304	0.267	0.246	0.232	0.170	0.144	0.123	0.212 d
C0.025	0.359	0.305	0.274	0.265	0.213	0.181	0.150	0.250 b
P50	0.286	0.250	0.232	0.205	0.187	0.163	0.136	0.209 d
P25	0.317	0.268	0.257	0.240	0.212	0.168	0.156	0.231 c
P10	0.347	0.284	0.272	0.253	0.228	0.190	0.173	0.250 b
M50	0.255	0.238	0.216	0.195	0.150	0.135	0.088	0.182 f
M25	0.272	0.256	0.237	0.225	0.185	0.167	0.119	0.209 d
M10	0.327	0.277	0.262	0.243	0.204	0.179	0.136	0.233 c
Mean	0.3111 a	0.269 b	0.252 c	0.235 d	0.196 e	0.170 f	0.139 g	

Mean in the same column with different superscripts are significantly different (p< 0.05).

Regarding to colour attributes, treatments showed significantly different L values over the storage period as shown in Table (5). Control treatment and the lowest enzyme concentrations; C0.025, P10 and M10 had significantly ($p < 0.05$) the highest means of L values over the six months storage period which were 35.62, 35.29, 35.79 and 35.39, respectively. While the highest

enzyme concentrations; C0.1, C.05, P50, M50 and M25 had the lowest means which were 34.76, 34.81, 34.84, 34.76 and 34.76, respectively. Voragen *et al.* (1986) indicated that pectin lyase rich preparation enzymes showed the darkest clear apple juice which characterized by its high amount of AUA (anhydrogalacturonic acid) which released by pectin lyase, as in our case of Macer which contain high level of pectin lyase. Also Verstrate (1985) showed that oligogalacturonides, in particular the unsaturated compounds are very reactive in browning reactions in aerobic as well as in anaerobic conditions. The colour of cloudy apple drinks was influenced by the storage time as the mean of the L values of all treatments decreased significantly from 37.24 at zero-time to 32.87 at the sixth month.

The significant increasing of redness (a values) of cloudy apple drinks was recorded in Table (6). The mean of a values for different treatments elevated from 2.76 at zero time to 4.02 at the fifth month. M50 and P50 showed significantly the highest means of 3.51 and 3.48, respectively. Other treatments had insignificant means of a values which ranged from 3.23 to 3.39. Mean of a values increased significantly from 2.76 at zero-time to 4.02 at the fifth month, indicating occurrence of browning reactions.

Value "b" was the less susceptible colour attributes for enzymatic treatments since the mean values of b for treatments within the storage period ranged insignificantly from 4.29 to 4.66. The mean of b values of all treatments increased significantly from 3.97 at zero-time to 5.14 at the sixth month, as shown in Table (7). Mostafa (2004) found no significant differences between the colour values (L, a and b) for pulpy apple juices treated with PG and PL for 0.5 and 0.25 hr after three months at 8°C, and the colour was less deteriorated than the control. Alterations of L values (Lightness) and a values (redness) reflects the effect of storage on colour stability of cloudy apple drinks as Gui *et al*. (2005) noticed significant decrement of Lightness (L) and significant increment of the juice redness (a), during the stoage of cloudy apple juices. However, the yellowness (b value) of all samples did not shift significantly during storage at 4°C for 4 weeks.

Table (5): Value "L" at one month intervals of cloudy apple drinks produced by different concentratons of Cellulase 13L , Pectinase 444L and Macer8^{TM}FJ, during storage at refrigerator temperature (4°C).

Treatment	Storage (month)						Mean
	0	1	2	3	4	5	
Con	37.5	36.8	35.8	35.4	34.4	33.6	35.62 a
C0.1	36.9	35.5	35.1	34.3	33.4	33.3	34.76 c
C0.05	37.1	36.0	35.8	34.2	32.9	32.9	34.81 c
C0.025	37.5	36.5	36.0	35.0	33.5	33.3	35.29abc
P50	36.8	36.0	35.7	34.3	33.7	32.5	34.84 c
P25	37.4	36.6	35.7	34.3	33.1	31.4	35.04 bc
P10	37.8	37.5	36.8	35.4	33.9	33.4	35.79 a
M50	36.7	35.9	35.4	34.7	33.1	32.8	34.76 c
M25	37.4	36.6	35.7	34.3	33.1	31.4	34.76 c
M10	37.5	36.2	35.9	35.7	33.9	33.2	35.39 ab
Mean	37.24 a	36.33 b	35.82 c	34.84 d	33.54 e	32.87 f	

Mean in the same column with different superscripts are significantly different (p < 0.05).

Table (6): Value "a" at one month intervals of cloudy apple drinks produced by different concentratons of Cellulase 13L , Pectinase 444L and Macer8^{TM}FJ, for 5 months at refrigerator temperature (4°C).

Treatment	Storage (month)						Mean
	0	1	2	3	4	5	
Con	2.8	3.0	3.2	3.5	3.6	3.9	3.32 bc
C0.1	2.8	3.0	3.1	3.4	3.6	4.0	3.31 bc
C0.05	2.8	3.1	3.13	3.3	3.43	3.77	3.25 c
C0.025	2.67	2.93	3.2	3.43	3.6	3.93	3.29 bc
P50	2.93	3.13	3.36	3.47	3.8	4.17	3.48 ab
P25	2.7	2.83	3.1	3.23	3.6	3.9	3.29 bc
P10	2.7	3.1	3.2	3.3	3.4	3.77	3.24 c
M50	2.7	3.03	3.3	3.6	3.93	4.5	3.51 a
M25	2.77	2.9	3.07	3.5	3.77	4.2	3.39abc
M10	2.7	2.83	3.1	3.23	3.6	3.9	3.23 c
Mean	2.76 f	3.01 e	3.18 d	3.4 c	3.62 b	4.02 a	

Mean in the same column with different superscripts are significantly different (p < 0.05).

Table (7): Value "b" at one month intervals of cloudy apple drinks produced by different concentratons of Cellulase 13L , Pectinase 444L and Macer8^{TM}FJ, for 5 months at refrigerator temperature (4°C).

Treatment	Storage (month)						Mean
	0	1	2	3	4	5	
Con	4.13	4.17	4.33	4.3	4.43	5.1	4.41 a
C0.1	4.1	4.27	4.37	4.5	4.6	5.6	4.56 a
C0.05	4.1	4.2	4.23	4.47	4.87	5.67	4.59 a
C0.025	4.1	4.27	4.5	4.73	4.8	4.93	4.56 a
P50	3.97	4.07	4.3	4.73	5.1	5.8	4.66 a
P25	3.8	4.03	4.23	4.43	4.63	4.73	4.44 a
P10	3.9	4.1	4.23	4.37	4.53	4.63	4.29 a
M50	3.83	4.13	4.4	4.43	4.73	4.9	4.38 a
M25	3.8	4.03	4.23	4.43	4.63	4.73	4.31 a
M10	3.93	4.13	4.3	4.4	4.67	4.97	4.40 a
Mean	3.97 e	4.15 de	4.30 d	4.49 c	4.71 b	5.14 a	

Mean in the same column with different superscripts are significantly different (p < 0.05).

Acidity % of cloudy apple drinks were shown in Table (8). At zero-time, C0.1, P50 and M50 showed the highest acidity of 0.214, 0.216 and 0.213 %, respectively. Differences between treatments were significantly affected ($p < 0.05$) by the storage time since significant interaction occurred between treatments and storage time. The control, P50, C0.1 and M50 showed the highest means of acidity within the six months of storage which were o.234, 0.233, 0.23 and 0.231 %, respectively. The lowest significant acidity mean was 0.227% for M25.

The storage time affected the acidity % of cloudy apple drinks significantly ($p < 0.05$). The means of different treatments were significantly stable at zero time and throughout the first month which were 0.210 and 0.212 %, respectively. Then significant increase in acidity mean to o.237% declared at the third month and continued up to the last month (mean=0.25%).

Values of pH of apple drinks were falling with progressing of the storage time (Table 9). Values of pH underwent significant ulterations within the storage period. At zero-time the mean of pH value was 3.75 which had significant decrement each month up to 3.6 at the sixth month.

The pH mean of the treatments showed significant differences throughout the storage time. Control, C0.025 and M10 showed the highest mean value of pH which were 3.69, 3.689 and 3.696, respectively. While P50, P25, M50 and M25 had significantly the lowest means of 3.663, 3.661, 3.663 and 3.661, respectively.

Table (8): Acidity (as citric acid %) at one month intervals of cloudy apple drinks produced by different concentratons of Cellulase 13L, Pectinase 444L and Macer8^{TM}FJ, during storage at refrigerator temperature (4°C).

Treatment	Storage (month)							Mean
	0	1	2	3	4	5	6	
Con	0.211	0.213	0.217	0.243	0.246	0.250	0.256	0.234 a
C0.1	0.214	0.216	0.223	0.235	0.243	0.245	0.248	0.23 ab
C0.05	0.209	0.211	0.218	0.239	0.239	0.241	0.243	0.229 bcd
C0.025	0.207	0.209	0.209	0.237	0.237	0.245	0.250	0.228 cd
P50	0.216	0.218	0.218	0.239	0.243	0.245	0.250	0.233 a
P25	0.211	0.211	0.213	0.239	0.241	0.248	0.252	0.231 abc
P10	0.207	0.209	0.209	0.237	0.239	0.245	0.248	0.228 cd
M50	0.213	0.213	0.216	0.235	0.243	0.248	0.250	0.231 ab
M25	0.207	0.207	0.211	0.232	0.241	0.245	0.248	0.227 d
M10	0.205	0.209	0.209	0.237	0.237	0.241	0.256	0.228 cd
mean	0.210 f	0.212 f	0.214 e	0.237 d	0.241 c	0.245 b	0.25 a	

Mean in the same column with different superscripts are significantly different (p < 0.05).

Table (9): pH at one month intervals of cloudy apple drinks produced by different concentratons of Cellulase 13L , Pectinase 444L and Macer8^{TM}FJ, for 6 months at refrigerator temperature (4°C).

Treatment	Storage (month)							Mean
	0	1	2	3	4	5	6	
Con	3.75	3.76	3.74	3.68	3.66	3.64	3.63	3.69 a
C0.1	3.76	3.74	3.73	3.66	3.64	3.63	3.59	3.679 ab
C0.05	3.76	3.74	3.71	3.67	3.65	3.61	3.60	3.679 ab
C0.025	3.76	3.76	3.72	3.71	3.65	3.61	3.62	3.689 a
P50	3.75	3.71	3.67	3.66	3.63	3.62	3.59	3.663 b
P25	3.74	3.72	3.65	3.64	3.63	3.63	3.59	3.661b
P10	3.74	3.74	3.70	3.69	3.64	3.63	3.61	3.678 ab
M50	3.74	3.72	3.71	3.65	3.64	3.60	3.58	3.663 b
M25	3.74	3.73	3.67	3.66	3.63	3.60	3.59	3.661 b
M10	3.74	3.74	3.73	3.70	3.68	3.65	3.64	3.696 a
mean	3.751 a	3.737 b	3.703 c	3.672 d	3.645 e	3.624 f	3.604 g	

Mean in the same column with different superscripts are significantly different (p < 0.05).

Concerning the percent of reducing sugars presented in Table (10), M50 showed significantly the highest percent of reducing sugars (mean=3.59), in comparison with other treatments within the storage period. Control, C0.025 and P10 were significantly the lowest drinks in reducing sugars contents.

In all treatments percent of reducing sugars followed the same curve with progressing of the storage time. Mean of reducing sugars increased significantly from 2.45 % at zero-time to 4.6 % at the second month, then lowered significantly from 3.44 % at the third month to 1.89 % at the end of the storage period.

The total sugars reached its highest means in the highest concentration enzymatic treatments of C0.1, P50 and M50 which recorded means of 13.14, 13.39 and 13.32 %, respectively. While the control had significantly the lowest mean which was 11.24%. Other treatments exhibited lower insignificant means during the storage time (Table 11). Resultes showed that the increase of total sugars was related to the enzyme treatment and was proportional to the increase of enzyme concentration.

Table (10): Percent of reducing sugars at one month intervals of cloudy apple drinks produced by different concentratons of Cellulase 13L , Pectinase 444L and Macer8^{TM}FJ, during storage at refrigerator temperature (4°C).

Treatment	Storage (month)							Mean
	0	1	2	3	4	5	6	
Con	2.2	3.55	4.26	3.10	2.64	2.56	1.59	2.85 c
C0.1	2.6	4.44	4.65	3.78	2.6	2.98	2.19	3.37 ab
C0.05	2.26	3.74	4.76	3.38	2.93	2.75	1.89	3.10 bc
C0.025	1.87	3.96	4.43	2.93	2.75	2.52	1.54	2.87 c
P50	2.76	4.37	4.73	3.62	3.21	3.05	2.18	3.42 ab
P25	2.69	4.32	4.61	3.34	2.83	3.05	2.01	3.25 abc
P10	2.01	3.32	4.55	3.20	2.70	2.70	1.67	2.88 c
M50	2.9	4.66	4.79	4.20	3.34	2.92	2.34	3.59 a
M25	2.89	4.5	4.76	3.73	3.02	2.81	1.90	3.37 ab
M10	2.33	4.0	4.49	3.14	2.71	2.62	1.59	2.98 bc
Mean	2.45 e	4.09 b	4.60 a	3.44 c	2.9 d	2.80 d	1.89 f	

Mean in the same column with different superscripts are significantly different (p < 0.05).

Table (11): Percent of total sugars at one month intervals of cloudy apple drinks produced by different concentratons of Cellulase 13L , Pectinase 444L and Macer8^{TM}FJ, during storage at refrigerator temperature (4°C).

Treatment	Storage (month)							Mean
	0	1	2	3	4	5	6	
Con	11.75	12.34	11.62	11.06	10.74	10.69	10.47	11.24 c
C0.1	13.59	13.6	12.62	13.46	13.59	13.6	12.02	13.14 a
C0.05	12.62	13.32	12.25	12.05	12.01	11.99	11.58	12.26abc
C0.025	11.13	12.72	11.98	11.02	11.19	11.03	10.75	11.40 bc
P50	13.75	13.98	12.62	13.47	13.66	13.45	12.78	13.39 a
P25	12.7	13.6	12.26	12.30	12.88	12.52	12.43	12.67 ab
P10	11.76	13.06	12.01	11.28	11.83	11.70	10.07	11.69 bc
M50	13.92	14.01	12.52	12.81	13.58	13.40	12.98	13.32 a
M25	12.97	13.77	12.14	12.13	13.12	12.56	12.36	12.71 ab
M10	13.02	13.2	12.01	11.73	12.49	12.13	11.67	12.23abc
Mean	12.7ab	13.3a	12.20b	12.06b	12.5ab	12.26b	11.70b	

Mean in the same column with different superscripts are significantly different (p < 0.05).

In respect of storage time, total sugars underwent significant and insignificant alterations within the six months storage. As the mean of the

total sugars percent of different treatments raised insignificantly from 12.7 % at zero-time to 13.37 % at the first month, then decreased significantly to 12.2 % at the second month and remained insignificant changes to the end of the storage period.

Concerning ascorbic acid (AA) contents of cloudy apple drinks recorded in Table (12), the treatments showed very low contents of AA, ranged from 1.16 to 1.39 mg/100 mL at zero-time.Contents of AA decreased significantly ($p < 0.05$) with progressing of the storage time as the mean of AA contents of all treatments reduced significantly from 1.21 mg/100mL at zero-time to 0.481 mg/100mL at the sixth month. According to Mostafa (2004) , ascorbic acid (AA) contents ranged between 61.3- 62.4 mg/ 100mL in pulpy apple juices which were fortified with 50 mg/ 100mL.

Table (12): Ascorbic acid contents (mg/100mL) at one month intervals of cloudy apple drinks produced by different concentratons of Cellulase 13L , Pectinase 444L and Macer8^{TM}FJ, for 6 months at refrigerator temperature (4°C).

Treatment	Storage (month)							Mean
	0	1	2	3	4	5	6	
Con	1.16	0.93	0.87	0.780	0.677	0.551	0.522	0.783 ab
C0.1	1.39	1.16	0.95	0.760	0.677	0.551	0.540	0.861 a
C0.05	1.16	1.04	0.930	0.740	0.580	0.551	0.480	0.783 ab
C0.025	1.16	1.04	0.930	0.790	0.580	0.551	0.522	0.796 ab
P50	1.16	1.04	0.970	0.730	0.580	0.551	0.483	0.787 ab
P25	1.16	0.93	0.87	0.750	0.580	0.540	0.480	0.759 b
P10	1.16	0.93	0.87	0.770	0.551	0.480	0.425	0.741 b
M50	1.39	1.04	0.970	0.780	0.599	0.530	0.474	0.826 ab
M25	1.27	0.93	0.87	0.750	0.580	0.522	0.435	0.766 b
M10	1.16	0.93	0.87	0.750	0.580	0.522	0.445	0.75 b
Mean	1.21a	0.995b	0.910c	0.760d	0.598e	0.536f	0.481g	

Mean in the same column with different superscripts are significantly different (p < 0.05).

Microbiological evaluation

The log numbers of total bacterial count for cloudy apple drinks were less than one (< 1) till the end of the storage period as shown in Table (13). For yeast and molds, no microbial growths were detected at zero-time, and for periodical assessments it was less than 30 CFU/mL, till the end of the storage time (Table 14) which indicated the good microbiological quality of cloudy drinks stored for six months at refrigerator temperature.

Table (13): The log numbers of total bacterial count of cloudy apple drinks stored at refrigerator temperature (4°C) for six months.

Treatment	Time of assessment (month)				
	0	1.5	3	4.5	6
Con	< 1	< 1	< 1	< 1	< 1
C0.1	< 1	< 1	< 1	< 1	< 1
C0.05	< 1	< 1	< 1	< 1	< 1
C0.025	< 1	< 1	< 1	< 1	< 1
P50	< 1	< 1	< 1	< 1	< 1
P25	< 1	< 1	< 1	< 1	< 1
P10	< 1	< 1	< 1	< 1	< 1
M50	< 1	< 1	< 1	< 1	< 1
M25	< 1	< 1	< 1	< 1	< 1
M10	< 1	< 1	< 1	< 1	< 1

Table (14): Total count of molds and yeasts (CFU/mL) for cloudy apple drinks stored at refrigerator temperature (4°C) for six months.

Treatment	Time of assessment (month)				
	0	1.5	3	4.5	6
Con	-	< 30	< 30	< 30	< 30
C0.1	-	< 30	< 30	< 30	< 30
C0.05	-	< 30	< 30	< 30	< 30
C0.025	-	< 30	< 30	< 30	< 30
P50	-	< 30	< 30	< 30	< 30
P25	-	< 30	< 30	< 30	< 30
P10	-	< 30	< 30	< 30	< 30
M50	-	< 30	< 30	< 30	< 30
M25	-	< 30	< 30	< 30	< 30
M10	-	< 30	< 30	< 30	< 30

Sensory evaluation

Means of periodical sensory evaluations of cloudy apple drinks during storage at refrigerator temperature (4°C) for six months and their evaluations in comparison with flavoured treatment, are presented in the Tables from (15) to (20). Because of the diversity of the treatments it is hard to discuss their sensory evaluations in details but results could be summarized in the following comment:

Colour assessment of cloudy apple drinks processed by different pectinases and cellulase treatments showed no visual differences in their colour, in comparison with the control. Colour quality indicated "very good" for all treatments at zero-time which lowered significantly to "satisfactory" by the end of the storage period. Taste of the treatments was good at zero-time. With progressing of the storage period, taste became better. By the sixth month, assessment mean of all treatments reduced significantly to "satisfied". Aroma of the treatments including the control were insignificant. With extension of the storage period the aroma weakened significantly to "satisfactory". All treatments showed "very good" appearance quality. By the end of the sixth month appearance quality decreased significantly to "good" which could be due to the colour deterioration by the end of the storage period. All treatments exhibited "very good" to "good" mouthfeel quality, then decreased significantly by the sixth month. This could be explained by the reduction in taste and aroma quality by the end of the storage period. Addition of apple flavouring agent to the drinks improved taste, flavour and mouthfeel and increased the overal acceptability of the drinks.

Table (15): Mean of periodical sensory evaluations of cloudy apple drinks from juices extracted by different cellulase concentrations, stored at refrigerator temperature (4°C) for six months.

Treatment	Character				
	colour	taste	aroma	Appearance	mouthfeel
C0.1	5.66a	4.92a	4.62 a	5.58 a	5.3 a
C0.05	5.66a	4.8 a	4.28 a	5.44 a	5.3 a
C0.025	5.42a	4.86 a	4.24 a	5.38 a	5.3 a
Cont	5.5a	4.92 a	4.3 a	5.44 a	5.3 a

Mean in the same column with different superscripts are significantly different (p < 0.05).

Table (16): Sensory evaluation of cloudy apple drinks from juices extracted by different cellulase concentrations in comparison with flavoured treatment.

Treatment	Character					Overall acceptability
	colour	taste	aroma	appearance	Mouth-feel	
C0.1	4.9	4.4	4.8	4.7	4.8	23.6 ab
C0.05	4.4	3.8	3.7	4.4	4.4	20.7 b
C0.025	4.4	3.9	3.9	4.5	4.7	21.4 b
Con	4.5	4.0	4.1	4.2	4.7	21.5 b
C0.1+F	4.6	5.9	5.9	4.8	6.1	27.3 a

Mean in the same column with different superscripts are significantly different (p < 0.05).

Table (17): Mean of periodical sensory evaluations of cloudy apple drinks from juices extracted by different Pectinase 444L concentrations, stored at refrigerator temperature (4°C) for six months.

Treatment	Character				
	colour	taste	aroma	appearance	mouthfeel
P50	5.68 a	5.14 a	4.68 a	5.66 a	5.38 a
P25	5.62 a	4.84 a	4.42 a	5.56 a	5.32 a
P10	5.56 a	5.02 a	4.62 a	5.62 a	5.3 a
Con	5.42 a	4.64 a	4.46 a	5.38 a	5.0 a

Mean in the same column with different superscripts are significantly different (p < 0.05).

Table (18): Sensory evaluation of cloudy apple drinks processed by different Pectinase 444L concentrations in comparison with flavoured treatment.

treatment	Character					Overall acceptability
	colour	taste	aroma	appearance	mouthfeel	
P50	**5.5**	**4.6**	**4.3**	**4.8**	**4.4**	**23.6 ab**
P25	5.3	3.9	3.8	4.8	4.5	22.3 b
P10	4.9	4.2	3.8	5.0	4.4	22.3 b
Con	4.5	3.2	3.4	4.4	3.8	19.3 c
P50+F	4.8	5.3	5.5	5.2	5.4	26.2 a

Mean in the same column with different superscripts are significantly different (p < 0.05).

Table (19): Mean of periodical sensory evaluations of cloudy apple drinks from juices extracted by different Macer8^{TM}FJ concentrations, stored at refrigerator temperature (4°C) for six months.

Treatment	Character				
	colour	taste	aroma	appearance	mouthfeel
M50	5.62 a	5.22 a	4.4 a	5.68 a	5.30 a
M25	5.52 a	5.04 a	4.4 a	5.56 a	5.38 a
M10	5.26 a	5.18 a	4.56 a	5.32 a	5.36 a
Con	5.34 a	4.92 a	4.36 a	5.38 a	5.34 a

Mean in the same column with different superscripts are significantly different (p < 0.05).

Table (20): Sensory evaluation of cloudy apple drinks processed by different macer concentrations in comparison with flavoured treatment.

Treatment	Character					Overall acceptability
	colour	taste	aroma	appearance	mouthfeel	
M50	**4.7**	**4.6**	**4.5**	**5.3**	**4.5**	**23.6 b**
M25	4.8	4.2	4.2	5.3	4.8	23.3 b
M10	4.3	4.9	4.6	4.7	4.8	23.3 b
Con	4.4	4.3	4.0	4.9	4.7	22.3 b
M50+F	4.6	5.9	5.9	5.3	5.6	27.3 a

Mean in the same column with different superscripts are significantly different (p < 0.05).

CONCLUSION

Addition of pectinases and cellulase increased TSS and juice yield on the expenses of clould stability. Conducted concentrations of the enzymes showed good balance between yield, TSS and cloud stability. All treatments including the control were highly accepted by the panelists and showed not less than "satisficatory" by the end of the storage period for any quality aspects. Also drinks remained good microbiological quality till the end of the storage period.

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إنتاج عصير التفاح غير الرائق باستخدام الأنزيمات البكتينية

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إستخدمت ثمار تفاح صنف آنا (*Malus spp*., Anna cultivar) و التى تمت زراعتها فى مدينة رشيد – مخافظة البحيرة- مصر لإنتاج مشروب التفاح غير الرائق بإستخدام مستحضرات تجارية مختلفة من الإنزيمات البكتينية وكذلك إستخدم إنزيم سليولاز كمعاملة مقارنة. إجرى سلق لب التفاح فى فرن الميكروويف لمدة ٤ ق لمنع التلون ثم إضيفت الإنزيمات إلى مهروس اللب بنسب مختلفة على ٤٥° م لمدة ٣٠ ق.

خزنت مشروبات التفاح الغير رائقة لمدة ستة أشهر على درجة حرارة الثلاجة و أجريت التحليلات التالية لها : تقدير درجة البركس، نسبة الحموضة كحمض ستريك، قيم الـ pH ، ثبات العكارة (الإمتصاص على ٦٦٠ نانوميتر)، و قيم اللون (L ,a, b) و نسبة السكريات الكلية والمختزلة شهريا.

و قد أوضحت النتائج أن إضافة الإنزيمات البكتينية و السليولوزية تعمل على زيادة الريع و المواد الصلبة الذائبة على حساب درجة ثبات العكارة، و لكن أمكن إستخدام تركيزات مناسبة من الإنزيمات للحصول على ريع جيد و صوالب ذائبة أعلى بالمقارنة بمعاملة الكونترول والحفاظ على عكارة جيدة. أعطت جميع المعاملات قبول عالى فى التحكيم الحسى و ظلت مقبولة حتى نهاية فترة التخزين. كذلك إستمرت جميع المعاملات صالحة للإستهلاك من الناحية الميكروبيولوجية حتى نهاية التخزين.