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EFFECT OF NATURAL EXTRACTS FROM THE FRUIT OF DOUM PALM, CAROB AND LICORICE ON THE QUALITY AND SAFETY OF APPLE SLICES

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ABSTRACT

In this study, attempts were carried out, to investigate the possibility of using fruit extractives from carob, licorice and doum as both anti-browning and anti-microbial agents in apple slices during storage at room temperature (25 °C) for 24 hours and in a refrigerator (4 °C) for 4 weeks. Sliced apples were immersed in different cold and hot extracts of carob, licorice and doum (browning inhibitors) and evaluated for color and microbiological change during storage. The most effective extracts pre-treatments were cold and hot extracts of doum and carob in apple slices stored for 24 hours at 25 °C and the cold and hot extracts of doum, carob and licorice in apple slices stored for 4 weeks at 4 °C. Also, the results showed that the apple slices pretreated with different cold and hot extracts of carob, licorice and doum showed no browning and lowest microbial count (yeast, mold and total plate count) during storage at 4 °C for 4 weeks. The results showed that different cold and hot extracts of carob, licorice and doum are considered to be anti-browning and anti-microbial agents, in controlling both enzymatic and non-enzymatic browning reactions and microorganisms, respectively.

Keywords: apple, slices, carob, licorice, doum, extracts, quality, color, browning, yeast, mold, bacteria.

INTRODUCTION

Conventionally, fruits, vegetables and their products are preserved by different methods to inactivate degradative enzymes and kill spoilage microorganisms. The food industry has been given increasing attention in recent years to meet consumer demands for "freshness" in processed foods by pursing the development of minimally processed products. Most recent reviews have concentrated on technology for improvement of fruit and vegetable product quality without adding chemicals, without effecting on nutrition value of them and safety products.

Refrigerated apples have an established market for institutional utilization and to consumers as an intermediate ingredient. For minimally processed apples, proper packaging, the use of controlled atmosphere, anti microbial and anti browning agents, and refrigeration may reduce the incidence of apple spoilage to extent shelf life (Monsalve, *et al.* 1995).

The shelf-life of minimally processed fruits and vegetables may be limited by enzymatic browning during storage (Sapers, *et al.*, 1994). Huxsoll and Bolin (1989) have indicated that a fundamental problem in shelf life extension of minimally processed fruits is loss of firmness during distribution due to action of endogenous enzymes related to cell wall degradation, and growth of microorganisms. Both enzymes and microorganisms may have caused softening of apple slices observed. The browning reaction of fruit results from polyphenoloxidase (PPO)-catalyzed oxidation of phenolic compounds to o-quinons that subsequently polymerize to form dark-colored

pigments (Mayer and Harel, 1979, Vamos-Vigazo, 1981 and Son *et al*.., 2001).

To evaluate the effectiveness of experimental treatments in controlling enzymatic browning and to compare them with conventional treatments, accurate measurements of the extent of browning are required. Tristimulus reflectance colorimetry (usually the measurement of Rd or Hunter a*- values) has been used to follow the extent of enzymatic browning in apple slices (Ponting, *et al.*, 1972; Sapers and Douglas, 1987 and Monsalve, *et al.*, 1993).

The low pH of apple products restricts the growth of a wide variety of microorganisms. Only yeasts, molds and lactic acid bacteria are capable of prolific growth in apple products. Significant organisms present in processed apple products fall into three categories- spoilage organisms, pathogenic organisms and fermentation organisms. Growth of yeast, mold and bacteria may lead to production of off-flavors and unacceptable filamentous structures in processed apple products (Katherine, 1980).

Hence, use of alternative anti-browning from fruit extracts (like: carob, licorice and doum) is extremely limited. Natural fruit extracts are proper materials to search for safe preservatives in such a time of worldwide tendency for consumers to prefer natural additives. Kim *et al*.., (2005) investigated that the effects of onion extract was prepared by extracting onion with water on pear browning. Therefore, the inhibitory effect of onion extract against pear browning seems to be due to the inhibitory effect of onion extract against pear polyphenol oxidase. Doum *(Hyphaene thebaica*) is a desert palm native to Egypt, sub-Saharan Africa and West India. It is known in Egypt as the Doum or gingerbread palm which grows to 6 or 9 m and usually has forked stems with fan-shaped leaves, with a flat surface of approximately 65–75 cm long. It is listed as one of the useful plants of the world (Fletcher, 1997). Previous studies on Doum had focussed on the fruit because, besides its nutritional value, the fruit drink brewed from hot water infusion of the dried fruit pulp is widely consumed as a health tonic and has been valued in the region, for its many anecdotal medicinal properties, for centuries (Martin, 1999). Research on the fruit pulp showed that it contains nutritional trace minerals, proteins and fatty acids, in particular the nutritionally essential linoleic acid (Cook *et al*.., 2000). Identification of compounds, by thin-layer chromatography, showed that the fruit contains significant amounts of saponins, coumarins, hydroxycinnamates, essential oils and flavonoids (unpublished data), and the fruit also lowers blood pressure in animal models. Doum fruit could play an important nutritional role in the diet of adults and children alike in some of the poorest regions of the world (Sharaf *et al*.., 1972 and Hsu *et al*.., 2006).

At a time when consumers are demanding the partial or complete removal of chemically synthesized preservatives from foods, there is also an increased demand for convenience foods with long shelf-lives. These consumer-led trends have fuelled a renewed interest in the development of 'more natural' preservatives for extending the shelf-life and maintaining the safety of foods. Although the antimicrobial properties of many compounds from plant, animal and microbial sources have been reported, their potential for use as natural food preservatives has not been fully exploited (Roller,

1995). At the end the last century, anti-microbial activities of carob, licorice and doum extracts had already been examined and their oils were known to retard microbial spoilage in foods. Carob, licorice and doum extracts also have been known to possess anti microbial properties toward bacteria, yeasts and molds. Lester *et al..*, (1983), Toshio *et al..*, (2002) and Vivek *et al..*, (2008) investigated that the licorice extracts as antimicrobial agents. Also, Andrés-Elias *et al..*, (2007) investigated that the carob extracts as antimicrobial agents

The objective of this study was to extend the hurdle approach to the preservation of apple fruit slices by using three common cold and hot extracts of Carob, Licorice and Doum and storage at room temperature (25 °C) for 24 hours and in refrigerator (4 °C) for 4 weeks. Also, the objective was to develop technology to produce high quality and long shelf life of apple slices to maintain color, to control enzymatic and non-enzymatic browning, to delay their spoilage and to produce highly quality accepted apple slices.

MATERIALS AND METHODS

Preparation of apple slices:

Apple *(Red delicious)* samples representing common cultivars were obtained from local food stores during the summer of 2008 and stored at 4 °C until needed. One hour prior to use, fruits were removed from the refrigerator and equilibrated to room temperature. Each apple was rinsed with water, sectioned to slices at least 1cm.from the skin end (to exclude the effects of bruising), exposing fresh surface.

Preparation of fruits:

Licorice or liquorice:

(*Glycyrrhiza glabra*), Carob (*Ceratonia siliqua L.*) and Doum *(Hyphaene thebaica)* were purchased from the store of the Ministry of Agriculture, Cairo, Egypt and stored at 4 °C until used in about 2-3 days. All fruits (licorice, carob and doum) were sliced, gently powdered and homogenized with a homogenizer in model MORAT-Motor-Stirrer R 270 (Franz MORAT KG, GmbH & Co., Frano ® - Geratetechnik, Germany) and then ground in an electric mill (Wiley, model 4) with a 1mm mesh. The amount of powder used for each aqueous extraction corresponds to the typical mass of herbs found in the commercial bags for home-made tisanes until used.

Different natural extraction methods:

Cold of Carob, licorice and Doum fruit extract:

Ten grams of Carob, licorice and Doum fruit powder were put into a glass bottle, containing 600ml deionized water, with continuous stirring for 5 hours. The aqueous extract was allowed to cool to 25 °C and filtered by three layers of cheese cloth, as described by the method of Asehraou *et al..*, (1997). The solution was centrifuged (3000 rpm for 10 min) and the supernatant was retained. Under the above conditions, the extent of extraction of Carob, licorice and Doum reached up to 90%. The hot extracts were kept at 20 °C until used. Sample extract was warmed to redissolve

precipitated materials prior to use. The filtered material was used as a source of PPO and browning inhibitors.

Hot of Carob, licorice and Doum fruit extract

Ten grams of Carob, licorice and Doum fruit powder were put into a glass bottle, containing 600ml boiling deionized water, with continuous stirring for 30 min. The aqueous extract was allowed to cool to 25 °C and filtered by three layers of cheese cloth, as described by the method of Asehraou *et al.*., (1997). The solution was centrifuged (3000 rpm for 10 min) and the supernatant was retained. Under the above conditions, the extent of extraction of Carob, licorice and Doum reached up to 90%. The hot extracts were kept at 20 °C until used. Sample extract was warmed to redissolve precipitated materials prior to use. The filtered material was used as a source of PPO and browning inhibitors.

Physical and Chemical analyses:

The pH of samples was measured using a digital pH-meter (HANNA, HI 902 meter, Germany). The percent of Total Soluble Solids (TSS), expressed as °Brix (0-32), was determined with a Hand refractometer (ATAGO, Japan). Titratable acidity was determined according to the method reported by Tung-Sun, *et al.*., (1995). The viscosity measurements were carried out using a HAAKE viscometers (HAAKE, Mess-Technik Gmbhu. Co., Germany) with thermostatic bath to control the working temperature within the temperature of 25°C. Results of viscosity were expressed in centipoise (cP) according to the method of Ibarz *et al.* (1994). Fat, carbohydrate and ash were determined according to AOAC (2000). Also, protein was determined according to the macro Kjeldahl method indicated by AOAC (2000).Total phenols were determined by the method of Amerine and Ough (1980). Results of Total phenols were expressed as milligrams of gallic acid equivalent per 100ml extract by constructing a calibration plot for different amounts of gallic acid using the same conditions as in the total phenols analysis. Vitamin C (ascorbic acid) was determined by AOAC (2000) using 2,6 di-chlorophenolindophenol.. Results of Vitamin C were expressed as milligrams of L ascorbic acid equivalent per 100ml extract by constructing a calibration plot for different amounts of L ascorbic acid using the same conditions as in the Vitamin C analysis.

Non-enzymatic browning determination:

Non-enzymatic browning was measured spectrophotometrically by 4054 UV/Visible spectrophotometer, (LKB-Biochrom Comp., London, England), as absorbance at 420 nm using ethanol as blank according to the method of Birk *et al.*, (1998).

Color determinations:

Hunter a*, b* and L* parameters were measured with a color difference meter and the color of apple slices samples was measured using a spectro-colorimeter (Tristimulus Color Machine) with the CIE lab color scale (Hunter, Lab Scan XE - Reston VA, USA) in the reflection mode. The instrument was standardized each time with white tile of Hunter Lab Color Standard (LX No.16379): X= 72.26, Y= 81.94 and Z= 88.14 (L*= 92.46; a*= -0.86; b*= -0.16) (Sapers and Douglas, 1987). Color difference, Delta E, was calculated

from a*, b* and L* parameters, using Hunter-Scotfield's equation (Hunter., 1975) as follows.

Delta E = (delta a^2+ delta b^2 + delta L^2) $^{1/2}$

where : a-a_o, b-b_o and L-L_o; subscript "$_o$" indicates color of control or untreated sample.

Evaluation of brown inhibitors:

Colorimetry was performed with spectro-colorimeter (Tristimulus Color Machine) using the CIE lab color scale. This color assessment system is based on the Hunter L*-, a*- and b*- coordinates. L*- representing lightness and darkness, + a*- redness, -a*- greenness, + b*- yellowness and - b*- blueness (Hunter, LabScan XE - Reston VA, USA). The transversely cut surface of a slice was centered over the aperture, oriented so that the arrow cut in the opposite end pointed away from the colorimeter operator.

To determine the suitability of the tristimulus reflectance procedure for evaluating browning inhibitors applied to cut surface of Red delicious apple slices. Treatments were applied to 2 slices and 2 slices as a control, using only one apple fruit. Treatments consisted of 5min. dips in freshly prepared of cold and hot of Carob, Licorice and Doum extracts. After dipping, the slices were drained, blotted dry with absorbent tissue and then held in covered glass dishes to minimize dehydration at the cut surface for 24 hours at room temperature (25 °C) during which time tristimulus reflectance measurements were made at intervals. Values of the tristimulus coordinates in the L*, a* and b* values were recorded at 0, 10, 30, 60, 120, 180, 210min. and after 24 hours. The tristimulus coordinates were plotted against time, and the slopes of linear portions of these curves were obtained by linear regression. Changes in the color due to the treatment cold and hot of Carob, Licorice and Doum extracts and during storage at 4 °C for 4 weeks were recorded at 1 day, 2 and 4 weeks using a tristimulus color analyzer (Hunter Lab Scan XE - Reston VA, USA).

Microbiological experiments:

The populations of total bacteria, yeast and molds were determined by the method of Sadler, *et al.* (1992). The counts of total bacteria (TPC), yeast and molds (Y&M) calculated per one gram of all apple slices using plate count agar and malt extract agar (Merck KGaA, Darmstadt, Germany), respectively. The number of colonies (TPC or Y and M) that appeared on the plates was counted and expressed as Colony Forming Unit (CFU/g).

Statistical analysis:

The results of sensory evaluation were analyzed statistically using the analysis of variance and the Least Significant Difference (LSD) as described by Richard and Gouri, (1987). Both the treatments and the samples were repeated at least in duplicate.

Results and Discussion:

Gross chemical composition of carob, licorice and doum fruits

Data presented in Table (1) show that the licorice and doum fruits could not be considered as a source of protein, where, the percentage of protein was 3.39 and 4.91%, respectively, while the percentage of protein was high

9.63% in carob fruit. Total carbohydrates were also very low in carob fruit (80.09%) and increased in licorice and doum fruits 87.54 and 85.33 % respectively. Lipids percentage was approximately at similar levels (4.27 and 5.26%) in licorice and doum fruit, respectively and it increased to 6.53 in carob fruit. Ash percentage was approximately at similar levels (4.81 and 4.50%) in licorice and doum fruit, respectively and decreased to 3.75% in carob fruit. These results in agreement with the results of Avallone *et al.*., (1997); Drake and Eisele (1999) and Bengoechea *et al.*., (2008).

Table (1): Gross chemical composition (% Dray weight bases) of doum, carob and licorice fruit.

Fruit samples	Protein	Fat	Ash	Total Carbohydrate
Carob fruit	9.63	6.53	3.75	80.09
Licorice fruit	3.39	4.27	4.81	87.54
Doum Fruit	4.91	5.26	4.50	85.33

Physico - Chemical composition of carob, licorice and doum cold and hot extracts:

The pH of licorice and doum cold and hot extracts ranged from 3.81-4.59, and carob cold and hot extracts showed increased pH values to 5.96-6.39. TSS (°Brix) of carob, licorice and doum hot extracts were greater 5 times than the TSS (°Brix) of carob, licorice and doum cold extracts, as shown in Table 2. Whereas, the increase of TSS was obvious with increasing of extraction and concentration of extracts. This increasing of TSS was attributed to the greater degree of tissue breakdown, releasing more components that contribute to soluble solids (Tung-Sun, *et al.*, 1995). The TSS / acid ratio is the major analytical measurement for quality in natural extracts. The TSS / acid ratio of carob, licorice and doum hot extracts were greater 4 times than the ratio of carob, licorice and doum cold extracts, as seen in Table 2. TSS / acid ratio was shown to be correlated with sweetness but not so closely with flavour (Guyer, *et al.*, 1993).

Table (2): Physico-chemical properties of cold and hot extracts from doum, carob and licorice fruit.

Different Extracts	PH	TSS	% acidity*	TSS / acid ratio	Vitamin C (mg/100ml)	T.phenols** (mg/100ml)	Viscosity (cP)
Cold Carob	5.96	1.7	0.038	44.27	19	85.88	0.22
Hot Carob	6.39	3.6	0.067	53.57	9.5	162.39	0.22
Cold Licorice	3.81	3.6	0.250	14.42	15.2	107.74	0.209
Hot Licorice	4.44	14.7	0.230	63.80	3.8	184.25	0.198
Cold Doum	4.15	4.8	0.288	16.67	22.8	235.78	0.242
Hot Doum	4.59	17.8	0.269	66.22	7.6	259.20	0.198

*** % acid value as a citric acid ** expressed as gallic acid**

Titratable acidity of licorice and doum cold and hot extracts (0.230 - 0.288) was higher than carob cold and hot extracts (0.038-0.067) as reported in Table2, which may due to degradation of pectin resulting in an increasee of total acid. Results in table 2, reveal that the carob, licorice and doum cold extracts contained a high level of vitamin C (15.2 – 22.8 mg/100 ml). It is of importance to mention that pectinase enzyme treated PPJ maintain vitamin C

more than that of hot extracts. However, vitamin C was decreased in carob, licorice and doum hot extracts to 3.8 – 9.5 mg/100ml due to the breakdown of vitamin C by boiling process of extracts, as recorded in Table 2. The high content of vitamin C in cold extracts may suggest a good source for such vitamin. These results are in accordance with those of Tung-Sun, *et al.*, (1995) and Hsu *et al..*, (2006).

The amount of materials that can be extracted from a plant depends on the vigour of the extraction procedure and the possibility exists of sample-to-sample variation in extracted materials. We employed a double extraction (cold and hot) of carob, licorice and Doum fruit as an example extraction for the study. For a hot water infusing procedure that is commonly employed in preparing drink from the dried Doum fruit (30 min infusing with boiling water but with continuous stirring), bulk material was extracted from the fruit. Since most antioxidant activities from plant sources are derived from phenolic-type compounds (Bravo, 1998), the total phenolic content of the fruits and extracts was measured and was expressed as gallic acid. A gallic acid standard curve was obtained with a linear coefficient value of 0.9891 and was used for the calculation of total phenol cotents (mg/100ml) as shown in Table 2. Total phenol compounds contents in carob, licorice and doum hot extracts was greater than in cold extracts. However, this increasing by the boiling process attributed to the greater degree of tissue breakdown, releasing more phenol components that contribute to total phenol content. This was not unexpected because the extract comes from the mesocarp of the fruit. A more vigorous by hot water extraction procedure at boiling temperature for 30 min increased the amount of phenolic materials. These results are in accordance with those of Tung-Sun, *et al.*, (1995) and Hsu *et al..*, (2006). The viscosity reduced from 0.22cP in cold extracts to 1.98cP in hot extracts for licorice and doum dut to the efficient of boiling process, while it was 0.22cP in carob cold and hot extracts, as reported in table 2.

Effect of cold and hot extracts of doum, carob and licorice on microbiological counts in of apple slices during storage:

Total microbial count of different apple slices treatments with carob, licorice and doum cold and hot extracts, and of untreated apple slices were followed up through four weeks at 4oC. Results that show the effect of treating apple slices with the studied various carob, licorice and doum cold and hot extracts and stored at 4 °C for 4 weeks on inhibiting the microbial counts are showed in Tables 3 & 4.

It can be observed that the apple slices pretreated with cold carob and licorice extracts have the highest inhibition of yeast & molds (Y&M), but cold and hot licorice extracts of total plate count bacteria (TPC) followed by those pretreated with hot doum extract after 4 weeks storage at 4 °C. Untreated apple slices were 1.08 log (CFU/g) of TPC compared to 2.12 log (CFU/g) in case of those Y&M after 4 weeks storage at 4 °C. Whereas, the apple slices treated with cold carob and licorice was 0.72 and 1.10 log (CFU/g) of Y&M but with cold and hot licorice 0.95 and 0.85 log (CFU/g) of TPC after 4 weeks storage at 4 °C. But those pretreated with cold doum extract and untreated samples had the lowest reduction of Y&M and TPC for 4 weeks stored at

4°C. These results are partially confirmed by those of Lester *et al.*., (1983), Toshio *et al.*., (2002) and Vivek *et al.*., (2008) They found that the licorice extracts as antimicrobial agents. Also, Andrés-Elias *et al.*., (2007) investigated that the carob extracts as antimicrobial agents. Also, results from Tables (3&4) showed that the refrigeration temperature 4 °C could enhance the inhibitory effect of cold carob and licorice extracts but not hot carob, licorice and doum extracts. These results nearly consistent with results given by Ting and Deibel (1992) who indicated that refrigeration temperature (4°C) could enhance the inhibitory effect of sage.

Table (3): Effect of doum, carob and licorice extracts (cold and hot) on the log (CFU/g) of growth yeast and molds (Y&M) in apple slices during storage at refrigerator (4°C).

Treatments	Zero time		Two Weeks		Four Weeks	
	Y &M	SD*	Y &M	SD	Y &M	SD
Control	2.30	0.22	2.08	0.08	2.12	0.41
Cold Carob	1.79	1.06	1.64	1.02	0.72	0.22
Hot Carob	0.90	0.23	1.08	1.00	1.34	1.01
Cold Licorice	1.00	0.65	0.99	0.19	1.10	1.04
Hot Licorice	1.18	0.84	1.60	1.05	1.56	0.74
Cold Doum	0.80	0.10	1.53	1.32	1.79	1.74
Hot Doum	0.30	1.11	0.84	0.45	1.00	0.71

* SD = Standard Deviation (σ_{n-1}) of the log values of yeast and molds.

Table (4): Effect of doum, carob and licorice extracts (cold and hot) on the log (CFU/g) of growth bacteria (TPC) in apple slices during storage at refrigerator (4°C).

Treatments	Zero time		Two Weeks		Four Weeks	
	TPC	SD*	TPC	SD	TPC	SD
Control	2.30	0.29	1.24	1.07	1.08	0.23
Cold Carob	2.30	0.31	1.62	0.41	1.00	0.31
Hot Carob	0.66	0.44	1.23	0.45	1.00	0.28
Cold Licorice	2.30	1.00	1.34	0.24	0.95	1.00
Hot Licorice	2.30	1.11	0.96	1.08	0.85	1.45
Cold Doum	1.66	0.21	1.51	0.19	1.00	1.00
Hot Doum	2.30	0.34	1.19	1.04	1.00	0.17

* SD = Standard Deviation (σ_{n-1}) of the log values of bacteria or total plate counts.

Effect of cold and hot extracts of doum, carob and licorice on non-enzymatic browning and color characteristics of apple slices during storage :

The non-enzymatic browning is the major analytical measurement for quality in fruit. The non-enzymatic browning (OD 420nm) was slightly close (3 - 25.6) for all cold and hot of carob, licorice and doum extracts treated apple slices and also untreated sample, directly after treatment. Hot extracts of carob, licorice and doum treated sample showed very little browning (3 - 9.5) after 24 hours storage at room temperature, but untreated and cold extracts treated showed increased browning (4 – 25.6) after 24 hours storage at 22 °C, as indicated in Fig 1.

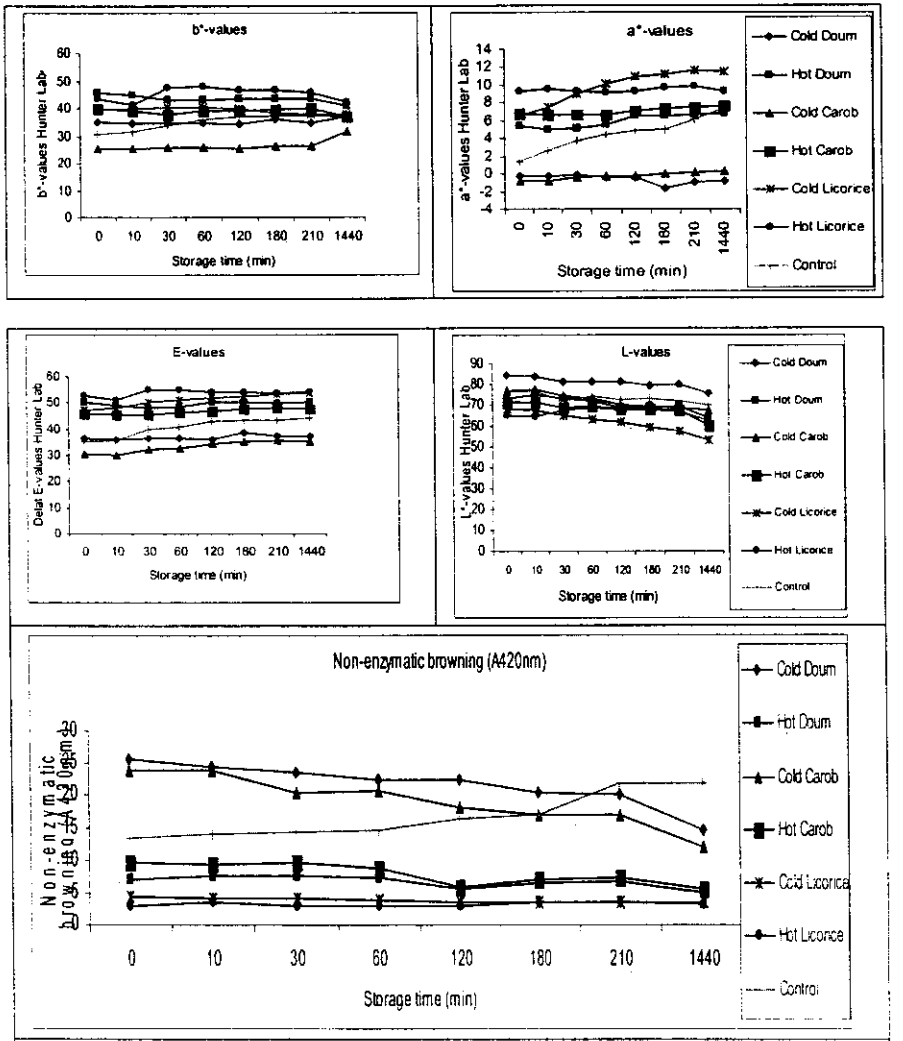

Fig 1: Effect of cold and hot extracts of doum (CD, HD), carob (CC, H licorice (CL, HL) on color characteristics and non-enzymatic bro of apple slices during storage at room temperature (23 °C) hours..

From figure (1), it could be seen that the non-enzymatic browning (OD 420nm) reduced to 3-4.5% after hot and cold licorice extract treatment at zero time and to 3.3-3.5% after 24 hours storage at room temperature. The hot doum and hot carob extract treatment was reduced non-enzymatic browning (OD 420nm) to 5 – 5.5% after 24 hours at 25 °C, while all other extracts treatments were not efficient on the non-enzymatic browning (OD 420nm).

From figure (2), it could be seen that the non-enzymatic browning (OD 420nm) reduced to 6.49% after hot licorice extract treatment at zero time and to 6.05% after 4 weeks storage in a refrigerator (4 °C). The cold carob extract treatment reduced non-enzymatic browning (OD 420nm) to 9.73% after 4 weeks at 4oC, while all other extracts treatments were not efficient on the non-enzymatic browning (OD 420nm).

Browning of the apple slices was measured by a* (green-red) and L* (lightness-darkness). A decrease in L*-value and an increase in a*-value are indicative of browning (Monsalve *et al.*., 1993). No heat treatment was given to the apple slices; thus enzymatic activity of polyphenoloxidase was assumed. The results show the effect of treating apple slices with the different cold and hot extracts of doum, carob and lacorice as antibrowning agents and stored at room temperature for 24 hours at (25 °C) and for 4 weeks at 4 °C on inhibiting the browning reactions. All these results are graphically represented in Figs 1 & 2. These figures illustrate the change in the color of apple slices in terms of a*-values over storage periods after slicing and dipping in different cold and hot extracts of doum, carob and lacorice for each one. Also, the color a*values were recorded for the untreated apple slices over storage periods after slicing.

It can be observed that the apple slices pre-treated with cold extracts of doum and carob have no browning or the lowest a*-value (-0.7 and 0.32) followed by those pretreated with hot extracts of doum and carob and licorice (7.6 and 9.4) then with cold extract of licorice (11.6) after 24 hours stored at room temperature (25 °C). While a*-value of untreated apple slices was too high after 24 hours stored at room temperature (25 °C), as shown in Fig 1.

On other hand, both cold and hot extracts of carob and doum are considered to be stronger anti-browning agents than lacorice extracts and untreated sample, in controlling both enzymatic and non-enzymatic browning reactions.

With long term storage at 4 °C for 4 weeks, results showed that the increase of a*-values and the decrease in L*-values were high in the untreated apple slices. While, the increase in a*-values and the decrease in L*-values during 4 weeks were slight for samples treated with cold and hot extracts of doum and carob, as shown in Fig. 2. The most effective extracts pre-treatments were cold and hot extracts of doum and carob in apple slices stored for 24 hours at 25 °C, while cold and hot extracts of doum, carob and licorice in apple slices stored for 4 weeks at 4 °C were not so effective (Figs 1 & 2). These results indicated that the cold and hot extracts of doum, carob and licorice pre-treatment inhibited browning of refrigerated apple slices compared with that of the untreated samples. This result is considered as an addition to those of Vijay, (1991) and Monsalve, *et al.*, (1993), who proved the effectiveness of some extracts and 4-hexylresorcinol as anti-browning agents in tomato juice and apple slices, respectively. Research on the fruit pulp of doum showed that it contains nutritional trace minerals, proteins and fatty acids, in particular the nutritionally essential linoleic acid (Cook *et al.*., 2000). Identification of compounds, by thin-layer chromatography, showed that the fruit contains significant amounts of saponins, coumarins, hydroxycinnamates, essential oils and flavonoids (Hsu *et al.*., 2006)

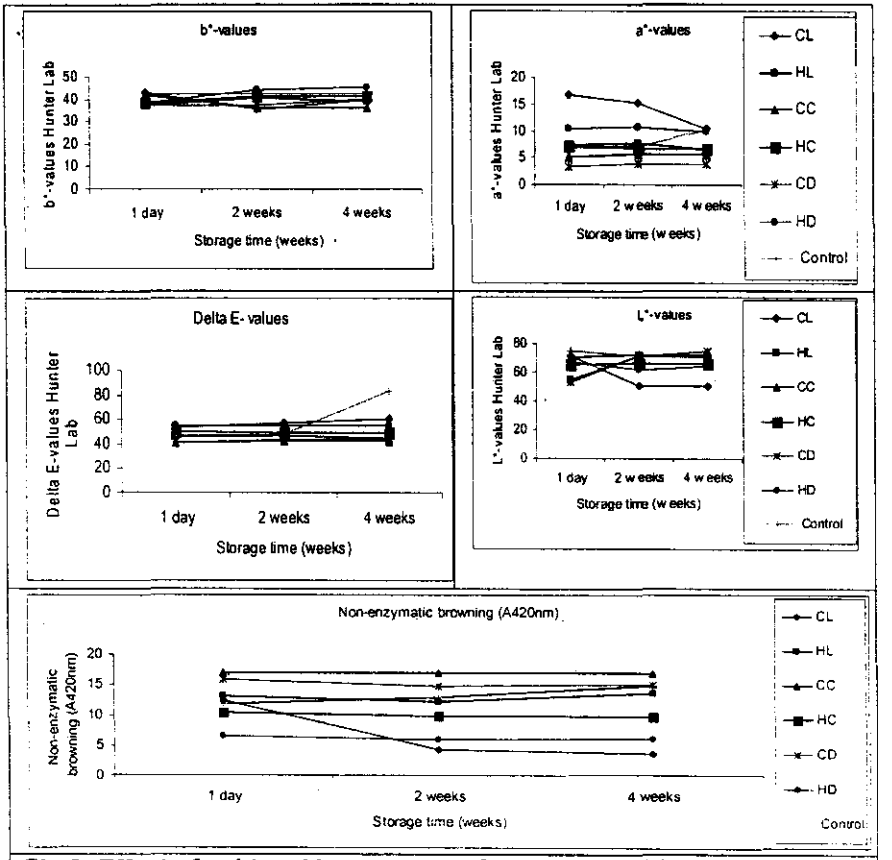

Fig 2: Effect of cold and hot extracts of doum (CD, HD), carob (CC, HC) a licorice (CL, HL) on color characteristics and non-enzymatic bro of apple slices during storage at refrigerator (4 °C) for 4 weeks.

Cold and hot extracts of doum, carob and licorice as preservatives may be due to its content of aldehydes, phenol compounds, organic acids and volatile compounds that are efficient on inhibition of browning and inhibition of growth microorganisms. These results are confirmed by Nakatani (1994), who found that the essential oil of extracts like eugenol and isoeugenol and other phenolic volatile components exhibited appreciable effect while they have too strong characteristic odors to be used as food additives. As to be colorless, odorless and tasteless, these diterpene compounds are suitable for food additives.

Delta E values for L* and a* both appeared to be related to the extent of browning, changes in a*-value. Because of occasional inconsistencies between L* and a*- values, perhaps due to determine the extent of browning in apple slices, then measurements should be made at times appropriate to

the samples and treatments, as shown in Figs 1 & 2. The results in Figs 1 & 2 showed that the Delta E values were closely constant during storage at refrigeratore (4 °C) in hot extracts of carob, doum and licorice, while increased during storage at refrigeratore (4 °C) in cold extracts of carob, doum and licorice.

In general, refrigeration effects caused an increase in the a*-values and a decrease in the L*-values. Furthermore, for long-term storage at 4 °C, the results showed that the apple slices pre-treated with cold and hot extracts of doum and carob had no browning or the lowest a*-value followed by those pretreated with cold and hot extracts of licorice after 4 weeks stored at refrigerator (4 °C). While the a*-value of untreated apple slices was too high after 4 weeks stored at refrigerator (4 °C), as shown in Figs 2. The results showed that application of a browning inhibitor solution containing cold and hot extracts of doum, carob and licorice could control the enzymatic browning of apple slices. The use of these pre-treatments, especially cold and hot extracts of doum and carob constituted an effective method of quality improvement and shelf life extension.

In general, the refrigeration effects prevent browning (an increase in the a* and b*-values and a decrease in the L*-values) and increase the inhibition of total plate count, yeast and mold counts. The refrigeration of apple slices effects an increase in the a* and b*-values and a decrease in the L*-values. Also, the results showed that in the apple slices pretreated with cold extracts of doum and carob showed no browning and lowest microbial count (Y&M and TPC) with cold carob and licorice during storage at 4 °C for 4 weeks. Licorice and doum as preservatives may be due to their aldehydes, organic acids, phenol compounds and volatile compounds that are efficient on inhibition of browning and inhibition of growth microorganisms. These results confirmed by the results of Nakatani (1994) who found that the essential oil of spices like eugenol and isoeugenol in Clove and other phenolic volatile components exhibited appreciable effect while, they have too strong characteristic odors to be used as food additives. They suggested that the inhibitory mechanism and the anti-fungal action of the aldehydes were due to the ability to form charge transfer complexes with electron donors and reactivity with SH group in cystein or glutathione moieties. Shelef (1983) studied the inhibition of gram positive and gram negative food borne bacteria, yeast and mold by garlic, onion, cinnamon, cloves and other spices in pickles, rice and meat products. He found that spices have also been known to posses antimicrobial properties toward bacteria, yeasts and molds. Eugenol carvacrol and thymol had been identified as the major anti-microbial compounds in cloves and cinnamon.

Conclusion:

It can be concluded that the treatment of apple slices with different cold and Hot extracts of carob and doum greatly inhibits the non-enzymatic and enzymatic browning (a*-values) reaction. The most effective extracts pre-treatments were cold and hot extracts of doum and carob in apple slices stored for 24 hours at 25 °C. The cold and hot extracts of carob and licorice pretreatment of apple slices increased the inhibition of total plate count, yeast

and mold counts for 4 weeks stored at 4oC. In general, the results showed that. different cold and Hot extracts of carob, licorice and doum are considered to be anti-browning and anti-microbial agents, in controlling both enzymatic and non-enzymatic browning reactions and microorganisms, respectively. The use of these pre-treatments, especially cold and hot extracts of doum and carob constitutes an effective method of quality improvement and shelf life extension. Results of this work could indicate that the cold and hot extracts of carob, licorice and doum may serve as alternative to conventional chemical preservatives in the preservation of fruit slices by hurdle technology. A practical application of cold and hot extracts of carob, licorice and doum in food as natural inhibitors against browning and microorganisms has to be further studied.

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تأثير المستخلصات الطبيعية الناتجة من كل من الدوم و الخروب و العرقسوس على جودة وسلامة شرائح التفاح

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فى هذا البحث أجريت محاولات لدراسة امكانية استخدام مستخلص الخروب و العرقسوس و الدوم كمضادات للتلون البنى و للكائنات الحية الدقيقة و ذلك على شرائح التفاح المخزنة على درجة حرارة الغرفة (٢٥ م) لمدة ٢٤ ساعة و الشرائح المخزنة بالثلاجة على درجة حرارة ٤ م لمدة ٤ أسابيع.

حيث أن شرائح التفاح التى تم نقعها فى مستخلصات كل من الخروب و العرقسوس و الدوم الباردة و الساخنة و المستخدمة كمضادات للتلون قد تم تقدير اللون بها و التغيرات الميكروبية اثناء التخزين. وقد أوضحت النتائج أن أكبر تأثير للمعاملة الأولية بمستخلصات الخروب الدوم البارد و الساخن كانت على شرائح التفاح المخزنة لمدة ٢٤ ساعة على ٢٥ م. وأن أكبر تأثير للمعاملة الأولية بمستخلصات الخروب و العرقسوس و الدوم البارد و الساخن كانت على شرائح التفاح المخزنة لمدة ٤اسابيع على ٤ م .

أيضا النتائج اوضحت أن شرائح التفاح المعالجة بمستخلصات الخروب و العرقسوس و الدوم البارد و الساخن كانت غير ملونة باللون البنى و بها أقل حمل ميكروبى من الخميرة و الفطر و البكتريا و ذلك عند التخزين بالثلاجة لمدة ٤اسابيع على ٤ م.

كما أثبت أن المستخلصات الثلاثة (الخروب و العرقسوس و الدوم) سواء البارد أو الساخن تعتبر مواد مثبطة للتلون البنى و للنمو الميكروبى فى التحكم لكل من التفاعلات البنية الغير انزيمية و الانزيمية و الكائنات الحية الدقيقة.