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INHIBITORY EFFECT OF THIOL CONTAINING COMPOUNDS ON ENZYMATIC BROWNING IN APPLE JUICE.

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ABSTRACT

A comparative study concerning the inhibition of the enzymatic browning in Red delicious apple juice stored for 24 h at 4, 25, and 35 °C was carried out between the thiol containing compounds; cysteine , glutathione and Maillard reaction products (MRPs) derived by heating aqueous solutions containing cysteine with glucose or ribose (Cys/G or Cys/R) and those commercially applied such as: sodium metabisulfite , ascorbic acid and 4-hexyl resorcinol. The efficiency of all antibrowning agents was determined in terms of absorbance and colour measurements. The obtained results confirmed the presence of a proportional relation between concentration of antibrowning agents and their inhibitory effect, whereas the increase in storage temperatures showed an opposite trend. The inhibitory effect of the thiol containing compounds was comparable with 4-hexyl resorcinol and being significantly ($P < 0.05$) higher than ascorbic acid under all experimental conditions. The MRPs derived from cysteine / glucose model system were more active than their counterpart derived from cysteine / ribose model system. However, each of them could be considered as potential natural inhibitor having technological and commercial benefits to be applied in processed fruits. The degree of browning was determined by measurement of soluble (A_{420}) and nonsoluble brown pigments, showing high correlation ($r = 0.9454$) with the percent of degraded phenolic compousnd during storage of apple juice samples treated with various antibrowning agents.

Keywords: Apple juice, Enzymatic browning, Antibrowning, Maillarad reaction products (MRPs), Phenolic degradation.

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INTRODUCTION

Apple is one of the most popular fruits, consumed all over the world and thus susceptibility of apples to enzymatic browning during post-harvest, handling and processing operations is an important topic from the standpoint of Food Science and Technology.

Enzymatic browning usually starts with the oxidation of phenols to quinones by the enzyme polyphenol oxidase (PPO) in the presence of oxygen. These quinones are subjected to further reactions catalyzed either enzymatically leading to the formation of pigments (Murata *et al.*, 1995) or non-enzymatically with amino acids or proteins to yield dark-coloured pigments or complexes generally called melanines or melanin-proteins (Walker and Ferrar, 1998). Apple juice is very sensitive to enzymatic browning since it contains considerable quantities of polyphenols and polyphenol oxidase that are bound to suspended particles (Trone *et al* ., 1998). The control of enzymatic browning has always been a challenge to the

food industry and so , various physical and chemical methods have been used to control the PPO activity in fruits and vegetable. For instance, sulfites are commercially used as effective inhibitors for PPO but these compounds have been restricted by the Food and Drug Administration (FDA) due to the possibility of its associated potential hazards (Sapers *et al.*, 1989). Thereby several studies have been devoted to the nonsulfite antibrowning agents, and among these browning inhibitors, ascorbic acid and its derivatives and 4-hexyl resorcinol (4HR) have been used commercially with limited success (Sapers and Miller, 1992).

Due to the consumer's demand for natural food additives, recently many studies have been devoted to search for natural inhibitors of enzymatic browning such as honey (Oszminaski and Lee, 1990), sulfur containing amino acids and their derivatives (Friedman and Molnar-Perl, 1990 and Richard-Forget *et al* ., 1992) and mixtures of sugars and thiol compounds (Roux *et al* ., 2003). Many studies showed that sulfhydryl (SH or thiol) compounds such as cysteine , N-acetyl-L-cysteine and reduced glutathione are good inhibitors of the enzyme polyphenol oxidase (PPO) which catalyze enzymatic browning in fruits and vegetables (Friedman and Bautista, 1995).

It has also been shown that Maillard reaction products (MRPs) might inhibit enzymatic browning initiated by PPO; (Tan and Harris, 1995). The key intermediate of the early stage of the Maillard reaction is the Amadori rearrangement products, which is a type of amino reductone that has chelating, reducing and oxygen-scavenging properties. Billaud *et al.*(2003) and (Billaud *et al.* (2004) added further support to the possible involvement of Maillard reaction products derived from thiol precursors , they extended their study to the inhibitory potency of various amounts of MRPs derived from hexose (glucose or fructose) /glutathione model mixture. They concluded that the MRPs derived from glucose with sulfhydryl amino components (cysteine or the tripeptide , glutathione) could be considered as potential natural inhibitors.

The objectives of the present study was to evaluate the inhibitory pattern of the thiol containing compounds that well documented to have antioxidant and antitoxic effects *in-vivo* against the enzymatic browning of cloudy apple juice in comparison with the commercially used antibrowning agents. Since the enzymatic browning in apples is mainly due to the oxidation of phenolic compounds by PPOs, therefore the present study will be extended to estimate the relation between the degree of browning and degradation of phenolic compounds.

MATERIALS AND METHODS

Materials:

Apples: Red delicious (*Malus Domestica* c.v *Borkh*) were purchased from local market at the commercial maturity and stored at 4 °C until used.
Antibrowning agents: The tested antibrowning agents included thiol containing compounds and those commercially applied. Each antibrowning agent was used at three concentrations as shown in Table (1):

Table (1): Antibrowning agents and their applied concentrations.

Antibrowning agents*	concentrations ** mg / 100 ml apple juice		
	C_1	C_2	C_3
1- Sodium metabisulfite	0.01	0.02	0.05
2- 4-Hexyl resorcinol	0.01	0.05	0.1
3- Ascorbic acid	0.1	0.2	0.5
4- L-Cysteine	0.1	0.2	0.5
5- Reduced glutathione	0.1	0.2	0.5
6- MRPs (Cysteine/Glucose)	100	200	500
	(ul / 100 ml apple juice)		
7- MRPs (Cysteine/Ribose)	100	200	500
	(ul / 100 ml apple juice)		

*** All these compounds were obtained from Sigma –Aldrich Chemical Co. (St. Louis No, USA). The other chemicals and solvents were of analytical grade.**

****: C_1: Least, C_2: Medium, C_3: Highest amount added of each antibrowning agents /100 ml apple juice**

Methods:

1- Preparation of MRPs containing thiol group:

Model mixtures containing cysteine and glucose or cysteine and ribose at molar ratio, 1:1, 1.2 and 2:1 M/M, respectively , were used for preparation of model aqueous MRPs containing thiol group. Each mixture was dissolved in phosphate buffer (100 ml, 0.5 M, pH 5) and allowed to react at 90 °C under efficient reflux for heating time 0, 1, 2 h. The flasks containing the aqueous solutions of MRPs were immediately cooled in ice. Aliquots of these solutions (5 ml) were withdrawn and analysed for thiol content. Analysis was carried out spectrophotometrically at 412 nm with Vis Shimadzu Spectrophotometer (UV-1601 PC) using 5,5'-dithiobis(2-nitrobezoic acid) (DTNB, Elman's reagent) according to the method described by (Molero-Vilchez and Wedzicha, 1997).

2- Antibrowning treatment:

The investigated apple samples were washed under water current and juiced with ordinary domestic food processor. Samples of juice (100 ml) were poured into beakers containing the antibrowning agents to adjust their concentrations as shown in Table 1 and immediately stirred with a magnetic stirrer for 10 S. A juice sample without antibrowning agents was considered as control. Each prepared sample was immediately subjected to absorbance and colour measurements as well as estimation of phenolic content before and after storage at 4, 25 and 35 °C for 24 h. All experiments were carried out in three replicates.

2-1: Absorbance measurement:

Absorbance values at A_{420} nm were recorded with 4054 U.V/ visible spectrophotometer (LKB-Biochrom) after centrifugation of apple juice mixture with inhibitory agents at 12000 rpm for 10 min according to the method of (Ozoglu and Bayindirh ,2002)

2-2: Colour measurement:

L (lightness), a (red to green colour dimension) and b (yellow to blue colour dimension) values of the tested apple juice samples were measured with a tristimulus colour analyzer (Hunter Lab scan XE- Reston VA; USA). The instrument was calibrated using a standard white tile: X=72.26, Y=81.94 and Z=88.14 (L*=92.46; a*= -0.86; b*= -0.16).

2-3: Estimation of inhibition:

The browning inhibition based on L-mesurments and A_{420} was calculated using the equations of Iyidogan and Bayindirh, (2004) :

Inhibition (%) = $(\Delta L_{control} - \Delta L_{treatment}) \times 100 / \Delta L_{control}$ .....Equation [1]

Inhibition (%) = $(\Delta A_{420,\ control} - \Delta A_{420,\ treatment}) \times 100 / \Delta A_{420,\ control}$ .....Equation [2]

Where Δ in Eqs. (1) And (2) shows the change in L or A_{420} between time t (24 h) and the initial time t_o at different tempratures.

Total colour change (Δ E) was also used to evaluate browning potential according to the following formula:

$\Delta E = [(L_t - L_{to})^2 + (a_t - a_{to})^2 + (b_t - b_{to})^2]^{0.5}$ ..... Equation [3]

2-4: Degree of browning:

Degree of browning was performed according to Amiot *et al.*, (1992). In such a case the following equations were applied

A_{420} and L* were first normalized:

Normalized $A_{420} = (A_{420} - A_{420min} / A_{420\ max} - A_{420min})$

Normalized $L = (L - L_{min} / L_{max} - L_{min})$

Sum (s) = $[A_{420}]n + [L]n$

Degree of browning = normalized sum(s) $n = s - s_{min} / s_{max} - s_{min}$

3-: Extraction and determination of total phenolic compounds:

The total phenolic compounds in the tested apple juice that exhibited the highest inhibitory effect for each antibrowning agents and those in the control sample were extracted and estimated before and after storage for 24 h. The extraction was carried out according to the method described by Bengoechea *et al.* (1997). To each 20 ml of the juice sample; 15 ml of methanol/hydrochloric acid (1000:1, v/v) was added and stirred with a magnetic stirrer for one min. Methanol was evaporated under vacuum and the residue was redissolved in 25 ml of water / ethanol (80:20,v/v) and extracted four times with ethyl acetate (25 ml). The organic fractions were combined , dried for 30 min with anhydrous sodium sulfate, filtered through a Wahtman-40 filter paper (Whatman International Ltd., Kent, England), and evaporated to dryness in a rotary evaporator, keeping the water bath temperature under 35 °C. Each residue was diluted 5-10 times with methanol to obtain a final absorbance below 0.5. The total phenolic compounds were estimated colorimetrically at 760 nm with Vis Shimadzu Spectrophotometer (UV-1601 PC) by the Folin-Ciocalteu method (Scalbert *et al.*, 1989). The results were expressed as milligram per liter gallic acid equivalent (GAE). Calibration curve was carried out with gallic acid aqueous solutions (8-80 ug / ml).

The percent of degraded phenolic compounds during storage of each sample was calculated as follows:

Degraded phenolic (%) = phenolic $_{in}$ – phenolic $_{r}$ /phenolic $_{in}$ X100 .....Equation [4]

Where phenolic $_{in}$ (initial) = total phenolics (mg / L .... GAE) before storage

phenolic $_{r}$ (residual)= total phenolics (mg / L ...GAE) after storage

4- Statistical analysis:

Statistical analysis was carried out using SPSS statistical package (Version 9.05) according to (Rattanathanalerk *et al.*, 2005), analysis of variance (ANOVA) and least significant difference (LSD) was performed to determine any significant difference among various treatments. $P < 0.05$ was selected as the level decision for significant differences.

RESULTS AND DISCUSSIONS

Inhibitory effect of various antibrowning agents:

Quantification of enzymatic browning in cloudy apple juice stored for 24 h at different temperatures 4, 25 and 35 °C was followed either by absorbance measurements (A_{420}) or reflectance methods (L-measurements). The inhibitory effect of the antibrowning agents at different concentrations (Table1) based on A_{420} measurements (Eq.2) and L-measurements (Eq. 1) were calculated and cited in Table 2. The total colour changes (Δ E) for all tested samples were calculated (Eq.3) from the hunter colour lab values and given also in Table 2. It is obvious that for all antibrowning agents there were some differences between percent inhibition based on measurement of A_{420} and L-values. Under all tested conditions, ascorbic acid showed much higher inhibition values based on L than A_{420} measurements, whereas most of the other antnibrowning behaved an opposite trend. Fig. 1 illustrates the inhibitory effect of antibrowning agents; based on L-measurements, at different concentrations on the enzymatic browning in cloudy apple juice stored for 24 h at 4, 25 and 35 °C. For all tested samples the increase in the concentration of the antibrowning agents revealed significant increase ($P < 0.05$) in the inhibition percent. Such trend is in agreement with previous studies of Janovitz-Klapp *et al.* (1990) , and Ozoglu and Bayindirh , (2002) . Taking into consideration that the actual concentration used differed for each inhibitor (Table 1). The effectiveness of the antibrowning agents at 4 °C was in the decreasing order; sodium metabisulfite > 4-hexyl resorcinol > reduced glutathione > MRPs (Cys / G) > MRPs (Cys / R) > L-cysteine > ascorbic acid. This order showed noticeable variations at 25 °C and 35 °C. It is of important to refer to the opinion of Friedman and Bautista, (1995) who investigated the influence of temperature in the range 25 °C to 55 °C on the inhibitory effect of various antibrowning agents including cysteine and reduced glutathione on PPO activity. No uniform correlation was found between the temperature and inhibition activity. They attributed these findings to the possibility that, the antibrowning agents may undergo significant side reaction at high temperature.

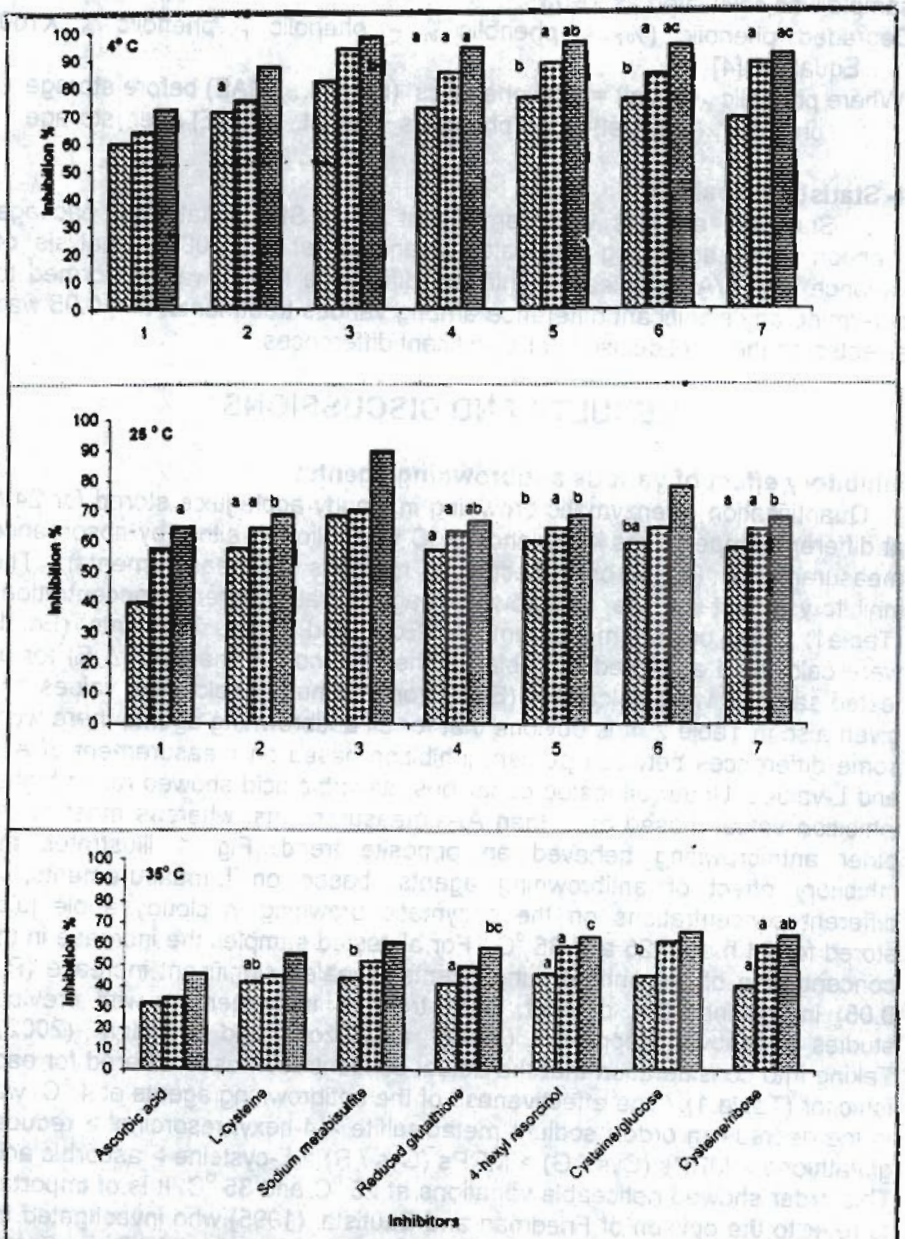


Fig (1) Inhibition of enzymatic browning (based on L-measurments) in Red delicious apple juice stored for 24 h at different temp. with different concentrations (Table 1) of each antibrowning agent . Inhibition (%) values with the same letters for the same added levels of each antibrowning agent at the same temprature are nonsignificant at $P < 0.05$

Table (2): Effect of varying concentration of the antibrowning agents on the inhibition of enzymatic browning reaction in cloudy apple Juice stored for 24 h at 4 °C, 25 °C and 35 °C.

Antibrowning concentrations(Added amount / 100ml juice)	4 °C			25 °C			35 °C		
	Inhibition of enzymatic browning (%) based on		Total colour change	Inhibition of enzymatic browning (%) based on		Total colour change	Inhibition of enzymatic browning (%) based on		Total colour change
	A_{420}	L	Δ E	A_{420}	L	Δ E	A_{420}	L	Δ E
Control	-	-	19.7 ± 2.2	-	-	21.9 ± 1.8	-	-	25.7 ± 1.4
Sodium metabisulfite									
0.01 mg	94.7 ± 1.5*	82.3 ± 2.2	10.0 ± 0.9	81.7 ± 1.6	68.7 ± 1.7	11.5 ± 1.3	80.6 ± 1.0	43.8 ± 0.8	17.9 ± 1.9
0.02 mg	97.5 ± 1.1	94.2 ± 2.7	9.3 ± 1.5	96.3 ± 0.8	70.4 ± 1.8	9.7 ± 0.6	94.6 ± 0.3	51.8 ± 0.4	16.8 ± 4.0
0.05 mg	98.7 ± 0.6	98.6 ± 0.2	8.9 ± 0.7	97.5 ± 1.0	89.6 ± 0.7	9.1 ± 0.9	96.7 ± 1.0	60.8 ± 0.8	14.3 ± 1.2
4-Hexyl resorcinol									
0.01 mg	87.6 ± 0.7	76.7 ± 1.2	10.03 ± 0.4	85.6 ± 0.8	60.5 ± 0.9	10.3 ± 1.2	83.7 ± 2.6	46 ± 1.0	17.6 ± 1.4
0.05 mg	89.4 ± 0.9	88.9 ± 1.5	8.6 ± 0.7	86.4 ± 0.6	65.8 ± 0.9	9.5 ± 1.1	84.6 ± 0.6	58.9 ± 1.0	15.4 ± 1.2
0.1 mg	91.3 ± 1.0	96.4 ± 0.9	8.1 ± 0.4	89.4 ± 1.1	68.9 ± 0.7	8.7 ± 0.6	87.9 ± 1.7	63.4 ± 2.5	14.6 ± 0.6
Ascorbic acid									
0.1 mg	36.9 ± 0.7	60.1 ± 1.9	13.3 ± 2.6	28.4 ± 0.9	40 ± 0.6	15.3 ± 0.6	19.8 ± 0.8	32.3 ± 1 3	22.4 ± 1.2
0.2 mg	48.8 ± 0.9	64.5 ± 2.5	11.7 ± 0.8	30.2 ± 1.8	57.6 ± 0.6	14.2 ± 1.7	24.6 ± 0.8	34.6 ± 0.7	20.6 ± 2.6
0.5 mg	51.6 ± 1.0	72.4 ± 3.3	10.5 ± 1.3	38.4 ± 2.4	64.7 ± 0.8	12.7 ± 1.6	29.4 ± 1.2	45.8 ± 0.7	15.9 ± 1.1
L-cysteine									
0.1 mg	87.4 ± 1.0	71.4 ± 2.5	10.4 ± 0.4	85.6 ± 1.0	57.6 ± 1.5	11.4 ± 0.5	83.7 ± 3.1	42 ± 0.2	20.3 ± 3.2
٠,٢mg	89.8 ± 1.0	75.7 ± 2.3	9.2 ± 1.3	87.8 ± 0.7	63.3 ± 0.3	10.8 ± 2.3	86.4 ± 1.2	44.9 ± 0.3	17.6 ± 1.0
٠,٥mg	90.2 ± 0.4	87.5 ± 2.2	9.0 ± 0.4	89.5 ± 1.5	66.7 ± 0.7	9.7 ± 1.6	88.9 ± 0.9	55.3 ± 0.9	15.2 ± 0.6
Reduced glutathione									
0.1 mg	92.2 ± 0.9	72.9 ± 0.4	11.4 ± 0.6	91.7 ± 0.8	57.2 ± 0.9	12.7 ± 0.6	90.6 ± 0.2	41.3 ± 2.1	16.2 ± 0.7
0.2 mg	94.5 ± 0.7	85.7 ± 1.7	10.2 ± 1.3	93.8 ± 0.2	64.3 ± 2.9	10.4 ± 1.2	92.5 ± 1.0	49.6 ± 0.9	14.8 ± 0.3
0.5 mg	96.4 ± 0.8	94.7 ± 2.2	9.4 ± 1.6	95.4 ± 1.2	68.7 ± 1.0	9.7 ± 1.6	94.8 ± 0.9	58.4 ± 0.7	14.1 ± 1.0
Cysteine / glucose									
100 uL	86.4 ± 0.8	75.6 ± 0.8	9.6 ± 0.3	81.4 ± 3.1	59.7 ± 0.5	10.2 ± 0.5	80.5 ± 0.9	45 ± 0.2	16.8 ± 0.7
200 uL	88.5 ± 1.0	84.6 ± 2.4	8.5 ± 0.8	85.6 ± 0.5	64.8 ± 0.4	9.4 ± 1.3	81.2 ± 1.1	61.4 ± 1.1	15.4 ± 1.6
500 uL	89.4 ± 1.5	94.6 ± 1.0	7.7 ± 0.3	88.9 ± 2.2	77.8 ± 1.4	٥.7 ±0.5	84 ± 0.3	65.8 ± 0.3	14.3 ± 1.5
Cysteine / ribose									
100 uL	84.6 ± 0.6	68.7 ± 1.1	10.4 ± 1.6	82.4 ± 1.0	57.6 ± 1.4	11.4 ± 0.5	81.3 ± 0.8	40.5 ± 1.0	17.6 ± 1.2
200uL	87.3 ± 0.4	88.4 ± 1.4	9 6 ± 1.3	85.5 ± 0.8	63.7 ± 0.4	10.2 ± 0.5	82.8 ± 2.0	59.3 ± 0.6	16.8 ± 3.1
500 uL	88 ± 1.2	91.7 ± 1.4	8 7 ± 2.0	86 ± 0.7	67.7 ± 0.4	9.6 ± 0.6	85.4 ± 0.8	64.3 ± 0.7	15.4 ± 1.4

* Values are mean of three experiments ± S. D.

As shown in Fig. 1 among the antibrowning agents, ascorbic acid exhibited the least inhibition ($P < 0.05$) activity. Ascorbic acid has been widely used as antibrowning agents for processing of fruits and vegetables. It prevents enzymatic browning by reducing the quinone products to their original polyphenol compounds Son *et al.*, 2000. The low inhibitory activity of ascorbic acid in the present study is going with the findings of Ozoglu and Bayindirh, (2002) who reported that the effectiveness of ascorbic acid in cloudy apple juice at 1.8 mM concentration lasted for about 4 h. only. They correlated this short and temporary effect of ascorbic acid to the fact that this compound is oxidized irreversibly by reaction with pigments intermediates, endogenous enzymes and metal such as copper.

The phenolic competitive inhibitor of PPO, 4-hexyl resorcinol (4-HR) is the most effective of a group of synthetic analogues of natural browning inhibitors derived from 2,4-dihydroxy dihydrocinnamic acid as reported by McEvily *et al* . (1992). The increase in storage temperature revealed high significant ($P < 0.05$) decrease in inhibitory effect of 4-HR based on L-measurements at all tested concentrations (Table 2 and Fig. 1). Compared to sodium meatbisulfite , 4-HR showed less significant inhibitory effect ($P < 0.05$) under all tested concentrations , whereas the highest inhibitory effect of 4-HR (4 °C , 0.1 mg /100 ml juice) being of higher effective by 57 % than ascorbic acid (4 °C , 0.5 mg / 100 ml juice). Such aforementioned level increased to 88 % at 35 °C for the same sample .These results are in accordance with Son *et al* ., 2001, who evaluated the effect of various antibrowning agents on apple slices. Four antibrowning compounds containing SH-moiety that examined and including L-cysteine, reduced glutathione (GSH), MRPs derived from (Cys /G) and (Cys/R) aqueous model systems (Table 2). L-cysteine and reduced glutathione were considered to be effective inhibitors of PPO (Buta *et al.*, 1999; and Gacche *et al.*, 2004). These compounds prevent enzymatic browning by reacting with o-quinone to produce stable, colourless adducts instead of the brown pigments (McEvily *et al.*, 1992). L-cysteine was found to form a product with catechol and these products inhibit the enzyme activity (Dudley and Hotchkiss, 1989).

The high significant increase in the inhibitory effect ($P < 0.05$) associated with increasing concentration of either L-cysteine or glutathione based on L-measurements (Fig. 1) may be attributed to the fact that , at low concentrations, the o-quinones that formed in excess can cooxidize the L-cysteine –quinone addition compounds leading to phenol regeneration with deep colour formation (Richard-Forget *et al.*, 1992). However, at high concentration the addition products formed by L-cysteine with o-quinones could be a slight inhibitor of the enzymatic reaction as mentioned by Janovitz-Klapp *et al.* (1990). In comparison to ascorbic acid, L-cysteine and GSH were more effective ($P < 0.05$) at the same concentration and storage temperature. These results are in agreements with previous study of Ozoglu and Bayindirh , (2002) who examined the inhibition of enzymatic browning in cloudy apple juice with various antibrowning agents including L-cysteine and ascorbic acid .

Compared to sodium metabisulfite , L-cysteine was less effective towards browning, however there was nonsignificant difference between the effectiveness of sodium metabisulfite and GSH at concentrations 0 2, and 0.5 mg/100 ml apple juice at storage temperatures 25 and 35 °C . Fig. 1 illustrates that at low concentration (0.1 mg/100 ml apple juice) L-cysteine and GSH exhibited approximately the same inhibitory activity at all storage temperatures. On the other hand, increasing concentration to 0.2 and 0.5 mg/100 ml apple juice resulted in significant improvement ($P < 0.05$) in effectiveness of GSH compared with L-cysteine. Recently Billaud *et al* (2004) reported that in comparison with L-cysteine , GSH exhibited a much higher effect on 4-methyl catechol oxidation catalyzed by PPO. They correlated this finding to the fact that, raising the concentration of GSH added to the substrate solution were accompanied by a drop in the pH of the solution to acidic values. Compared to the cysteine structure (1-COOH , 1SH ,1 NH_2 free function) , GSH possesses an additional COOH function which is responsible for lowering the pH of the substrate solution, in concentration-dependent way.

It is obvious from Fig. 2 that the MRPs derived by heating the two aqueous model mixtures Cys/G or Cys/R at molar ratio 2.1 M/M for each, heated for 1 h at 90 °C possessed the highest thiol content (31.18 , 35.49 u mole, respectively). Therefore in the present study, these two samples were used as natural antibrowning agents.

The coloured pigment formation in the Maillard reaction systems indicates the formation of the melanoidines. Lee and Park, (2005) reported that, the reductone moiety in the melanoidines in addition to their antioxidant activity may also prevent browning by reducing the copper of PPO. Tan and Harris, (1995) attributed the high inhibitory effects of the MRPs derived from cysteine-glucose solution heated for 1 h , to both the melanoidine structure and presence of SH group. As related by Billaud *et al.* (2003) heating cysteine with glucose in solution led to production of MRPs which inhibited the enzymatic browning more than that with fructose. As shown in Table 2 and Fig. 1, the MRPs derived from Cys/G were more effective than those of Cys/R under similar conditions. In comparison with the other thiol containing antibrowning agents, L-cysteine and GSH , MRPs derived from Cys / R exhibited similar inhibitory effect on enzymatic browning reaction in cloudy apple juice stored at 25 °C, whereas MRPs derived from Cys/G were more significantly better ($P< 0.05$) under the same conditions. As early reported by Lingert and Waller, (1983); Maillard reaction mixture is a dynamic system containing several derivative products including functional groups susceptible to rapid oxidation by direct attack of oxygen.

As shown in Table 2 the variation in the total colour change (ΔE , Eq 3), for all samples under investigation was significantly ($P < 0.05$) increased by prolonging storage temperature of the cloudy apple juice whereas increment of the antibrowning agents showed an opposite trend. A good correlation (r = 0.8474, 0.8668 and 0.8593 at 4, 25 and 35 °C respectively) was found between ΔE and inhibition of browning based on L-measurements at all storage temperatures.

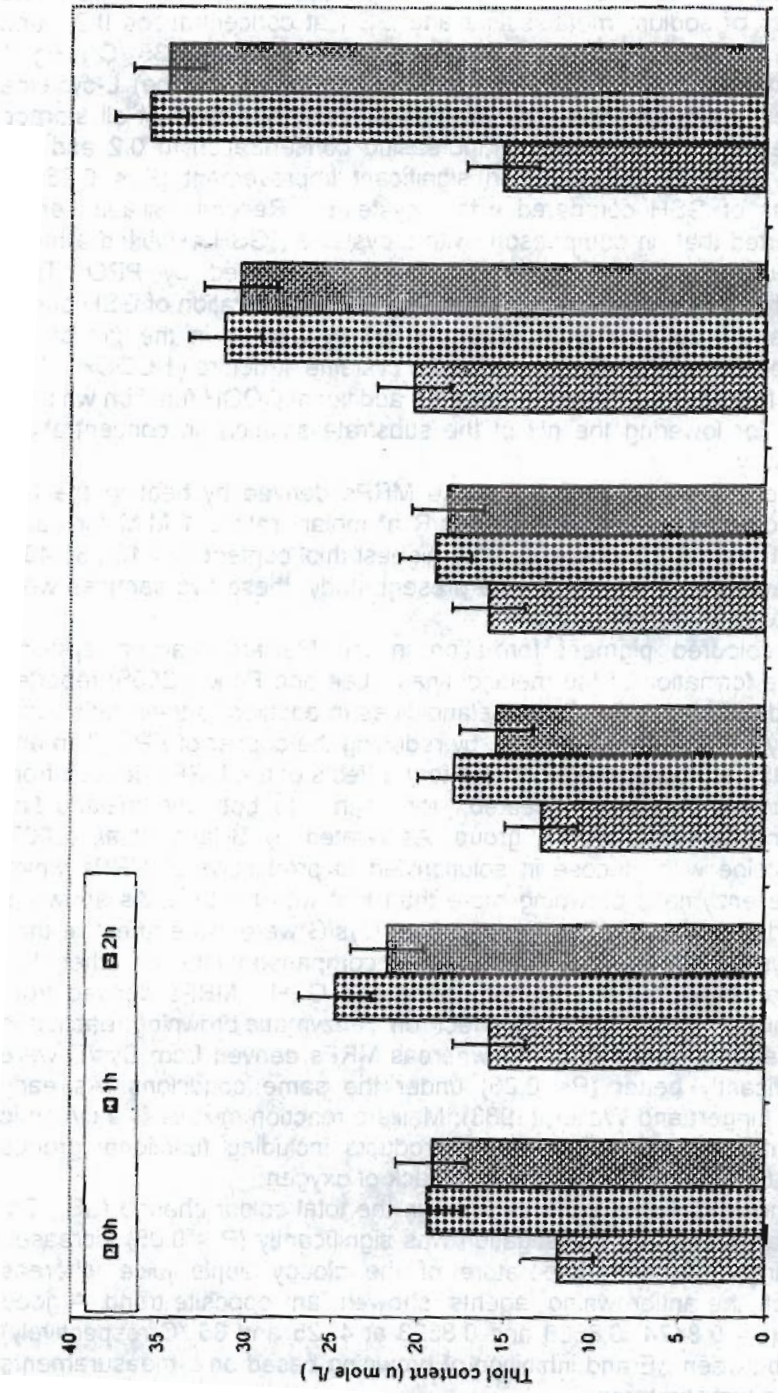


Fig (2) Thiol content obtained in MRPs generated by heating at 90 °C for 1-2 h aquoeus, model mixtures of Cys/G or Cys/R at variable molar ratios (1:1, 1:2, and 2:1 M/M)

These results confirm those reported by Ozoglu and Bayindirh, (2002) who compared the effectiveness of a series of compounds for the inhibition of browning in the apple juice.

Effect of antibrownlng agents on the degradation of phenolic compounds:

According to the results in Table 2; the highest concentration of each antibrowning agents (Table 1) and storage temperature 4 °C lead to the most potent inhibition of the enzymatic browning in cloudy apple juice. As previously reported (Burda *et al* ., 1990) the tendency of enzymatic browning in apples was closely related to the content of phenolic compounds during storage. Therefore, the total phenolics in the samples showed the highest antibrowning inhibition was determined before and after storage, and the percentage of the degraded phenolic compounds in each sample was calculated according to eq.[4].

It is apparently from (Fig.3) that there was a linear contradictory correlation between the degraded phenolic level in stored apple juice and inhibitory effect of the antibrowning agents. Such variation in the degraded phenolic percent among the investigated samples is mainly correlated to the mode of action of each antibrowning agent.

Since browning susceptibility resulted from both soluble and insoluble pigments, the degree of browning could be expressed as normalized sum of the two parameters (A_{420} and L *). After normalization as illustrated in Fig. 4 , the degree of browning in the present study was expressed as a function of the degradation percentage of the total phenolic content. The results revealed a high correlation ($r = 0.9454$) between the degree of browning and the degraded phenolics. These results are in quite agreement with those of Amiot *et al.* (1992). They stated that the soluble and insoluble phenloic compounds seemed equally involved in the apple browning susceptibility.

They explained these findings by the presence of coupled oxidation mechanisms. It has been demonstrated that o-quinones enzymatically produced from the soluble phenolics represented by o-dihydroxy cinnamic acids were able to oxidize insoluble phenolics mainly flavans by nonenzymatic coupled reaction mechanisms (Cheynier *et al* ., 1989 and Oszmianski and Lee , 1990).

Conclusion:

There are numerous compounds capable of reducing the enzymatic browning; therefore the use of natural antibrowning agents is still stimulated to meet the demands for production of healthy fruit products having high quality. As far as the authors are aware to study and evaluate the efficiency of soluble MRPs derived from monosaccharide with thiol containing compounds to inhibit the enzymatic browning in apple fruit juice. On the other, hand the MRPs derived from cysteine / glucose or cysteine / ribose showed comparable results with cysteine alone, so the use of these MRPs is more economical. Furthermore from a technological point of view, it would be conceivable to use these natural antibrowning in processed fruits provided that their safety is assessed and their commercial feasibility demonstrated.

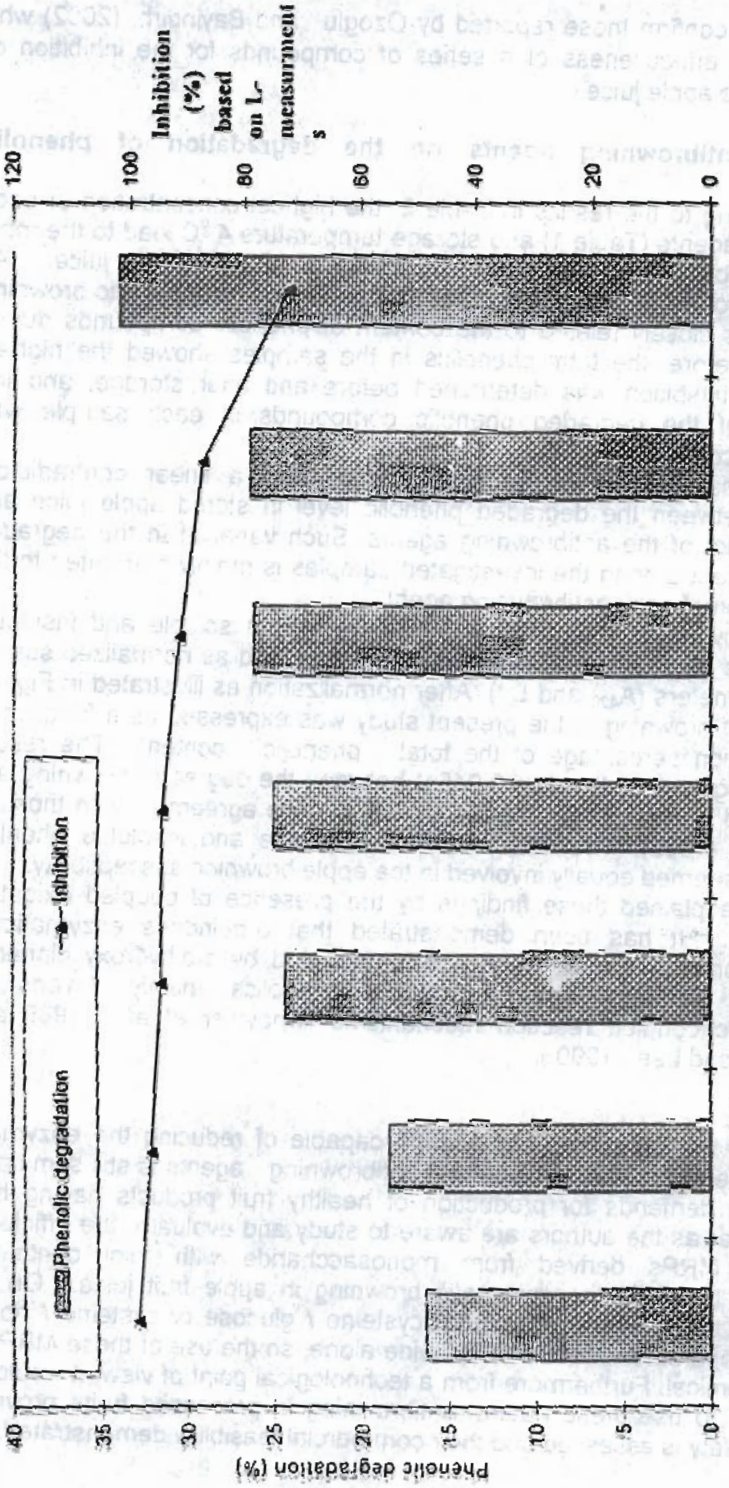
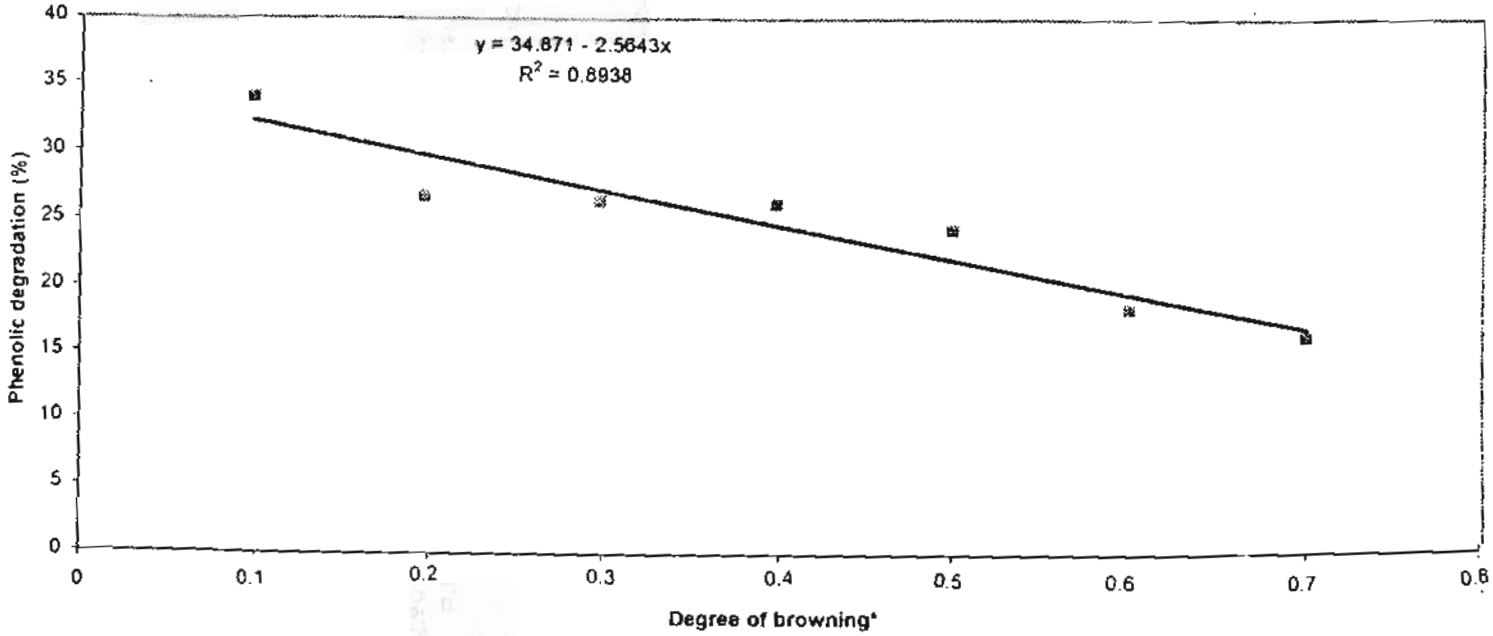


Fig (3) Correlation between the effect of inhibition activity (%)of the antibrowning agents and degarded phenolic (%) in "Red delicious " apple juice

Fig (4) Relationship between phenolic degradation (%) and degree of browning in "Red delicious "apple juice after treatment with different inhibitors



Degree of browning calculated: A_{420} and L were first normalized:

Normalized A_{420} = (A_{420} - A_{420min} / $A_{420\,max}$ - A_{420min})

Normalized L = (L - L_{min} / L_{max} - L_{min})

sum(s) | A_{420} | $_n$ + [L] $_n$

Degree of browning = normalized sum(s) $_n$ = s- s_{min} / s_{max} -s_{min}

High correlation (r = 0.9454) was found between the degree of browning in apple juice determined by measurements of soluble (A_{420}) and insoluble (L^*) brown pigments and percent of degraded phenolic compounds during storage of juice samples treated with various antibrowning agents. This finding confirms the importance of the phenolic compounds in the susceptibility of apple juice to the enzymatic browning.

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التأثير المثبط لبعض المركبات المحتوية علي الثايول للتلون البني الإنزيمي في عصير التفاح

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يتزايد الطلب علي عصائر التفاح غير المصفاة نظرا لخصائصها الحسية و التغذوية القيمة. و علي هذا الأساس أجريت هذه الدراسة علي تثبيط التلون البني الإنزيمي في عصير التفاح الاحمر "Red Delicious" و المخزن لمدة ٢٤ ساعة علي درجات حرارة ٤ ،٢٥، ٣٥°م لمقارنة تأثير بعض المركبات المحتوية علي الثايول مثل السيستئين و الجلوتاثيون و نواتج تفاعل ميلارد الناتجة من تسخين محاليل تحتوي علي السيستئين مع الجلوكوز أو الريبوز مع بعض المركبات التجارية مثل حمض الأسكوربيك و صوديوم ميتاباي سلفيت و ٤-هكسيل ريزورسينول . وتم تقدير كفاءة هذه المثبطات عن طريق الامتصاص بجهاز الاسبكتروفوتوميتر و قياسات اللون بجهاز هانتر ووجد أن زيادة تركيز جميع هذه المثبطات أدي إلي فروق معنوية في التأثير المثبط بينما كان لزيادة درجة الحرارة تأثير عكسي . أظهرت مركبات الثايول تثبيط مماثل لمركب ٤-هكسيل ريزورسينول و لكن هذا الإتجاه كان أكثر وضوحا و بصورة معنوية عن حمض الأسكوربيك تحت جميع ظروف التجربة . علي الرغم من أن نواتج تفاعل ميلارد الناتجة من تسخين السيستئين مع الجلوكوز كانت أكثر كفاءة من مثيلتها مع الريبوز إلا أنه يمكن إعتبار أن كلاهما من المثبطات الطبيعية الفعالة للتلون البني الإنزيمي كما أن لها خصائص تكنولوجية و تجارية عند الإستخدام مع الفواكه المصنعه. أوضحت الدراسة أن هناك إرتباط قوي بين درجة التلون البني عند قياسها بتقدير كلا من الصبغات الذائبة بجهاز الاسبكتروفوتوميتر و تلك غير الذائبة بجهاز هانتر (r=0.9454) مع نسبة الفينولات المستهلكة أثناء تخزين عينات العصير المعامل بمثبطات التلون البني الانزيمي المختلفة.