Mesopotamia J. of Agric. ISSN:2224-9796(Online) مـــجـلة زراعـــة الـرافـدين
Vol. (41) No. (4) 2013 ISSN: 1815-316x (Print) المجلد (41) العدد (4) 2013

EFFECT OF *FUNGAL* PECTINASES AND GELATIN ON APPLE JUICE CLARIFICATION

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ABSTRACT

Clarifying of raw apple juice using different concentrations of fungal pectinases (5–30 IU) and gelatin (0.00–0.02%) was investigated. Different temperatures (30-60 ^{o}C) and holding times (1-7 hours) were studied. Combined mixture of *Aspergillus niger* pectinases and gelatin had more efficiency on juice clarification than using the enzyme alone. The clarified apple juice samples were examined for transmittance at 690 nm ($\%T_{690}$). The highest clarification effect ($\%T_{690}$=85.5) ($p<0.001$) was achieved with 25 IU/mL and 0.015 % gelatin at 50 ^{o}C and 5 hour holding time.

Keywords: apple juice, pectinases, polygalacturonase, gelatin, clarification.

Received: 1/2/2012 Accepted: 18/6/2012

INTRODUCTION

Raw apple juice is characterized as a complex colloid containing various components. It has been reported that the cloudy apple juice acquires relatively high quantities (4.4g/l) of polymers such as polysaccharides, pectin, protein, etc (Lukanin *et al*., 2003). Benitez and Lozano (2007) mentioned that the cloudy apple juice is a colloidal suspension where the continuous medium is a solution of pectin, sugars, and malic acid, and the dispersed materials are mainly formed by cellular tissue comminuted during fruit processing. These colloidal materials affect the juice cloudiness and stability and consumer's preference of juice of which the clarification of apple juice has been considerably focused on. The haze component of apple juice is considered to be a protein-carbohydrate complex containing 36% protein (Yamasaki *et al*., 1994). This colloidal complex which is suspended in a dispersed phase, is very small in size (~ 1 – 1000 nm) on which the gravitational forces are negligible (Benitez and Lozano, 2007). However, the surfaces of the protein molecules as well as other carbohydrates in the apple juice are negatively charged at pH 3.5. Consequently, there are positively charged proteins beneath the negative charged coat. When a partial hydrolysis of the negative charged coats occurs, the positively charged molecules are exposed and lead to the attraction between different charged molecules and eventually cause flock formation. Enzymatic treatment using specific enzymes (pectinases) cause breaking of pectin, so the positively charged proteins become partially exposed on the particle surface, promoting flocculation (Lozano *et al*., 2007; Tajchakavit *et al*., 2001). Enzymatic treatment also allows an efficient use of clarifying agents to assist cloud removal (Singh and Gupta, 2004). Addition of fining or clarifying agents is intended to modify clarity, color, flavor and/or stability of juices. They are grouped according to their general nature into (i) Earths (bentonite, kaolin); (ii) Proteins (gelatin, isinglass, casein, albumin); (iii) Polysaccharides (agars); (iv) Carbons; (v) Synthetic polymers (PVPP, nylon); (vi) Silicone dioxide (kieselsols); and (vii) Others,

including metal chelators, enzymes, etc. (Zoecklein, 1988). These fining agents work either by sticking to the particles or by using charged ions to cause particles to stick to each other, making them heavy enough to sink to the bottom by the action of gravity. What is left is a transparent though not a clear juice. Subsequent filtration operations are needed to obtain a crystal clear product. Differences in the nature of ionic charges of protein, polyphenols and the fining agents, induce flocculation and sedimentation and result in the removal of these potential haze precursors from solution. However, clarification of apple juice using gelatin is a common industrial practice ((Stocké, 1998). Gelatin forms gelatin-tannin complexes causing more clarification. In this work, effect of gelatin on the efficiency of a fungal pectinases has been studied for the clarification of apple juice.

MATERIALS AND METHODS

Apple juice preparation: Green apples produced in Chile were purchased from a local market and washed with tap water to remove any adhering substances. Peeled apples were introduced in a centrifugal juice extractor (AL-165 JE, ALONSA, Japan) in order to obtain the raw apple juice. The juice enzymes were inactivated by heat (90 ^{0}C for 5 min) and the juice was filtered through a cheese cloth in order to remove the remaining pulp before the clarification step.

Pectinases production: pectinases were produced from *Aspergillus niger* (local isolate). Production media contained in gL^{-1}: KH_2PO_4, 4; Na_2HPO4, 6; $FeSO_4.7H_2O$, 0.1, $CaCl_2$, 0.01; $MgSO_4.7H_2O$, 0.2; $(NH_4)_2SO_4$, 2; pectin, 12 (pH 4.5) (Maldonado and Saad, 1998).

Polygalacturonase (PG) activity: It was determined by the method of Maldonado and Saad (1998) using the cultural filtrate. International Unit (IU) was expressed as the micromoles of galacturonic acid released/mL per minute at 50 ^{0}C.

Apple juice clarification: Raw apple juice samples were clarified by adding 15 IU of enzyme to 10 mL of juice at temperature range (30–60 ^{0}C) at 1 hour time intervals up to 7 hours in the presence of 0.01% gelatin. The resulted samples were centrifuged at 2500 rpm for 15 min and filtered through Whatman paper no. 2 . The filtrate was examined for transmittance at 690 nm ($\%T_{690}$) using spectrophotometer (APEL UV-VIS Spectrophotometer PD-303, Japan) of which the $\%T_{690}$ values were expressed as juice clarification level. Both the enzyme and gelatin were combined at various enzyme levels (5–30 IU) and gelatin (0.00 – 0.02%) where they were added to 10 mL of juice. The polygalacturonase was used in the form of filtered broth and each 1 mL was equivalent to 18 IU of enzyme.

Statistical Analysis: The study was designed as C.R.D with three replicates and the date were subjected to analysis of variance using SAS computer program (1997).

RESULTS AND DISCUSSION

Table 1 shows the effect of different temperatures and holding times the in presence of 0.01% gelatin and 15 IU of PG on the apple juice clarification expressed as the transmittance value at 650 nm ($\%T_{650}$). This experiment was set to find out the best combination ratio of PG and gelatin that can be selected for best holding time (hr) and temperature (^{0}C). In general, it can be observed that the apple Juice clarification increased with increasing temperatures implemented in this study. However, the maximum $\%T_{690}$ (83.1, $P< 0.001$) was observed at 50 ^{0}C after

Table (1): Effect of temperature and holding time on the clarification of apple juice

holding time (hr)	$\%T_{650}$			
	30 ^{o}C	40 ^{o}C	50 ^{o}C	60 ^{o}C
0	12.32 t	12.30 t	12.34 t	12.31 t
1	14.24 s	18.81 q	22.72 p	16.73 r
2	18.41 q	35.81 o	47.31 l	34.90 o
3	36.22 o	51.92 k	68.21 f	41.52 n
4	43.91 m	66.73 f	74.30 d	55.13 j
5	57.33 i	76.80 c	83.05 a	61.44 h
6	71.90 d	80.65 b	81.60 ab	63.81 g

Figures with different letters are significant ($p<0.05$)

5 hr. It was observed that the enzymatic clarification of juice with adding 0.01 % of gelatin had reduced to half time of juice clarification (Hsu *et al*., 1989; Kristenov and Dimitrova, 1993). Nevertheless, other studies which were carried out using enzymatic treatment have recorded different optimum temperature and holding time for fruit juice clarification. A temperature of 45 ^{0}C and holding time of 1 hr were found optimum for apple juice clarification (Gupta *et al*., 2003). Whereas, Ishii and Yokotsuka (1972) reported that 45-50 ^{0}C was optimum temperature for apple juice clarification using pectin trans-eliminase. The variation in these results as compared with this study concerning the optimum conditions of apple juice clarification could be referred to variety, origin and/or to enzyme preparation method.

According to the results obtained in Table (1) which states the optimum temperature (50 ^{0}C) and holding time (5 hr) for apple juice clarification, an experiment of using various concentrations of enzyme and gelatin was conducted to find out the best level of juice clarification. The best ($p<0.001$) juice clarification (85.5 $\%T_{690}$) was obtained with 25 IU of enzyme and 0.015 % gelatin (Table 2).

Table (2): % transmittance at 690 nm of different combinations of enzyme /gelatin

Enzyme Conc. (IU/10 mL juice)	% T_{690} nm at different gelatin conc. (%)				
	0.00	0.005	0.01	0.015	0.02
5	18.00 r	30.45 p	55.05 k	62.20 i	72.05 g
10	28.40 q	52.10 l	72.90 g	75.60 f	80.25 e
15	35.40 o	58.90 j	80.10 e	81.40 d	82.50 c
20	43.65 n	70.70 h	82.85 c	83.45 bc	84.25 b
25	46.60 m	79.75 e	84.25 b	85.50 a	83.30 bc
30	46.80 m	80.20 e	83.70 bc	84.10 b	83.40 bc

Figures with different letters are significant ($p<0.05$)

Table 2 shows that the increasing enzyme and gelatin concentrations caused increase in % T_{690} values (i.e. juice clarification values). This could be due to that the increase of enzyme concentration had led to exposure of higher positively charged molecules (particles) which were attracted by negatively charged molecules leading to flock formation. However, increasing gelatin concentration induced more sticking affinity of particles causing them to stick to each other and making them

heavy enough to sink in the bottom by the action of gravity (Benitez and Lozano, 2007). It can be also noticed that at the same enzyme concentration (25 IU) but with less gelatin (0.01%) has given good result ($p< 0.05$) for juice clarification (% T_{690} nm). Commercial apple juice production does not encourage excessive concentration of gelatin because it is considered to be the main cause of after-bottling haze (Van Buren, 1989). Additionally, it has been reported that the risk of haze by free gelatin in apple juice required at least 10 times more gelatin than the optimum dosage for clarification (Benitez and Lozano, 2007). It can be concluded that the highest apple juice clarification ($\%T_{690}$, 85.5) ($p<0.001$) was obtained at 50 ^{0}C and 5 hr holding time and the enzymatic treatment of apple juice was more effective when enzyme/gelatin combination was adopted.

تأثير البكتينزات الفطرية والجلاتين في ترويق عصير التفاح

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الخلاصة

تم ترويق عصير التفاح الخام باستخدام تراكيز مختلفة من مستخلص أنزيم البولي كالاكتيورونيز الفطري (5-30 وحدة إنزيمية) ومسحوق الجلاتين (0-0.02 %). تم اختبار درجات حرارية مختلفة (30-60 ^{0}م) وفترات مختلفة من الحضن (1-7 ساعة) في هذه الدراسة. وجد أن استخدام خليط من أنزيم البولي كالاكتيورونيز المستخلص من فطر *Aspergillus niger* كان أكثر كفاءة في ترويق العصير من استخدام الأنزيم لوحده. ارتفعت درجة ترويق عصير التفاح بارتفاع درجات الحرارة والفترة المستعملة في الترويق. قدرت درجة ترويق نماذج عصير التفاح بقياس نسبة الشفافية على طول موجي 690 نانوميتر ($\%T_{690}$). كان أعلى نسبة ترويق للعصير ($\%T_{690}$= 85.5) ($p<0.001$) عند استعمال تركيز للأنزيم قدره 25 وحدة أنزيمية/مللتر وتركيز للجلاتين قدره 15% عند درجة حرارة 50 ^{5}م ولفترة 5 ساعات.

كلمات داله: عصير التفاح، البكتينزات، كالاكتيورونيز، جيلاتين، ترويق.

تاريخ تسليم البحث: 2012/2/1 وقبوله: 2012/6/18

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