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Effect of Enzymatic Treatment on Viscosity and on Pectic Substances of Tomato Juice

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ABSTRACT

Effect of treatment of hot break single strength tomato juice (SSTJ) with different concentrations of exogenous pectin methylesterase (0, 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 U/ 100 ml tomato juice) (PME) produced by *Aspergillus niger* under optimum conditions (45°C and pH= 4 for 10 min in a water bath) selected based on a preliminary experiment was investigated in this study. The maximum viscosity value (342 cP) for tomato juice was obtained when pectin methylesterase concentration was added as (25 U/ 100 ml juice). The changes in some properties of (SSTJ) were evaluated after pasteurization and bottling in amber glass bottles and during storage at 4 and 22°C for 6 weeks. It was found that there was a significant increasing in viscosity for enzymatic treated (SSTJ) samples after pasteurization, comparatively to the viscosity value of enzymatic untreated SSTJ (control), storage for 6 weeks at two respective temperatures, caused an insignificant decreasing in the viscosity of enzymatic treated (SSTJ) samples. A significant increasing in GalA for WSP was observed in juices after their pasteurization and storage at 4 and 22°C for 6 weeks. The results indicated that there were significant decreasing in GalA for CSP whereas, the decreasing of GalA for NSP was insignificant for enzymatic treated tomato juices as compared with untreated tomato juices. The DM for WSP and CSP in treated samples were significantly lower than that for untreated samples after pasteurization and bottling and during storage for 6 weeks at 4 and 22°C. The DM of NSP could not be assaying due to the saponification of methylesters during the alkali extraction of this fraction.

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INTRODUCTION

Tomato consumption has greatly increased over the past twenty years, mostly because of a growing necessity for tomato products such as tomato juices (Queralt *et al.*, 2013). Thermal processing is the more method to extend shelf life of tomato juice by killing microorganisms and inactivation of enzymes. Pectins are a largest component of plant walls and affect the quality of many based plant products. While great variability in pectin composition exists across the plant; in fruits and vegetables galacturonic acid comprises (>80)% pectin carbohydrate (Ridley *et al.*, 2001). Pectin galacturonic acid is found in many; polymeric forms, homogalacturonan (HG); a linear α , 1-4 linked galacturonic acids containing different levels of methyl esterification of the (COOH) group and acetylation on C-2; rhamnogalacturonan I (RG-I), a repeating galacturonic and rhamnose; and rhamnogalacturonan II (RG-II), a homogalacturonic backbone with side chains containing; rhamnose and sugars. Pectin methylesterase (PME, E. C. 3.1.1.11 class eight of carbohydrate esterases) is an enzyme of either plant or microbial origin that catalysis of the methylester bond at sixth carbon atom of a galacturonic

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acid residue in the linear homogalacturonan domain of pectin, thus altering the degree and pattern of methyl esterification and releasing methanol and protons (Jolie *et al.*, 2010). Changes in different properties during steps of processing of tomatoes are nearly related with modifications in the pectin; firstly by the action of endogenous pectin methylesterase; (PME) and polygalacturonase; (PG) (Ridley *et al.*, 2001). The presence of both active PME; PG; during processing of tomato results deesterified pectin chains and; as a consequence, in drastic changes of the end products; (e.g. decrease in viscosity). It is proclaimed the literature that, in order to improve the quality characteristics of processed juices, the inactivation of endogenous PME; and PG, are critical factor. The objective of the present work was to determine the effect on some properties of single strength tomato juice (SSTJ) treated by exogenous fungal PME under identical conditions.

MATERIALS AND METHODS

Materials: Red ripe Roma tomatoes (*Lycopersicon esculentum*) were purchased from a local supermarket (Arkansas state; USA); and storage at (4)°C for processing. Pectin methylesterase from *Aspergillus niger* was obtained from DSM company (U/ 100 ml) (Delft, Netherlands). All other materials (chemical and reagent) used were of analytical grade.

Experimental treatments: The surfaces of the tomatoes were washed with tap water, and fruits were left to dryness at room temperature for (15) minutes, and chopped by; mill (Chicago, IL., U.S.A.). The chopped tomatoes were treated in a heat exchanger type; tubular (Kenosha, Wis., USA), for (3 min; at 93°C) as process of hot break. Single strength juice was obtained using screw extractor (CJED28, Niagara; N.Y.); by screen of (1.27) cm; diameter.

Enzymatic deesterification: A preliminary experiment was conducted to know the required added number of units of pectin methylesterase to get maximum value of viscosity of tomato juice through enzymatic deesterification for pectic substances in tomato juice. The experiment carried out by incubation enzyme with juice at 45°C and pH= 4 for 10 min in a water bath (Banjongsinsiri *et al.*, 2004). The concentrations of enzyme added as follows: 0, 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 U/ 100 ml tomato juice, respectively, then, the viscosity of tomato juice was determined for each concentration under identical conditions.

Thermal processing, Bottling and storage: both enzymatic treated (25 U/ 100 ml juice) and untreated tomato juices were held, (92°C for; 90 s) in a heat exchanger, hot filled into 250 ml amber glass bottles. They were quickly cooled with tap water until the temperature of the bottles was 25°C. Bottling juices were stored at 4 and 22°C for 6 weeks.

Analytical methods: viscosity, galacturonic acid content of water, chelator and sodium carbonate soluble pectin fractions, and degree of esterification of water and chelator soluble pectin fractions were determined after bottling in amber glass bottles and during storage at 4 and 22°C for 6 weeks.

Viscosity: the viscosity of juice tomato was determined using Brookfield viscometer (DV-II+) with a LV-3 spindle, and the viscosity was expressed as centipoise.

alcohol insoluble residue preparation: Tomato wall isolation was as alcohol insoluble residue (A.I.R.) as followed by Mcfeeters and Armstrong (1984). Approximately 30 g of sample was homogenized in 192 ml of ethanol 95% by a mixer at room temperature for 10 min. The mixture was filtered (Machery MN 615), and it was rehomogenized in 96 ml of ethanol (95)%. another filtration was required, the residue, was homogenized in 96 ml; acetone. A final step resulted in the alcohol insoluble residue, which was dried overnight at 40°C. A.I.R. was ground by mortar; then stored at - 20°C till future use.

Tomato cell fractionation: Tomato cell extracted as AIR was fractionated into different pectin fractions: the first fraction; water soluble pectin was obtained by incubation of 0.25 g AIR in (45) ml boiling water whereas; stirring (Sila *et al.*, 2006); the suspension was cooled to ambient temperature and filtered, After 5 min, the resulted water soluble pectin (W.S.P.); the residue was resuspended in (45) ml (0.05) M cyclohexane trans 1,2 diamine tetra acetic acid in (0.1) M potassium acetate pH 6.5 at 28°C for 6 hours (Chin *et al.*, 1999), the chelator soluble pectin (C.S.P.) was obtained, the residue was re incubated in 0.05 M Na₂CO₃ (45) ml containing 0.02 M NaBH₄ at 4 °C for 16 h and followed by at 28 °C (Chin *et al.*, 1999); the mixture was filtered which resulted fraction (NSP).

Galacturonic acid amount: All pectin fractions (WSP; CSP and; NSP) were analyzed for their galacturonic acid amount (GalA) according to the method described by Kintner and Vanburen (1982) in the presence of m- hydroxydiphenyl as reagent.

Esterification degree: The esterification degree (DM) was measured using a titrimetric method of Banjongsinsiri *et al.*, (2004). An amount of 1 g of AIR was extracted by (90) ml of distilled water at 60°C. The centrifugation for extract was conducted at 8000g at 4°C and filtered with Miracloth. The supernatant was collected and analyzed for % DE. A (20) ml of supernatant was titrated with (0.05) N NaOH, saponified at room temperature with 20 ml of 0.05 N NaOH, and neutralized with (0.05) N HCl. The carboxylic groups were measured by titration with (0.05) N NaOH. The end point lied in the presence of phenolphthalein, but was computed to pH between (8.0 and 8.5). The % esterification degree was determined as the ratio between free; total carboxylic acids; respectively;. The esterification degree of the fractions was determined whereas the DM of NSP has no able to check due to the saponification during the alkaline extraction.

Statistical analysis: the study was a completely randomized design (C.R.D.) with factorial experiments. Duncan's Multiple Range Test at 5% level of significance was used to compare between means. We are using the statistical analysis system (SAS); to Analyze variance; means (Anonymous, 2002).

RESULTS AND DISCUSSION

From the results of the preliminary experiment for the enzymatic deesterification of pectic substances of tomato juice (Fig. 1), the maximum viscosity value (342 cP) for tomato juice was obtained when pectin methylesterase concentration was added as (25 U/ 100 ml juice).

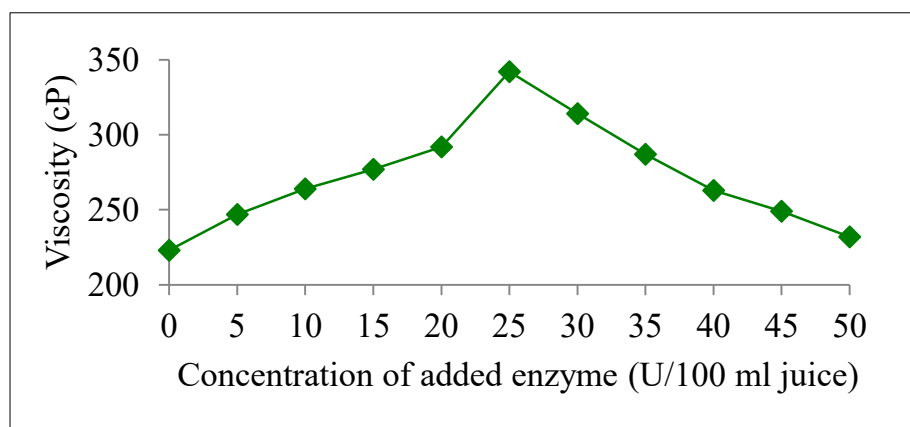


Fig (1): Effect of added concentrations of PME on viscosity of tomato juice.

Effect of enzymatic treatment (addition of PME) of SSTJ and its storage at 4 and 22°C for 6 weeks on its viscosity values are shown in Fig. 2. Enzymatic treated juice had a significantly higher viscosity (348 cP) than untreated juice (231 cP) ($P < 0.05$).

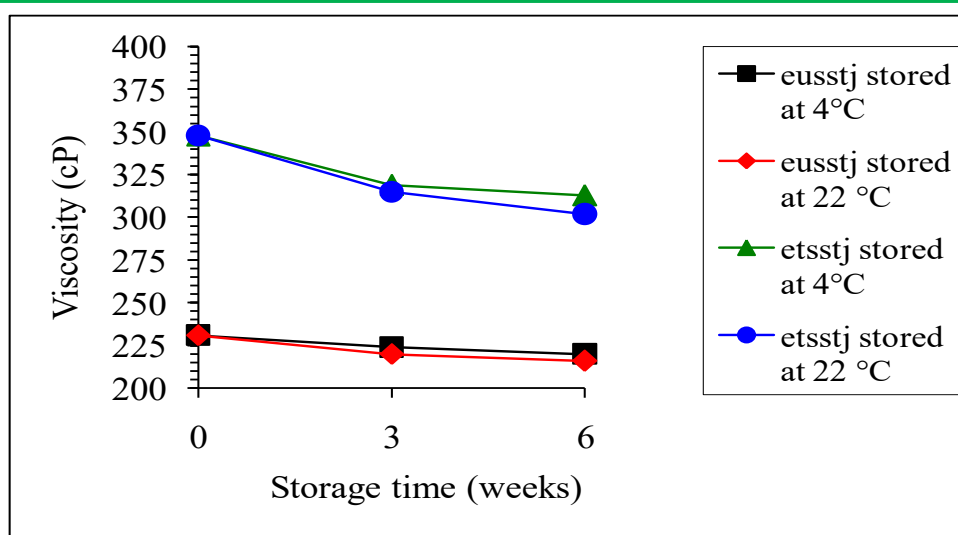


Fig. (2): Effect of enzymatic treatment (addition of PME) and storage at 4 and 22°C for 6 weeks on viscosity value of single strength tomato juice. (eusstj= enzymatic untreated single strength tomato juice, etsstj= enzymatic treated tomato juice).

The increasing of viscosity value of enzymatic treated juice can be explained throughout that activity of PME removes the methyl esters groups on the pectin molecules. This could in turn increase the regions for intermolecular complexes that facilitate interactions between the polysaccharide chains, and result in an increased viscosity (Schmelter *et al.*, 2002; Smits *et al.*, 2000). The results obtained also showed there were significant differences in viscosity values for enzymatic treated juices compared with that untreated juices ($P < 0.05$), after storage of both juices at 4 and 22°C for 6 weeks, enzymatic treated juices stored at 4 and 22°C for 6 weeks exhibited viscosity values of (313 and 302 cP), compared with (220 and 216 cP) for untreated juices stored at same conditions. Comparatively to the viscosity value of enzymatic untreated SSTJ (control), storage for 6 weeks caused an insignificant decreasing in the viscosity. Some published data talked about hot break step, the temperatures are high for pectinase inactivation, particularly polygalacturonase, the enzyme contributes pectin breakdown in the middle lamella of tomato fruit and hence, reduced the viscosity. The viscosity value in enzymatic treated SSTJ showed significantly decreasing compared with that in untreated juices. Some studies indicated that decreasing occurred in the viscosity of tomato juices even after pectolytic enzymes inactivation into highly levels, Gould (1992) reported that one of the most important factors affecting viscosity of tomato juice is the cultivar used, the study of Goodman *et al.*, (2002) confirmed that the cultivar of tomato had main role in the viscosity loss for juice in spite of they used different conditions for hot break and complete pectolytic enzymes was done. The same notices were obtained with Aguayo *et al.*, 2008 who studied the influences of juice storage at 4°C; for (77) days on some juice properties like viscosity. The galacturonic acid amount for water soluble pectin fraction (GalA for WSP) of untreated, enzymatic treated SSTJ samples are shown in Fig. 3. Enzymatic treatment of juice caused a significant change in the galacturonic acid amount (GalA) as compared to untreated juice, and had the following values (15.43 and 12.21 mg/100 ml) for three weeks, respectively. Enzymatic treated juices stored at 4 and 22°C for 6 weeks had significantly higher (GalA) (17.64 and 18.13 mg/100 ml) than that for untreated juices stored at same conditions (13.65 and 14.90 mg/100 ml).

The galacturonic acid amount for water soluble pectin fraction of enzymatic treated and untreated juices were significantly increased after storage at 4 and 22°C for 6 weeks (Fig. 3) as compared with juice at 0 day storage. The results obtained were in agreement with Hurtado *et al.*, (2002) who reported that increasing of (Gal for WSP) of hot breaking tomato juice. Significant differences were found in GalA between tomato juices stored at 4°C; and at 22°C; for 6 weeks for both samples (PME treated and untreated).

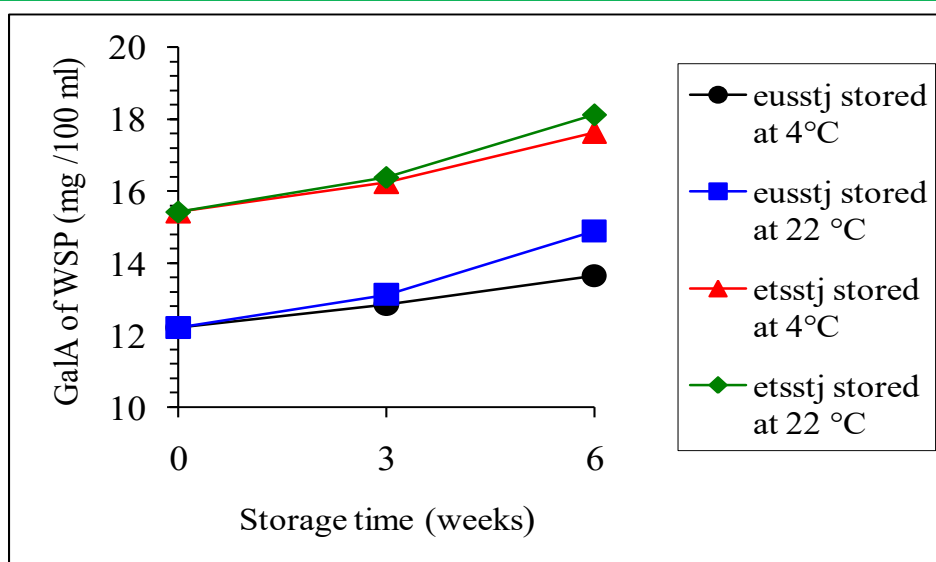


Fig. (3): Effect of enzymatic treatment (addition of PME) and storage at 4 and 22°C for 6 weeks on GalA of WSP of single strength tomato juice.

Fig. 4 shows the effect of enzymatic treatment (addition of PME) of SSTJ and its storage at 4 and 22°C for 6 weeks on galacturonic acid content for chelator soluble pectin fraction (GalA for CSP) of tomato juice. Enzymatic treated juice had a significantly higher GalA for CSP (9.61mg/100 ml) than that for untreated juice (9.36 mg/100 ml) ($P < 0.05$) at (0) day storage. Fig. 4 also shows that there were significant differences in GalA for CSP for enzymatic treated juices compared with that untreated juices ($P < 0.05$), after storage of both juices at 4 and 22°C for 6 weeks, enzymatic treated juices stored at 4 and 22°C for 6 weeks showed GalA for CSP of (8.82 and 8.66 mg/100 ml), compared with (8.69 and 8.32 mg/100 ml) for untreated juices stored at same conditions. In comparison with juice at 0 day storage, the GalA for CSP of enzymatic treated and untreated juices were significantly decreased after storage at 4 and 22°C for 6 weeks (Fig. 4).

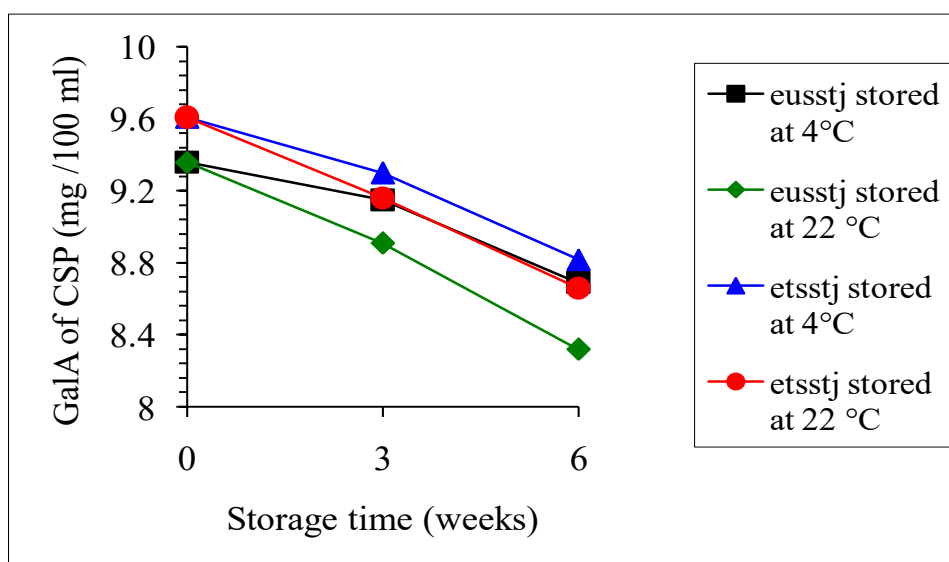


Fig. (4): Effect of enzymatic treatment (addition of PME) and storage at 4 and 22°C for 6 weeks on GalA of CSP of single strength tomato juice.

The same results were published by Hurtado *et al.*, (2002) who reported that decreasing in (GalA for CSP) for tomato juice subjected to hot break. As regarding of GalA for CSP, the significant differences were found between tomato juices stored at (4°C) and (22°C) for 6 weeks for both samples (PME treated and untreated).

Effect of enzymatic treatment (addition of PME) of SSTJ and its storage at 4 and 22°C for 6 weeks on galacturonic acid content for carbonate soluble pectin fractions (GalA for NSP) of tomato juices are shown in Fig. 5, statistically significant changes were observed after enzymatic treatment of tomato juices (4.48 mg/100 ml), compared with untreated juices (3.17 mg/100 ml). Enzymatic treated juices stored at 4 and 22°C for 6 weeks had significantly higher galacturonic acid content (3.77 and 3.51 mg/100 ml) than that for untreated juices stored at same conditions (2.25 and 2.28 mg/100 ml). The galacturonic acid content for carbonate soluble pectin fractions of enzymatic treated and untreated juices were significantly changed after storage at 4 and 22°C for 6 weeks (Fig. 5) as compared with juice at 0 day storage. The results obtained were in agreement with Hurtado *et al.*, (2002) who reported that changing of (GalA for NSP) of tomato juice produced by hot break. Significant changes were found in GalA between tomato juices stored at 4°C; and 22°C for 6 weeks for both samples (PME treated and untreated).

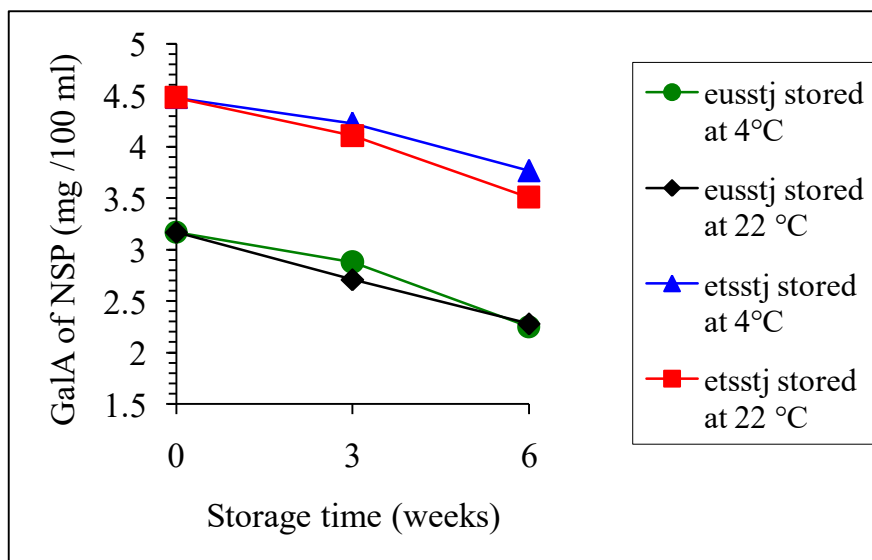


Fig. (5): Effect of enzymatic treatment (addition of PME) and storage at 4 and 22°C for 6 weeks on GalA of NSP of single strength tomato juice.

The DM for WSP of enzymatic treated, untreated SSTJ samples are shown in Fig. 6. Enzymatic treated juices had significantly lower DM for WSP (46.35%) than that for untreated juices (58.39%) at (0) day storage.

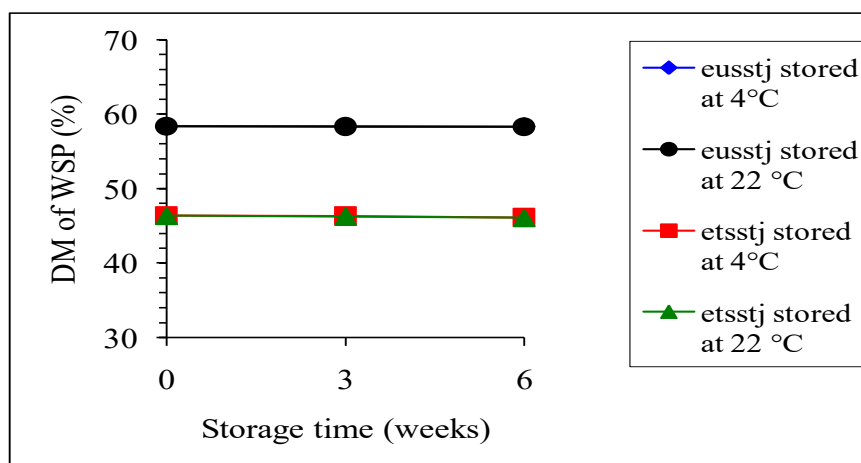


Fig. (6): Effect of enzymatic treatment (addition of PME) and storage at 4 and 22°C for 6 weeks on DM of WSP of single strength tomato juice.

The decreasing of DM for WSP of enzymatic treated juices might be due to elimination of methoxyl groups of pectic structure by action of added PME (Roeck *et al.*, 2008). Enzymatic treated juices stored at 4 and 22°C for 6 weeks had significantly lower DM for WSP (46.12 and 46.09%) than that for untreated juices stored at same conditions (58.36 and 58.32%). Insignificant decreasing in DM for WSP was observed between untreated tomato juice stored at 4 and 22°C for 6 weeks compared with juice (0 day storage), whereas significant decreasing was observed in DM for WSP of enzymatic treated juice stored for 6 weeks compared with treated juices stored for (0 and 3 weeks) at 4 and 22°C, respectively. Some studies indicated that same changes in DM with regard to effect of some thermal treatment conditions on carrot extracted pectins (Annie and Waldron, 1997; Sila *et al.*, 2005). Fig. 7 shows the effect of enzymatic treatment (addition of PME) of SSTJ and its storage at 4 and 22°C for 6 weeks on DM for CSP. Enzymatic treated tomato juices had significantly lower DM for CSP (43.94%) than that for untreated juices (53.33%).

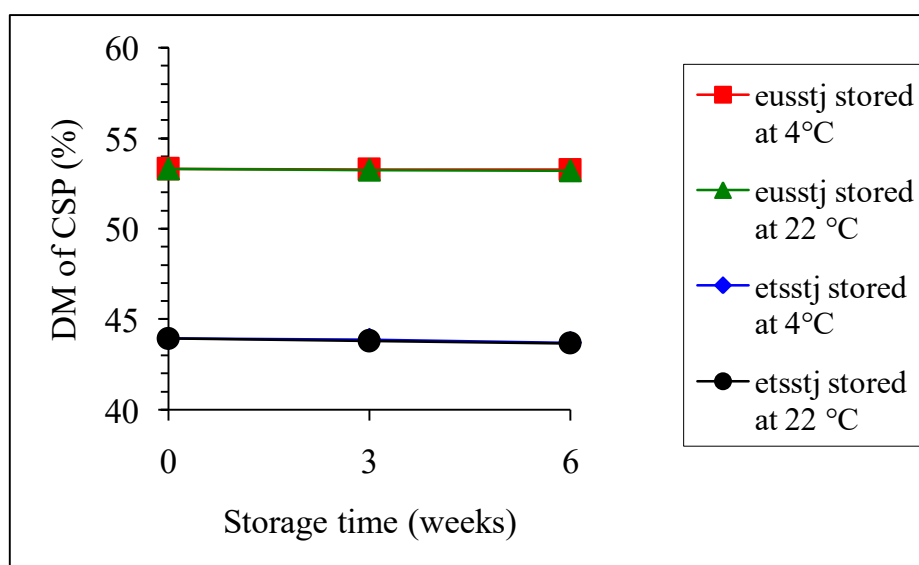


Fig. (7): Effect of enzymatic treatment (addition of PME) and storage at 4 and 22°C for 6 weeks on DM of CSP of single strength tomato juice.

The decreasing occurred might be due to elimination of methoxyl groups of pectic structure by action of added PME (Roeck *et al.*, 2008). Enzymatic treated juices stored at 4 and 22°C for 6 weeks had significantly lower DM for CSP (43.72 and 43.69%) than that for untreated juices stored at same conditions (53.27 and 53.22%). Insignificant decreasing in DM for CSP was observed between untreated tomato juice stored at 4 and 22°C for 6 weeks compared with juice (0 day storage), whereas significant decreasing was observed in DM for CSP of enzymatic treated juice stored for 6 weeks compared with treated juices stored for (0 and 3 weeks) at 4 and 22°C, respectively. Some studies indicated that same changes in degree of esterification with regard to effect of some thermal treatment conditions on carrot extracted pectins (Annie and Waldron, 1997; Sila *et al.*, 2005). No statistically significant effect were found in DM for CSP neither in samples stored at 4 nor at 22° for all treatments. The DM of NSP could not be assaying due to the saponification of methylesters during the alkali extraction of this fraction.

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تأثير المعاملة الإنزيمية في الزوجة والمواد البكتينية لعصير الطماطة

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المستخلص

هدفت هذه الدراسة إلى معرفة تأثير معاملة عصير الطماطة ذي التركيز الاعتيادي المصنع بالتهشيم الساخن بإنزيم البكتين مثل استريز المنتج من فطر *Aspergillus niger*، وتمت الإضافة الإنزيمية إلى العصير بالتركيز الأتية (0، 5، 10، 15، 20، 25، 30، 35، 40، 45 و 50 وحدة إنزيمية/100 مل من عصير الطماطة تحت ظروف مثلى (45°م و أس هيدروجيني يساوي 4 ولمدة 10 دقائق في حمام مائي) تم التوصل إليها بعد تطبيق تجربة أولية. إذ بلغت القيمة القصوى للزوجة (342 سنتيبوايز) لعصير الطماطة عند الإضافة الإنزيمية (25) وحدة/ 100 مل عصير. درست التغيرات في بعض صفات العصير بعد التعبئة في قناني زجاجية معتمة وأثناء الخزن في درجتي حرارة 4 و 22 °م لمدة 6 أسابيع. أظهرت النتائج حصول زيادة معنوية في لزوجة عينات العصير المعامل بالإنزيم بعد التعبئة مع ملاحظة حصول انخفاض غير معنوي في اللزوجة خلال فترة الخزن ولجميع المعاملات وعند درجتي الحرارة المحددة مقارنة مع عينات العصير غير المعامل بالإنزيم. أشارت نتائج الدراسة الى حصول زيادة معنوية في كمية حامض الكالاكتيورونيك لجزء البكتين الذائب في الماء في العصار بعد التعبئة والخزن في درجتي حرارة 4 و 22 °م لمدة 6 أسابيع، وحصول إنخفاض معنوي في كمية حامض الكالاكتيورونيك لجزء البكتين الذائب في المادة الخالصة في حين كان الإنخفاض غير معنوي في كمية حامض الكالاكتيورونيك لجزء البكتين الذائب في كاربونات الصوديوم في عينات عصير الطماطة المعامل بإضافة الإنزيم البكتين مثل استريز مقارنة مع عينات العصير غير المضاف إليها الإنزيم المذكور. كانت قيم درجة الأسترة لجزئي البكتين الذائبين في الماء وفي المادة الخالصة في عينات العصير المعامل إنزيميا أقل معنويا من قيمها في العينات غير المعاملة إنزيميا وذلك بعد التعبئة وخلال الخزن لمدة 6 أسابيع على 4 و 22°م. لا يمكن تقدير درجة استرة جزء البكتين الذائب في كاربونات الصوديوم وذلك لتصين استرات المثل أثناء الاستخلاص القاعدي للجزء البكتيني المذكور.

الكلمات المفتاحية: عصير الطماطة، محتوى البكتين، إزالة الأسترة الإنزيمية، اللزوجة.