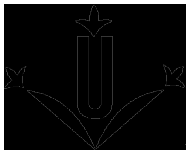

Universitat de Lleida

Escola Tècnica Superior d'Enginyeria Agrària

Departament de Tecnologia d'Aliments

Recubrimientos comestibles y sustancias de origen natural en manzana fresca cortada: Una nueva estrategia de conservación

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Tesis Doctoral

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BROWNING INHIBITION IN FRESH-CUT 'FUJI' APPLE SLICES BY NATURAL ANTIBROWNING AGENTS

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ABSTRACT

Response surface methodology was used to study the effect of antibrowning agents (4-hexylresorcinol, glutathione, N-acetylcysteine and ascorbic acid) and storage time (14 days) on the color of minimally processed Fuji apples. The selected color parameters were L^*, a^*, b^*, hue angle (h^*) and color difference (ΔE^*). Storage time had a significant effect on all the studied color parameters ($p \leq 0.05$). 4-hexylresorcinol showed the most effective individual effect on keeping constant a^* values ($p \leq 0.0001$). Besides, the interaction of N-acetylcysteine/glutathione was found to have a significant effect ($p \leq 0.05$) on maintaining a^* values over time. On the other hand, individual treatment with N-acetylcysteine in concentrations higher than 0.75% w/v may be used in order to preserve a^* and h^*. According to the *F*-test, 4-hexylresorcinol and N-acetylcysteine ($p \leq 0.05$) displayed a significant individual effect on ΔE^*, indicating that ΔE^* decreased when increasing the concentration of

these antibrowning agents. Nevertheless, color difference went down when 4-hexylresorcinol concentration increased up to 0.5%, but higher concentrations of this agent led to an increase in ΔE^* that indicates browning.

Key words: fresh-cut apples, antibrowning agents, color, response surface methodology

INTRODUCTION

'Fuji' apple (*Malus domestica* Borkh.), a cross between red "Delicious" and "Ralls Janet", was introduced in Japan in 1962. Nowadays it is extensively cultivated in the main apple-producing areas of the world. This apple cultivar stands out for its excellent quality and sensory characteristics such as juicy, firm, crispy, fine-grained with a sweet, spicy flavor that has high sugar and low acid content, as well as good storage potential (Stebbins and others 1991; Yoshida and others 1995).

Fresh-cut or minimally processed fruit is an important market in fast development because of their convenience and fresh-like quality. However, it is well known that minimally processed fruits and vegetables are generally more perishable than the original raw materials (Gorny and others 1998; Pittia and others 1999), being prone to surface browning usually caused by oxidase enzymes. Browning and its control have been extensively studied and reported in many fruits such as apples, pears and bananas (Rocha and others 1998; Abe and others 1998; Soliva-Fortuny and others 2001; Abbott and Buta 2002). Traditionally, sulphites have been used for prevention of browning. However, their use is limited because of their toxicity (Iyengar and McEvily 1992). On the other hand, consumers are demanding a reduction in the overall use of chemicals on fresh products and alternative methodologies are being investigated to extend shelf-life of fresh-cut fruits. The use of natural compounds and their derivatives has been found to be

effective in reducing browning in many fresh-cut fruits and vegetables (Ahvenainen 1996; Buta and others 1999; Son and others 2001; Gonzalez-Aguilar and others 2001; Gorny and others 2002). The most frequent alternative to sulphite is ascorbic acid which is generally recognized as safe (GRAS) antioxidant for being used in fruits and vegetable to prevent browning by the US Food and Drug Administration (FDA). However, its effect is temporary (Özoğlu and Bayındırlı 2002) so that other alternatives should be found to control browning. In this sense, several sulfur-containing amino acids and their derivates have been investigated as inhibitors of enzymatic browning (Molnar-Perl and Friedman 1990; Nicoli and others 1994; Son and others 2001; Gorny and others 2002). Cysteine and glutathione belong to this group. Both compounds act by reducing *o*-quinones back to their *o*-diphenols precursor, preventing the formation of pigments or reacting with *o*-quinones to yield colorless compounds (Richard and others 1991). In the case of 4-hexylresorcinol, it has been stated that it is effective in the control of enzymatic browning in several apple cultivars, although its effect is better when used in combination with other reducing compounds such as ascorbic acid (Monsalve-Gonzalez and others 1993; Luo and Barbosa-Canovas 1997; Dong and others 2000).

The use of an adequate experimental design is particularly important in assessing the effect of treatments on quality attributes. Response surface methodology (RSM) consists of mathematical and statistical procedures to study the relationships between one or more responses (dependent variables) and a number of factors (independent variables) (Diniz and Martin 1996). The multi-variate approach reduces the number of experiments, improves statistical interpretations and indicates whether parameters interact (Myers and Montgomery 2002). Central composite design is the most widely used response surface design.

The objective of this work was to evaluate the individual or combined effect of 4-hexylresorcinol, glutathione, N-acetylcysteine and ascorbic acid on the color of minimally processed Fuji apples in cold storage by a RSM.

MATERIALS AND METHODS

Apples

Fuji apple cultivar was chosen for this study because of its extensive use and the rapid browning of its tissue after cutting. The apples were provided by ACTEL, Lleida, Spain, at commercial maturity and stored at 4 ± 1 ºC prior to processing. A characterization of the raw material was carried out according to AOAC procedures (Horwitz 2000).

Fruit processing

Selected apples of uniform size were cleaned, peeled and cut into 4 mm thick slices. These were then dipped for 1 min in water (control) or different combinations of 4-hexylresorcinol, glutathione, N-acetylcysteine (Sigma-Aldrich Co., Steinhein, Germany) and ascorbic acid (Sigma-Aldrich Co., St. Louis, Mo., U.S.A.) aqueous solutions, according to the experimental design (Table 1). The excess of solution was then blotted off, and the samples were stored in plastic bags in contact with the air at 4 ± 1 ºC for 14 days.

Color measurement

Cut apple surface color was directly measured with a Macbeth Color-Eye 3000 colorimeter (Macbeth-Kollmorgen Inst. Corp., Newburgh, NY). The equipment was set up for illuminant D75 and 10º observer angle and calibrated using a standard white reflector plate. Three readings were obtained for each replicate by changing the position of the apple slices to get uniform color measurements. Color was recorded using a CIE-$L^*a^*b^*$ color space, where L^* indicates lightness, a^* indicates chromaticity on a green (-)

to red (+) axis, and b^* chromaticity on a blue (-) to yellow (+) axis. Total color difference (ΔE^*) was calculated as follows:

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad \text{Equation 1}$$

Numerical values of a^* and b^* were used to calculated hue angle (h^*):

$$h^* = \arctan b^*/a^* \quad \text{Equation 2}$$

Experimental design and statistical analysis

RSM was used to study the simultaneous effect of antibrowning agents and refrigerated storage time on fresh-cut apples. A central composite experimental design was chosen for this purpose (Khuri and Cornell 1996; Myers and Montgomery 2002). The experimental design yielded 44 experiments where the first 32 experiments were organized in factorial points, from 33 to 42 in star points and 43 and 44 involving the replications of the central points (Table 1). It was assumed that a mathematical function existed for each response variable Y_k in terms of 5 independent variables: 4-hexylresorcinol, glutathione, N-acetylcysteine, ascorbic acid, and storage time. Concentrations of anti-browning agents varied from 0.0 to 1.0% (w/v) and storage time from 0 to 14 days. Full design is shown in Table 1.

Table 1. Experimental central composite design used to study the effect of natural antibrowning agents on color of Fuji apple slices[a].

Run	*Variables*									
	Coded					Uncoded[b]				
	X_1	X_2	X_3	X_4	X_5	(4-HR)	(Glut)	(N-Cyst)	(AA)	(t)

1	-1	-1	-1	-1	-1	0	0	0	0	0
2	1	-1	-1	-1	-1	1	0	0	0	0
3	-1	1	-1	-1	-1	0	1	0	0	0
4	1	1	-1	-1	-1	1	1	0	0	0
5	-1	-1	1	-1	-1	0	0	1	0	0
6	1	-1	1	-1	-1	1	0	1	0	0
7	-1	1	1	-1	-1	0	1	1	0	0
8	1	1	1	-1	-1	1	1	1	0	0
9	-1	-1	-1	1	-1	0	0	0	1	0
10	1	-1	-1	1	-1	1	0	0	1	0
11	-1	1	-1	1	-1	0	1	0	1	0
12	1	1	-1	1	-1	1	1	0	1	0
13	-1	-1	1	1	-1	0	0	1	1	0
14	1	-1	1	1	-1	1	0	1	1	0
15	-1	1	1	1	-1	0	1	1	1	0
16	1	1	1	1	-1	1	1	1	1	0
17	-1	-1	-1	-1	1	0	0	0	0	14
18	1	-1	-1	-1	1	1	0	0	0	14
19	-1	1	-1	-1	1	0	1	0	0	14
20	1	1	-1	-1	1	1	1	0	0	14
21	-1	-1	1	-1	1	0	0	1	0	14
22	1	-1	1	-1	1	1	0	1	0	14
23	-1	1	1	-1	1	0	1	1	0	14
24	1	1	1	-1	1	1	1	1	0	14
25	-1	-1	-1	1	1	0	0	0	1	14
26	1	-1	-1	1	1	1	0	0	1	14
27	-1	1	-1	1	1	0	1	0	1	14
28	1	1	-1	1	1	1	1	0	1	14
29	-1	-1	1	1	1	0	0	1	1	14
30	1	-1	1	1	1	1	0	1	1	14
31	-1	1	1	1	1	0	1	1	1	14
32	1	1	1	1	1	1	1	1	1	14
33	-1	0	0	0	0	0	0.5	0.5	0.5	7

34	1	0	0	0	0	1	0.5	0.5	0.5	7
35	0	-1	0	0	0	0.5	0	0.5	0.5	7
36	0	1	0	0	0	0.5	1	0.5	0.5	7
37	0	0	-1	0	0	0.5	0.5	0	0.5	7
38	0	0	1	0	0	0.5	0.5	1	0.5	7
39	0	0	0	-1	0	0.5	0.5	0.5	0	7
40	0	0	0	1	0	0.5	0.5	0.5	1	7
41	0	0	0	0	-1	0.5	0.5	0.5	0.5	0
42	0	0	0	0	1	0.5	0.5	0.5	0.5	14
43	0	0	0	0	0	0.5	0.5	0.5	0.5	7
44	0	0	0	0	0	0.5	0.5	0.5	0.5	7

[a] Central composite with one block and with 44 assays.
[b] Uncoded variables: concentrations of antibrowning agents in % w/v and storage time in days. 4-HR = 4-hexylresorcinol, Glut = Glutathione, N-Cyst = N-acetylcysteine, AA = Ascorbic acid and t = days of storage.

The selected responses were L^*, a^*, b^*, h^* and ΔE^*. The second-order response function for our experiments was predicted by the Eq. 3

$$Y = \beta_\theta + \sum \beta_i \chi_i + \sum \beta_{ii} \chi_i^2 + \sum \beta_{ij} \chi_i \chi_j, \quad \text{Equation 3}$$

where Y is the dependent variable, β_θ is the constant, β_i, β_{ii} and β_{ij} represent the coefficients of the linear, quadratic and interactive effects, respectively; χ_i, χ_i^2 and $\chi_i\chi_j$ represent the linear, quadratic and interactive effects of the independent variables respectively. Three-dimensional surface plots and contour plots were drawn to illustrate the interactive effects of the two

factors on the dependent ones, while the other factors were kept constant. The analysis of variance (ANOVA) was used in order to determine significant effects of 4-HR (X_1), Glut (X_2), N-Cist (X_3), AA (X_4) and t (X_5) on L^*, a^*, b^*, h^* and ΔE^*. In order to find significant differences, *F*-values at probability levels of 0.0001 and 0.05 were studied. Design-Expert 6.0.1 software package (State-Ease Corp., Minneapolis, Minn., U.S.A.) was used to generate designs to fit the response surface model to the experimental data and drawn response surface plots.

RESULTS AND DISCUSSION

Characterization of flesh apples

A physicochemical characterization was carried out to offer detailed information about the fruits used in the study, such as firmness, soluble solids content, titrable acidity and color of non-processed apples (Table 2). Initial L^*, a^* and b^* values of the apples slices were 76.4 ± 0.7, -1.2 ± 0.2 and 6.3 ± 0.33, respectively. These values were considered as a reference to determine the changes in the color parameters during the storage.

Table 2. Analytical characteristics of flesh apples.

a_w	0.9845 ± 0.0008
pH	3.96 ± 0.18
Firmness (N)	9.46 ± 0.17
Soluble solids (ºBrix)	13.4 ± 0.5
Total acidity (g citric/100g)	0.23 ± 0.12
Dry content (%)	13.48 ± 0.03

Moisture content (%)	86.52 ± 0.02
Color parameters	
L^*	76.4 ± 0.7
a^*	-1.2 ± 0.2
b^*	6.13 ± 0.33

Color evolution

Results of the analysis of variance (*F*-test) for each dependent variable and their corresponding coefficients of determination (R^2) obtained by fitting the experimental data to the second order response model are shown in Table 3. The developed models for L^*, a^*, h^* and ΔE^* appeared to be adequate ($p>0.05$), showing no significant lack of fit ($p>0.05$). The regression coefficients for the quadratic models for L^*, a^*, h^* and ΔE^* are listed in Table 4. However, the developed model for b^* did not result to be significantly appropriate ($p>0.05$).

CIE L^*, a^*, b^* color parameters

The L^* value is a useful indicator of darkening during storage, either resulting from oxidative browning reactions or from increasing pigment concentrations (Rocha and Morais 2003). In the present study, all the observed changes in color parameters were found to be highly related to storage time, especially the decrease in L^* value (R^2 = 0.8217) (Table 3). Lightness of fresh-cut Fuji apples without treatment (control) decreased from 76.4 ± 0.7 to 66.44 ± 0.03 during 14 days of refrigerated storage, while L^* values of treated fresh-cut apples with antibrowning agents were maintained constant during all storage time without any significant

decrease. Similar effects in L^* values of apple slices (var. Liberty) dipped in a 1% solution of N-acetylcysteine or glutathione were observed previously by Son and others (2001). Storage time has been considered the limiting factor in the shelf-life of minimal processed fruits by various authors (Brecht 1995; Wiley 1997). Kim and others (1993) reported that the L^* value of 12 apples cultivars, different from Fuji, decreased sharply during storage time. They assumed that changes were due to enzymatic browning caused by tissue damage with consequent enhancement of the contact between enzymes and substrates.

Table 3. Analysis of variance of the second order model for color attributes.

		F-values				
Source	D.F.	L^*	a^*	b^*	h^*	ΔE^*
Model	20	5.30^{**}	5.86^{**}	1.79	2.18^{*}	11.03^{**}
Linear						
X_1 (4-HR)	1	0.41	23.16^{**}	2.58	4.19	12.06^{*}
X_2 (Glut)	1	0.12	3.67	0.091	1.18	3.79
X_3 (N-Cyst)	1	0.019	2.61	0.18	2.93	4.30^{*}
X_4 (AA)	1	0.15	1.69	1.75	2.71	1.73
X_5 (t)	1	24.11^{**}	22.28^{**}	9.75^{*}	7.19^{*}	152.84^{**}
Quadratic						
X_1^2	1	0.44	6.59^{*}	1.57	1.54	16.71^{*}
X_2^2	1	0.020	0.00016	0.011	0.036	0.13
X_3^2	1	0.037	0.014	0.21	0.14	0.87
X_4^2	1	0.061	0.020	0.029	0.079	0.78

X_5^2	1	28.98**	0.72	3.42	0.060	1.64
Interaction						
X_1X_2	1	0.39	0.74	0.00027	0.78	3.11
X_1 X_3	1	0.011	1.46	0.14	0.16	0.0029
X_1 X_4	1	0.13	0.38	2.10	0.15	1.94
X_1 X_5	1	1.78	0.13	0.011	0.68	12.25*
X_2 X_3	1	0.048	7.84*	0.050	3.22	0.10
X_2 X_4	1	0.11	0.064	0.56	0.0051	0.0050
X_2 X_5	1	0.25	6.46*	0.036	2.27	3.81
X_3 X_4	1	0.016	0.42	0.015	2.19	0.78
X_3 X_5	1	0.029	8.16*	0.45	4.29*	4.83*
X_4 X_5	1	0.60	0.57	0.76	2.32	1.83
Residual	23					
Lack of fit	22	14	111.21	2.33	3.15	15.20
Pure error	1					
R^2	-	0.8217	0.8359	0.6090	0.6549	0.9056

4-HR = 4-hexylresorcinol, Glut = Glutathione, N-Cyst = N-acetylcysteine, AA = Ascorbic acid and t = days of storage.
D.F.: degrees of freedom
* significant at $p \leq 0.05$
** significant at $p \leq 0.0001$

Table 4. Significant regression coefficients of the second order polynomial equations.

	Coefficients			
Model term	L*	a*	h*	ΔE*
X_1 (4-HR)	N.S	-5.5467**	34.9389	-10.5365*
X_3 (N-Cyst)	N.S	N.S	N.S	-2.8467*
X_5 (t)	-3.3238**	0.0669**	-1.6070*	0.7641**
X_1^2	N.S	4.1071*	N.S	10.4320*
X_5^2	0.2047**	N.S	N.S	N.S
X_1 X_5	N.S	N.S	N.S	-0.1768*
X_2 X_3	N.S	1.2419*	N.S	N.S

X_2 X_5	N.S	-0.0805*	N.S	N.S
X_3 X_5	N.S	-0.0905*	0.769*	-0.11109*

4-HR = 4-hexylresorcinol, N-Cyst = N-acetylcysteine and t = days of storage.
* significant at p≤0.05
** significant at p≤0.0001
N.S: no significant

The a^* value is a measure of greenness and is highly related to color changes of apple flesh (Goupy and others 1995). Changes in a^* values have previously been used in monitoring browning at cut apple surfaces (McHugh and Senesi 2000). Figure 1 shows the graphical representation (Pareto plot) of the size effect of each variable over a^* and any of these variables resulted significant (p≤0.05) when the size of its effect was greater than 2. The positive values of linear regression terms indicate that the higher the concentration of antibrowning agent or the higher the storage time, the higher the a^* value (Table 4). The analysis of the overall data set indicated that 4-hexylresorcinol (*F*=23.16) and storage time (*F*=22.28) showed the most pronounced individual effects on response (p≤0.0001). The negative sign of the effect of 4-hexylresorcinol (-4.61) indicated that an increase in the concentration of this compound lead to a decrease in a^* values. On the contrary, the positive (4.54) effect of the storage time indicated that a^* values increased over time (Figure 1). The interaction between glutathione and N-acetylcysteine (*F*=7.84) had the least significant influence on a^* (p≤0.05) (Table 3 and Figure 1). When N-acetylcysteine or glutathione were used individually, high concentrations (around to 1% w/v) were necessary to keep a^* values effectively during all storage time (Figures 2a and 2b).This is

in agreement with the results obtained by Son and others (2001) who observed no change in the color of Liberty apple slices dipped in a 1% solution of cysteine or glutathione. By contrast, Nicoli and others (1994) indicated that a treatment of 0.1% of cysteine in Golden Delicious apples was sufficient to keep the color at 0 ºC for no longer than 1 d. According to these authors, this ineffectiveness of the treatment was attributed to the fact that the employed concentrations were low. However, high concentrations of sulphur-containing compounds such as N-acetylcysteine and glutathione may produce an unpleasant odour in fruits and vegetables when used as dipping agents (İyidoğan and Bayındırlı 2004).

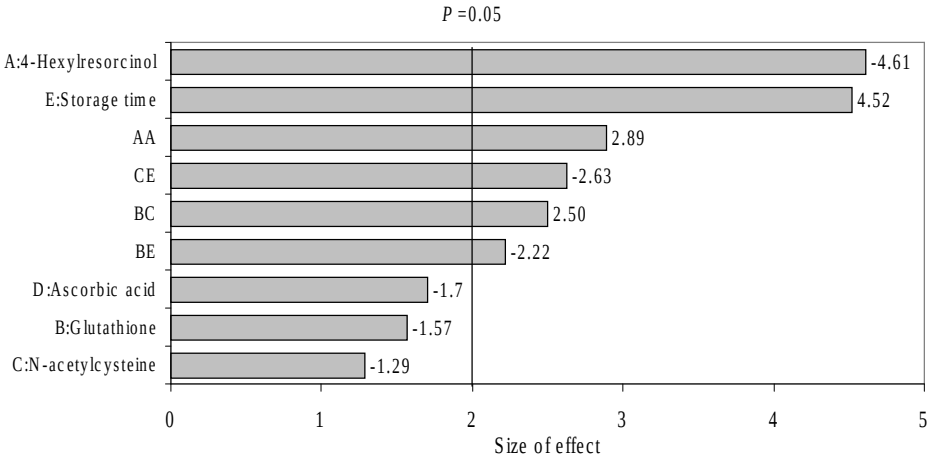
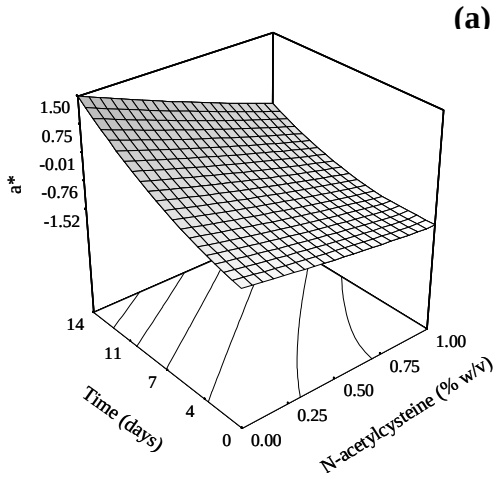


Figure 1. Pareto plot for the individual effects and significant interactions on a^* values of Fuji apple slices.

The combination of different agents in lower concentrations may prevent browning better than a specific chemical agent alone. L-cysteine at lower concentrations than 1 m*M* may be used together with different types of antibrowning agent to control enzymatic browning and this combination may be more suitable in terms of odour and bleaching effect (Özoğlu and Bayındırlı 2002). In our study, a synergistic effect was observed on the Fuji apple slices when coupling N-acetylcysteine and glutathione.



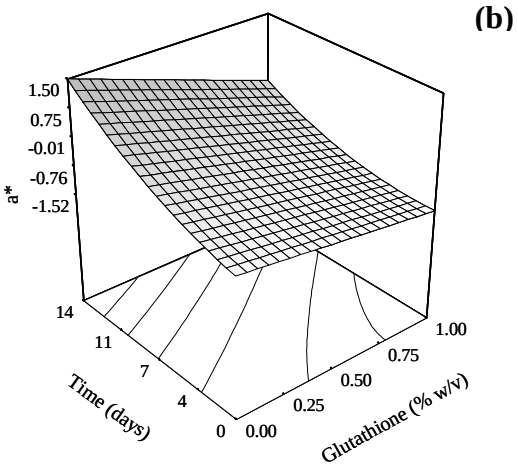


Figure 2. Response surface for the effect of N-acetylcysteine (% w/v) (a) and glutathione (% w/v) (b) with storage time (days) on a^* values of Fuji apple slices.

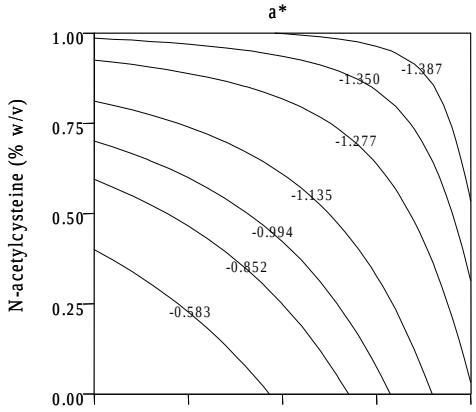
The effect of the interaction of both agents on a^* values are shown in Fig. 3a and 3b, corresponding to storage days 7 and 14, respectively. The N-acetylcysteine/glutathione combination gave the best a^* values ($p \leq 0.05$) during the first 7 days of storage (Figure 3a), being necessary concentrations around 0.60% w/v of each antioxidants to maintain a^* values near the initial one (-1.2 ± 0.2). However, higher concentrations of these agents were required to keep a^* values around initial value after 14 days of storage. Molnar-Perl and Friedman (1990) showed that the combination of N-acetylcysteine and glutathione, at concentrations of 25 or 50 m*M*, prevented browning of Golden and Red Delicious apple slices. Additionally, quadratic term of 4-hexylresorcinol (F=6.59 ; $p \leq 0.05$) had a significant effect on the response, indicating that a^* decreased when this antibrowning agent concentration was increased up to 0.7% w/v, but higher concentrations of

this agent led to an increase in a^* values and thus a darker product. This fact suggests the use of 4-hexylresorcinol in low concentrations. Son and others (2001) reported that 4-hexylresorcinol in low concentrations (0.1%) was the most effective antibrowning agent in preventing the browning of Liberty apple slices. Monsalve-González and others (1993) showed that concentrations of 4-hexylresorcinol beyond 0.03% may result in an increase of the residual content in the apple tissue and may also influence apple flavor. Nevertheless, the efficiency of 4-hexylresorcinol in the control of enzymatic browning has been demonstrated by several authors, its effectiveness is better when used in combination with other compounds (Dong and others 2000), as demonstrated by Luo and Barbosa-Canovas (1997) who found that the combination of 4-hexylresorcinol with ascorbic acid improved browning control in Fuji apples slices. Moreover, Monsalve-Gonzalez and others (1993) found that low concentrations of 4-hexylresorcinol and ascorbic acid in the 0.01-0.05 % and 0.2-0.5% ranges, respectively, were sufficient to control browning in apple quarters.

The b^* values indicates chromaticity on a blue (-) to yellow (+) axis. This value is a measure of yellowing in a lot of products, but it is not frequently associated to color changes of apple tissues. The ANOVA analyse for b^* values indicated that the term was not significant ($p \leq 0.05$) indicating that this value did not seem to be related to browning (Table 3). Similar results were obtained by Rocha and others (1998) on cut apple (cv. Jonagoned), who suggested that enzymatic browning at the cut surfaces of apples could be monitored by measuring changes in reflectance L^* and a^* values while b^* values seemed to be unrelated to the appearance of browning. As L^* and a^* values were adequate, these parameters were used to asses the color changes in Fuji apple slices. Usually, a decrease in L^* value and an increase in a^*

value are indicative of browning (Mastrocola and Lerici 1991; Monsalve-Gonzalez and others 1993).

(a)



(b)

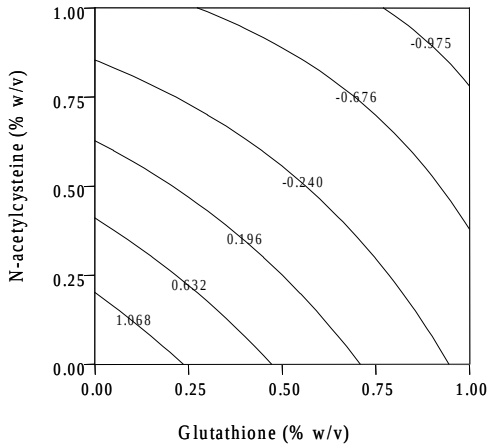


Figure 3. Contour plots for the effect of glutathione (% w/v) and N-acetylcysteine (% w/v) on a^* values of Fuji apple slices after (a) 7 and (b) 14 days of storage.

Color indexes (h^* and ΔE^*)

The hue angle and color difference are indexes frequently applied to represent changes in color of fruits over time. As h^* and ΔE^* color indexes are not only calculated from L^* and a^* but also from b^* parameters, b^* values were used to calculate them even though the model for b^* resulted not significant.

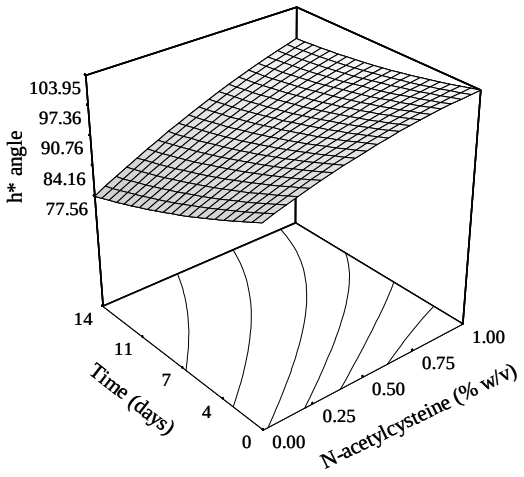


Figure 4. Response surface for the effect of N-acetylcysteine (% w/v) and storage time (days) on h^* angle of Fuji apple slices.

The h^* is an angle in a color wheel of 360º, with 0º, 90º, 180º and 270º representing the red, yellow, green and blue hue, respectively (Rocha and Morais 2003). The hue value represents true color, which is effective for visualizing the color appearance of food products (McGuire 1992). ANOVA and regression coefficients for this response are presented in Tables 3 and 4. The h^* values of apple slices without treatment decreased from 102.02 ± 0.72 to 63.87 ± 1.15 during the entire storage period, showing surface browning so that storage time had the greatest significant effect on the h^* angle ($F=7.19$; $p\leq0.05$), while the effect of N-acetylcysteine with storage time had a slight but significant effect ($F= 4.29$; $p\leq0.05$). Samples that were treated with N-acetylcysteine maintained higher hue values during storage time (Figure 4), indicating that N-acetylcysteine is an effective antibrowning agent. The effectiveness of N-acetylcysteine on h^* values is in agreement with the results obtained on a* values, since this index is calculated from numerical values of a^* and b^*, showing a directly relationship between a^* values and h^*.

Total color difference is used for determining color changes in vegetable products (Gnanasekharan and others 1992). Among the linear regression terms, storage time had the most pronounced effect on ΔE^* (F=152.84 ; $p\leq0.0001$). The positive values of linear regression terms indicated that ΔE^* increased when increasing storage time (Table 4). Figure 5 shows the graphical representation of the size effect of each variable investigated over

the response ΔE^* and any of these variables resulted significant ($p \leq 0.05$) when the size of its effect was greater than 2.

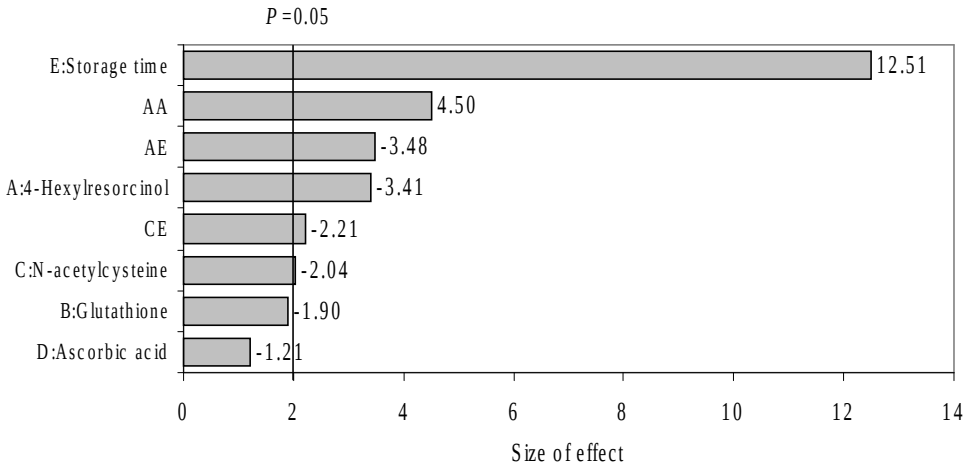


Figure 5. Pareto plot for the individual effects and significant interactions on color difference of Fuji apple slices.

Color changes at the fresh-cut fruit surface have been extensively reported, and most of the time browning has been considered the limiting factor in the shelf-life of minimal processed fruits (Brecht 1995; Wiley 1997). On the other hand, 4-hexylresorcinol (F=12.06 ; $p \leq 0.05$) and N-acetylcysteine (F=4.30 ; $p \leq 0.05$) showed a significant negative effect on ΔE^* (-3.41 and -2.04, respectively), meaning that ΔE^* decreased when these antibrowning agents concentrations increased (Table 3 and Figure 5). The positive quadratic coefficient values of 4-hexylresorcinol (F=16.71 ; $p \leq 0.05$)

indicated that ΔE^* decreased when this antibrowning agent concentration was increased up to 0.5%, but higher concentrations led to an increase in ΔE^*. No significant interactions between antibrowning agents were observed although there were significant interactions between 4-hexylresorcinol (F=12.25 ; p≤0.05) or N-acetylcysteine (F=4.83 ; p≤0.05) with storage time (Figures 6 and 7). As shown in Figure 6, minimum ΔE^* was obtained when the concentration of 4-hexylresorcinol was around 0.6% w/v. The effect of 4-hexylresorcinol is generally increased when used in combination with reducing compounds, such as ascorbic acid or glutathione. In this work, the interaction between 4-hexylresorcinol with both antibrowning agents had no significant effect in the preservation of the apple slices color during 14 days of storage at 4 ± 1ºC (Table 2).

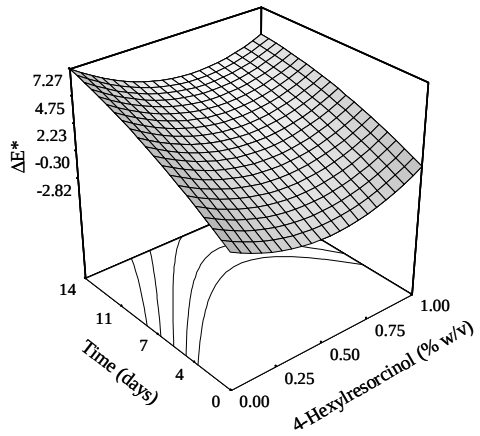


Figure 6. Response surface for the effect of 4-hexylresorcinol (% w/v) and storage time (days) on color difference (ΔE^*) of Fuji apple slices.

In contrast, N-acetylcysteine concentrations higher than 0.75% w/v were necessary to observe a lower change in color after a period of 14 days of storage (Figure 7). Özoğlu and Bayındırlı (2002) found that L-cysteine (1 m*M*) was the most effective antibrowning agent on maintaining total color difference on cloudy apple juice (Golden Delicious). Generally, the effect of cysteine appeared to be additive rather than synergistic. Red Delicious apple slices treated with a combined antibrowning dip (4-hexylresorcinol, isoascorbic acid, N-acetylcysteine and calcium propionate) and kept at 5 ºC maintained visual quality for 5 weeks when reflectance measurements of L^*, a^* and b^* values were made (Buta and others 1999). For browning estimation, the a^* value has been considered as one of the best color indexes. In our study, it can be said that a^* and ΔE^* values were highly related due to the negligible change in L^* and b^* values, that are included in the calculation of ΔE^*.

Although ascorbic acid is a usual browning inhibitor in fruits, this agent had not a significant effect on the color of fresh-cut Fuji apple. However, a progressive and lightly darkening in the apple slices was observed during storage time, indicating that its effect was not very lasting. Similar results were obtained by Luo and Barbosa-Canovas (1997) on various apple cultivars (Fuji, Gala, Jonagold, Criterion and Braeburn), which suggested that ascorbic acid is consumed during antibrowning reactions.

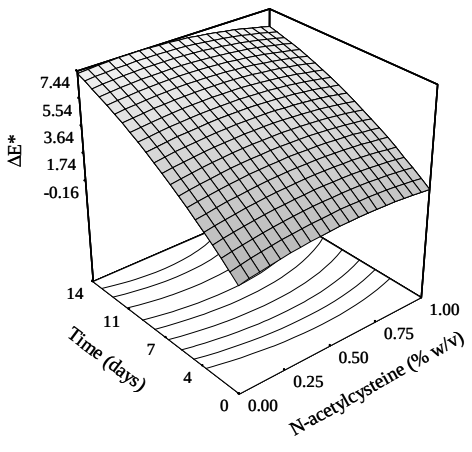


Figure 7. Response surface for the effect of N-acetylcysteine (% w/v) and storage time (days) on color difference (ΔE^*) of Fuji apple slices.

CONCLUSIONS

This study, using response surface methodology, demonstrated that browning may be avoided along refrigerated storage by an appropriate combination of natural compounds. a^* and ΔE^* values were the best parameters to monitoring browning fresh-cut Fuji apple surfaces. According to the obtained results, 4-hexylresorcinol concentrations lower than 0.5% w/v, N-acetylcysteine concentrations higher than 0.75% w/v and N-acetylcysteine/glutathione combination in concentrations higher than 0.60% w/v, were the most effective treatments in preventing browning during 14 days of storage at 4 ºC. Nevertheless, it is necessary to evaluate the effect of these antibrowning agents on sensory and nutritional quality attributes in order to determine not only the shelf-life of fresh-cut Fuji apples but also its acceptance by consumers.

REFERENCES

Abbott J, Buta G. 2002. Effect of antibrowning treatment on color and firmness of fresh-cut pears. J Food Qual 25(4): 333-41.

Abe K, Tanase M, Chachin K. 1998. Studies on physiological and chemical changes of fresh-cut bananas. I. Deterioration in fresh-cut green tip bananas. J. Jpn. Soc. Hortic. Sci. 67(1): 123-9.

Ahvenainen R. 1996. New approaches in improving the shelf-life of minimally processed fruit and vegetables. Trends in Food Sci & Technol 7(6):179-87.

Brecht JK. 1995. Physiology of lightly processed fruits and vegetables. HortScience 30(1): 18-22.

Buta JG, Moline HE, Spaulding DW, Wang CY. 1999. Extending storage life of fresh-cut apples using natural products and their derivatives. J Agric Food Chem 47(1):1-6.

Diniz FM, Martin AM. 1996. Use of response surface methodology to describe the combined effects of pH, temperature and E/S ratio on the hydrolysis of dogfish (*Squalus acanthias*) muscle. Intern J Food Sci Technol 31(5):419-26.

Dong X, Wrolstad RE, Sugar D. 2000. Extending shelf life of fresh-cut pears. J Food Sci 65(1):181-6.

Gnanasekharan V, Shewfelt RL, Chinnan MS. 1992. Detection of color changes in green vegetables. J Food Sci 57(1):149-54.

Gonzalez-Aguilar GA, Wang CY, Buta JG. 2001. Inhibition of browning and decay of fresh-cut radishes by natural compounds and their derivates. Lebensm Wiss Technol 34(5):324-8.

Gorny JR, Gill MI, Kader AA. 1998. Postharvest physiology and quality maintenance of fresh-cut pears. Acta Hortic 464:231-6.

Gorny JR, Hess-Pierce B, Cifuentes RA, Kader AA. 2002. Quality changes in fresh-cut pear slices as affected by controlled atmospheres and chemical preservatives. Postharvest Biol Technol 24(3):271-8.

Goupy P, Amiot MJ, Richard-Forget F, Duprat F, Aubert S, Nicolas J. 1995. Enzymatic browning of model solution and apple phenolic substrates by apple polyphenoloxidase. J Food Sci 60(3):497-501.

Horwitz W. 2000. Official methods of analysis of AOAC International. 17th ed. Gaithersburg: AOAC International.

Iyengar R, McEvily AJ. 1992. Antibrowning agents. Alternatives to use of sulfites in food. Trends Food Sci 3:60-4.

İyidoğan NF, Bayındırlı A. 2004. Effect of L-cysteine, kojic acid and 4-hexylresorcinol combination on inhibition of enzymatic browning in Amasya apple juice. J Food Eng 62(3):299-304.

Khuri AI, Cornell JA. 1996. Response Surface Designs and Analyses. 2nd ed. New York: Marcel Dekker Inc. 510p.

Kim DM, Smith NL, Lee CY. 1993. Quality of minimally processed apple slices from selected cultivars. J Food Sci 58(5):1115-7.

Luo Y, Barbosa-Canovas GV. 1997. Enzymatic browning and its inhibition in new apple cultivars slices using 4-hexylresorcinol in combination with ascorbic acid. Food Sci Tech Int 3(3):195-201.

Mastrocola D, Lerici CR. 1991. Colorimetric measurements of enzymatic and no-enzymatic browning in apple purees. Int J Food Sci 3:219-29.

McGuire RG. 1992. Reporting of objective color measurements. HortScience 27(12):1254-55.

McHugh TH, Senesi E. 2000. Apple wraps: a novel method to improve the quality and extend the shelf life of fresh-cut apples. J Food Sci 65(3):480-5.

Molnar-Perl I, Friedman M. 1990. Inhibition of browning by sulfur amino acids. 3. Apples and potatoes. J Agric Food Chem 38(8):1652-6.

Monsalve-Gonzalez A, Barbosa-Canovas GV, Cavalieri RP, McEvily AJ, Iyengar R. 1993. Control of browning during storage of apple slices preserved by combined methods. 4-hexylresorcinol as anti-browning agent. J Food Sci 58(4):797-800.

Myers RH, Montgomery C. 2002. Response Surface Methodology: process and product optimization using designed experiments. New York: John Wiley & Sons. 798p.

Nicoli MC, Anise M, Severinc C. 1994. Combined effects in preventing enzymatic browning reactions in minimally processed fruit. J Food Qual 17(3):221-9.

Özoğlu H, Bayındırlı A. 2002. Inhibition of enzymatic browning in cloudy apple juice with selected antibrowning agents. Food Control 13: 213-21.

Pittia P, Nicoli MC, Comi G, Massini R. 1999. Shelf-life extension of fresh-like ready-to-use pear cubes. J Sci Food Agric 79(7):955-60.

Richard FC, Goupy PM, Nicolas JJ, Lacombe JM, Pavia, AA. 1991. Cysteine as an inhibitor of enzymatic browning. 1.-Isolation and characterization of addition compounds formed during oxidation of phenolics by apple polyphenol oxidase. J Agric Food Chem 39(5):841-7.

Rocha AMCN, Brochado CM, Morais AMMB. 1998. Influence of chemical treatment on quality of cut apple (cv. Jonagored). J Food Qual 21(1):13-28.

Rocha AMCN, Morais AMMB. 2003. Shelf life of minimally processed apple (cv. Jonagored) determined by color changes. Food Control 14(1):13-20.

Soliva-Fortuny RC, Grigelmo-Miguel N, Odriozola-Serrano I, Gorinstein S, Martin-Belloso O. 2001. Browning evaluation of ready- to- eat apples as affected by modified atmosphere packaging. J Agric Food Chem 49(8):3685-90.

Son S, Moon K, Lee C. 2001. Inhibitory effects of various antibrowning agents on apple slices. Food Chem 73(1):23-30.

Stebbins RL, Duncan A, Compton C, Duncan D. 1991. Taste ratings of new apple cultivars. Fruit Var J 45(1):37-44.

Wiley RC. 1997. Preservation methods for minimally processed refrigerated fruits and vegetables. In: Wiley RC, editor. Minimally Processed Refrigerated Fruits and Vegetables. New York: Chapman & Hall, Inc. p 66-134.

Yoshida Y, Fan X, Patterson M. 1995. “Fuji” apple. Fruit Var J 49:194-7.

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EFFECT OF NATURAL ANTIBROWNING AGENTS ON COLOR RELATED ENZYMES IN FRESH-CUT FUJI APPLE SLICES

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Food Control (enviado)

ABSTRACT

Polyphenoloxidase (PPO) and peroxidase (POD) were evaluated in fresh-cut Fuji apple slices and the effect of the individual or combined use of ascorbic acid, 4-hexylresorcinol, N-acetylcysteine and glutathione on their respective activities was determined. Additionally, color changes during storage at 4ºC were measured through of 14 days of storage. PPO activity increased with storage time and was inhibited by the individual use of N-acetylcysteine and glutathione. POD activity in the apple slices was effectively inhibited by the combined use of ascorbic acid with any of the other antibrowning agents. On the other hand, an individual treatment with 1% N-acetylcysteine helped in maintaining the color of fresh-cut apples during 14 days of storage, whereas the use of ascorbic acid was not enough to prevent color deterioration of the apple slices from the first day of storage. Results obtained corroborated the effectiveness of other natural antibrowning agents over the traditional use of ascorbic acid in the control of the enzymatic browning in the fresh-cut fruit industry.

Key words: fresh-cut apple, polyphenol oxidase, peroxidase, color, antibrowning agents.

INTRODUCCION

Apple is a very popular fruit, consumed all over the world. Thus the susceptibility of apple and their products to enzymatic browning during post-harvest, handling and processing operations continues to be an important topic from the standpoint of food science and technology (Eissa, Fadel, Ibrahim, Hassan & Elrashid, 2006). Nowadays, ‘Fuji’ apple (*Malus domestica* Borkh.) is a variety extensively cultivated in the main apple-producing areas of the world. This apple cultivar stands out for its excellent

quality and sensory characteristics, as well as for its good storage potential (Brooks & Olmo, 1997; Yoshida, Fan & Patterson, 1995).

Fresh-cut fruit is an important market in fast development because of their convenience and fresh-like quality. However, it is well known that minimally processed fruit and vegetables are generally more perishable than the original raw materials (Gorny, Gill & Kader, 1998; Pittia, Nicoli, Comi & Massini, 1999). Mechanical stress during processing results in cellular delocalization of enzymes and their substrates, leading to biochemical deteriorations such as enzymatic browning, off-flavor and texture breakdown (Varoquaux, 1991). An important problem is the limited shelf-life of those commodities due to surface browning. The phenomenon is usually caused by the enzyme polyphenol oxidase (PPO) that, in the presence of oxygen, converts phenolic compounds into dark colored pigments (Zawistowski, Biliaderis & Eskin, 1991). The contribution of other enzymes such as peroxidase (POD) to total browning may also be relevant (Rolle & Chism, 1987). Valderrama and Clemente (2004) indicated that the control of the POD activity is important in processing and preservation of foods, since it could promote darkening in fruits and vegetables and their marketed products. Peroxidase is also intimately related to flavour loss and odour of stored foods, as well as a great variety of biodegradation reactions (Clemente, 1998).

Nowadays, numerous research efforts pursue the development of new ways of avoiding browning in fresh-cut fruit. Reducing agents play a relevant role in the prevention of enzymatic browning either by reducing o-quinones to colorless diphenols, or by reacting irreversibly with o-quinones to form

stable colorless products (Oms-Oliu, Aguiló-Aguayo & Martín-Belloso, 2006). Ascorbic acid is extensively used to avoid enzymatic browning of fruit due to the reduction of the o-quinones, generated by the action of the PPO enzymes, back to the phenolic substrates (Hsu, Shieh, Bill & White, 1988; McEvily, Iyengar & Otwell, 1992). It has been long applied in combination with organic acids and calcium salts to prevent enzymatic browning of fruits (Pizzocaro, Torregiani & Gilardi, 1993; Gorny et al., 1998; Soliva-Fortuny, Grigelmo-Miguel, Odriozola-Serrano, Gorinstein & Martin-Belloso, 2001; Soliva-Fortuny, Oms-Oliu & Martín-Belloso, 2002; Senesi, Galvis & Fumagalli, 1999). However, some authors have established that ascorbic acid is consumed during antibrowning reactions (Luo & Barbosa-Cánovas, 1997; Özoglu & Bayindirli, 2002; Rojas-Graü, Sobrino-López, Tapia & Martín-Belloso, 2006). Sapers (1993) reported that once the ascorbic acid has been completely oxidized to dehydroascorbic acid, quinones can again accumulate and undergo browning. Because of the individual effect of ascorbic acid is temporary, other alternatives should be searched to control browning. Several thiol-containing compounds such as cysteine, N-acetylcysteine and reduced glutathione have been investigated as inhibitors of enzymatic browning (Friedman & Bautista, 1995; Son, Moon & Lee, 2001; Gorny, Hess-Pierce, Cifuentes & Kader, 2002; Rojas-Graü et al., 2006; Oms-Oliu, et al., 2006). These compounds react with quinones formed during the initial phase of enzymatic browning reactions to yield the colorless addition products or to reduce o-quinones to o-diphenols (Richard, Goupy & Nicolas, 1991).

The use of other browning inhibitor such as 4-hexylresorcinol in fresh-cut apples has been also suggested in some works (Dong, Wrolstad & Sugar,

2000; Luo & Barbosa-Cánovas, 1997; Monsalve-González, Barbosa-Canovas, Cavalieri, McEvily & Iyengar, 1993; Son et al., 2001). 4-hexylresorcinol has structural resemblance to phenolic substrates and inhibits browning reactions by competitive inhibition of PPO. However, its effect is enhanced when used in combination with other compound such as ascorbic acid (Monsalve-Gonzalez et al., 1993; Luo & Barbosa-Canovas 1997; Dong et al., 2000). 4-hexylresorcinol may specifically interact with PPO, rendering it unable to catalyze the enzymatic reaction, while ascorbic acid reduces the quinones generated by PPO (Kahn & Andrawis, 1985).

The objective of this work was to evaluate the individual or combined effect of 4-hexylresocinol, glutathione and N-acetylcysteine on PPO and POD activity to avoid browning in fresh-cut Fuji apples slices, and compare it with the commercially used ascorbic acid.

MATERIALS AND METHODS

Fruit processing

Fuji apple cultivar was chosen for this study because of its extensive use and the rapid browning of its tissue after cutting. The apples were provided by ACTEL, Lleida, Spain, at commercial maturity, and stored at 4 ± 1 ºC prior to processing. Fruits were cleaned, peeled and cut into 4 mm thick slices. Apple slices were then dipped for 1 min in water (control) or in different solutions containing 4-hexylresorcinol (0.5% w/v), glutathione (1% w/v), N-acetylcysteine (1% w/v) (Sigma-Aldrich Co., Steinhein, Germany), or ascorbic acid (1% w/v) (Sigma-Aldrich Co., St. Louis, Mo., U.S.A.). Combinations of 1% ascorbic acid with 0.5% 4-hexylresocinol, 1% N-acetylcysteine or 1% glutathione were also tested. The excess of solution was then blotted off, and the samples were stored in plastic bags in contact with the air at 4 ± 1 ºC for 14 days. The concentrations of antibrowning agents used in this study were set up according to a previous work (Rojas-Graü et al., 2006).

Determination of PPO activity

Enzyme extraction

A portion of 50 g of apple slices was blended and mixed with a McIlvaine buffer solution (1:1) at pH = 6.5. This buffer contained 1 M NaCl (Riedel-de-Haën AG, Seelze, Germany) and 5% polyvinylpolypyrrolidone (Sigma-Aldrich Chemie, Steinheim, Germany). The homogenate was centrifuged at 12500 rpm for 30 minutes at 4ºC (Centrifuge AVANTI^{TM} J-25, Beckman Instruments Inc., Fullerton, CA, USA). The supernatant was collected and filtered through Whatman number 1 paper, and the resulting solution constituted the enzymatic extract, which was used for enzyme activity determination.

PPO activity measurement

Polyphenoloxidase activity was determined according to the method of Soliva-Fortuny et al., (2002). Enzyme activity in crude PPO extract was assayed spectrophotometrically by adding 3ml of 0.05 M catechol (Sigma-Aldrich, Steinheim, Germany) and 75 µl of extract to a 4.5ml quartz cuvette of 1cm pathlength. The changes in absorbance at 400nm were recorded every 5 sec up to 3 min from the time the enzyme extract was added using a Cecil CE 1010 spectrophotometer (Cecil Instruments Ltd., Cambridge, UK). One unit of PPO activity was defined as a change in absorbance of 0.001 per min and ml of enzymatic extract immediately after extract addition. The initial reaction rate was estimated from the linear portion of the plotted curve. All determinations were performed in triplicate.

Determination of POD activity

Enzyme extraction

The enzyme extracts were made by homogenization of 50g of apple slices with 50ml of 0.2 M sodium phosphate buffer (pH 6.5). The homogenate was centrifuged at 12500 rpm for 15 minutes at 4ºC (Centrifuge AVANTITM J-25, Beckman Instruments Inc., Fullerton, CA,

USA). The supernatant was collected and filtered through Whatman number 1 paper. The resulting solution constituted the enzymatic extract.

POD activity measurement

Peroxidase activity was determined according to the method of Cano, DeAncos & Lobo (1995). The reaction was started by adding 2,7ml of sodium phosphate buffer (0.05M, pH 6.5), 0.2ml of p-phenylenediamine 1% (w/v), 0.1ml of hydrogen peroxide 1.5% (w/v), and 0.1ml of extract to a 4.5 ml quartz cuvette of 1 cm pathlength. The changes in absorbance at 485nm were measured using a Cecil CE 1010 spectrophotometer (Cecil Instruments Ltd., Cambridge, UK). The enzyme activity unit was defined as the change in absorbance of 0.001 per min and ml of enzymatic extract immediately after extract addition. The initial reaction rate was estimated from the linear portion of the plotted curve. Each determination was run in triplicate.

Color measurement

Cut apple surface color values were directly measured with a color meter (Minolta Chroma Meter Model CR-400, Minolta, Tokyo, Japan). Color was measured using the CIE L^*, a^* coordinates. Illuminant D65 and 10º observer angle were used. The instrument was calibrated using a standard white reflector plate. Ten slices were evaluated from each bag. Three readings were made in each replicate by changing the position of the apple pieces.

Statistical analysis

Data were analyzed by analysis of covariance using statistical procedures of the Statgraphics Plus V.5.1. (Statistical Graphics Co., Rockville, MD, USA). Specific differences were determined by least significant difference (LSD). All comparisons were made at a 5% level of significance.

RESULTS AND DISCUSSION

Evolution of PPO activity

PPO activity of fresh-cut Fuji apples treated with each antibrowning agent increased from the early days after processing (Figure 1). Kahn (1977) indicated that an increase on PPO activity might be due to the activation of soluble tyrosinase forms existing in a latent state, which would be otherwise masked. Soliva-Fortuny et al., (2002) point out that the latent forms of the enzyme might be activated during storage by several factors such as proteolysis, detergents or denaturing agents, liberated by means of cell disruption and subsequent descompartmentalisation of enzymes, substrates and other substances present in cell vacuoles.

As can be seen in Figure 1, the lowest relative PPO activity of apple slices was obtained with the individual use of N-acetylcysteine or glutathione (1% w/v). Oms-Oliu et al., (2006) reported that both compounds completely inhibited the enzymatic activity of fresh-cut pears, although relative PPO activity increased from the 3rd wk of storage in pears treated with glutathione. As suggested by Molnar-Perl & Friedman (1990), Richard-Forget, Goupy, Nicolas, Lacombe & Pavia (1992) and Billaud, Brun-Mérimée, Louarme & Nicolas (2004) sulphur-containing antibrowning agents do not inhibit PPO by themselves although they give an apparent inhibition of activity due to their ability to conjugate with primary oxidation products to form stable colorless compounds, and this could be what happens in this study.

In contrast, the highest relative PPO activity was observed when the apple slices were dipped in an ascorbic acid solution. This agrees with Eissa, et al. (2006) who reported that ascorbic acid exhibited the least inhibition of PPO activity in Red Delicious apple slices treated with different antibrowning agents (sodium metabisulfite, ascorbic acid, 4-hexylresorcinol, cysteine, reduced glutathione and cysteine/ribose). Additionally, similar results on various apple cultivars (Fuji, Gala, Jonagold, Criterion and Braeburn) were observed by Luo and Barbosa-Canovas (1997) suggesting that ascorbic acid is consumed during antibrowning reactions. Ozoglu & Bayindirly (2002) correlated this temporary effect of ascorbic acid to the fact that this compound is oxidized irreversibly by reactions with intermediate pigments, endogenous enzymes and metals such as copper.

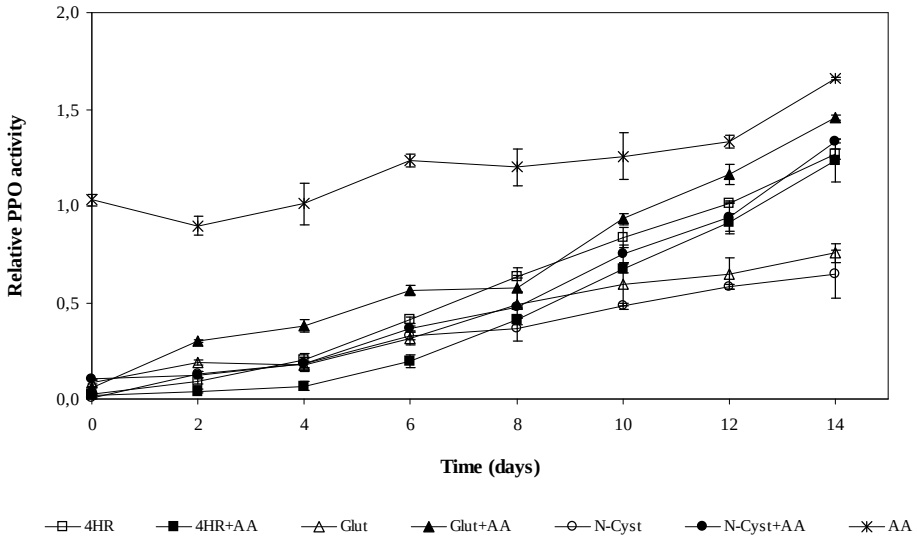

Figure 1.- Evolution of relative polyphenol oxidase (PPO) activity on fresh-cut apples treated with several antibrowning agents, containing or not ascorbic acid, during storage at 4ºC. Data shown are mean ± standard deviation.

On the other hand, some authors have indicated that the mechanism of enzymatic inhibition differs widely for each type of antibrowning agent and that the combination of different agents may be more effective in preventing browning than a specific antioxidant alone (Iyidogan & Bayindirli, 2004; Ozoglu & Bayindirly, 2002). In fact, most combinations of antibrowning agents cited in the literature or commercially available are ascorbic acid-based compositions (Pizzocaro, et al. 1993). In this work, the combination of ascorbic acid with other antibrowning agents reduced significantly ($p \leq 0.05$) the PPO activity (Table 1), although the presence of ascorbic acid did not enhance remarkably their effectiveness. In fact, samples containing a combination of 1% ascorbic acid with anyone of other compounds showed a higher PPO activity at the end of storage period. However, it was observed that the combined use of ascorbic acid and 4-hexylresorcinol effectively inhibited the enzymatic activity of fresh-cut apples during the first week of storage. Our results agree with several authors who have indicated that the effect of 4-hexylresorcinol is generally increased when used in combination with reducing compounds, such as ascorbic acid, avoiding discoloration on many fresh-cut fruits (Dong et al., 2000; Buta & Abbott, 2000; Moline, Buta & Newman, 1999). Luo & Barbosa-Cánovas (1997) found that the combination of 4-hexylresorcinol with ascorbic acid improved browning control in Fuji apple slices.

Table 1.- Analysis of covariance of the studied parameters.

	F-ratio

	PPO	POD	L^*	a^*
COVARIATE				
Time	212.98*	161.27*	647.85*	88.58*
MAIN EFFECTS				
A[a]: antibrowning treatments	7.32*	22.11*	81.41*	102.95*
B: addition of ascorbic acid	101.41*	5.54*	0.42NS	22.20*
INTERACTIONS				
AB	34.46*	123.51*	14.22*	25.40*

* $p \leq 0.05$
NS no significant
[a]: individual effect of 4-hexylresorcinol, N-acetylcysteine and glutathione.

Evolution of POD activity

Although, PPO is the main enzyme related to loss of quality on fresh-cut apples, POD can contribute to adverse changes in the flavor, color, texture or nutrient value (Fils, Sauvage & Nicolas, 1985). Analysis of covariance indicated that the type of dipping solution and the use of ascorbic acid had a significant effect ($p \leq 0.05$) on relative POD activity (Table 1). In fact, a different behavior was observed on POD activity when individual amounts of 4-hexylresolcinol, N-acetylcysteine, glutathione or their combinations with ascorbic acid were utilized (Figure 2). The individual use of ascorbic acid increased POD activity in apple slices whereas the presence of N-acetylcysteine and 4-hexylresolcinol in the dipping solution maintained constant the relative POD activity during storage time. In fruits where POD activity is more active such as melon, papaya, and strawberries, it has been observed that the effect of ascorbic acid is higher. Lamikanra and Watson (2001) reported that the presence of 1.25 and 2.5 mM ascorbic acid solutions effectively reduced POD activity in cantaloupe melon pieces.

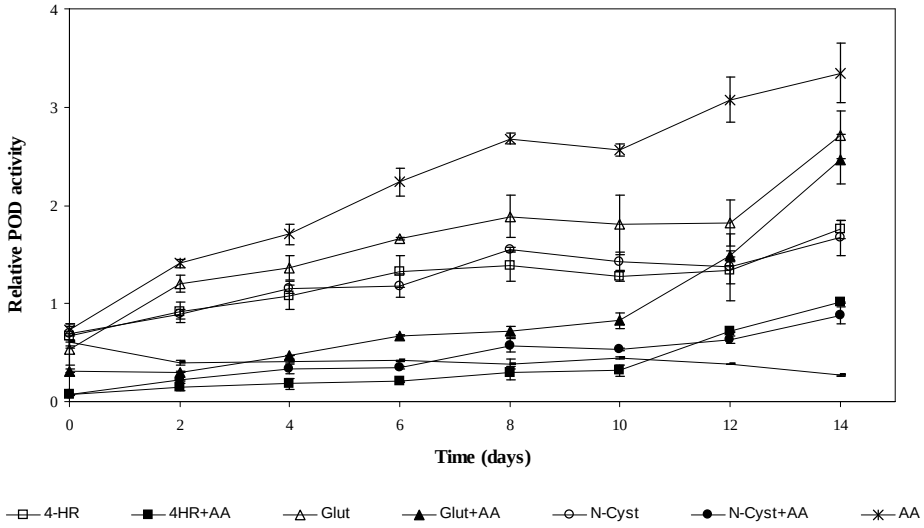

Figure 2.- Evolution of relative peroxidase (POD) activity on fresh-cut apples treated with several antibrowning agents, containing or not ascorbic acid, during storage at 4ºC. Data shown are mean ± standard deviation.

Additionally, Figure 2 shows that the combination of 1% of ascorbic acid with any of the studied antibrowning agents caused an inhibition of POD activity more efficiently that their individual uses. The lower POD activity was observed when using mixtures of 4-heylresolcinol and ascorbic acid, similar to results obtained on PPO activity. Padiglia, Cruciani, Pazzaglia, Medda and Floris (1995) indicated that POD activity may result in oxidative actions that involve any hydrogen donor component in foods. In effect, the ability of POD to contribute to enzymatic browning is related to its affinity to accept a wide range of hydrogen donors, such as polyphenols (Richard-Forget and Gauillard, 1997). POD is able to oxidize hydroxycinnamic derivatives and flavans (Robinson, 1991; Nicolas, Richard-Forget, Goupy,

Amiot & Aubert, 1994), i.e. the main phenolic structures implicated in enzymatic browning. POD also oxidizes flavonoids (Miller & Schreier, 1985; Richard & Nicolas, 1989), which are not PPO substrates but are found degraded in bruised fruits. Part of this degradation has been ascribed to co-oxidation reactions (Richard-Forget et al., 1997).

Color evolution

Color changes on the pulp of fresh-cut Fuji apples were determined by changes in lightness (L^*) and a* coordinate. Both parameters are frequently used in monitoring browning of cut apple surfaces. Usually, a decrease in L* value and an increase in a* value are indicative of browning (Mastrocola and Lerici 1991; Monsalve-Gonzalez and others 1993).

In general, L* values on fresh-cut apples decreased from the first day of storage (Figure 3). Kim, Smith and Lee (1993) reported that a rapid decrease in L* values of apples can be due to enzymatic browning caused by tissue damage with consequent enhanced contact between enzymes and substrates. However, the main depletion of L* values was observed after 1 week of storage in samples dipped with a solution containing 1% of ascorbic acid, 0.5% of 4-hexylresorcinol or a combination of both compounds, reaching values as low as 64.13 after 14 days of storage (Figure 3). According to Lozano-de-González, Barret, Wolstad and Durst (1993), the browning in apples (decrease in L* and increase of a* values) could be attributed to the consumption of substrates by PPO. The lower the L* values, the higher the relative PPO activity (Figure 1 and 3).

On the other hand, apple slices treated with 1% N-acetylcysteine solution or combination of N-acetylcysteine with ascorbic acid solution maintained higher L^* values during all the storage time (Figure 3). The antibrowning effect of *N*-acetylcysteine was observed previously by Rojas-Graü et al., (2006) in fresh-cut Fuji apple. Additionally, the lightness of samples treated with glutathione also decreased progressively during storage but this decrease was much lower when compared with those samples treated with ascorbic acid or 4-hexylresorcinol (Figure 3). Son et al., (2001) reported similar effects in L^* values of apple slices (var. Liberty) dipped in a 1% solution of N-acetylcysteine or glutathione.

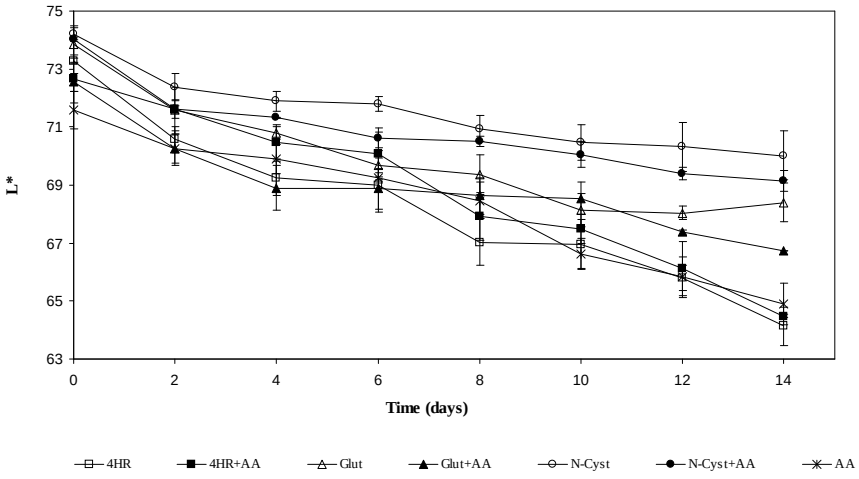

Figure 3.- Evolution of lightness (L^*) in fresh-cut apples treated with several antibrowning agents with or without ascorbic acid during storage at 4ºC. Data shown are mean ± standard deviation.

a^* initial values of fresh-cut Fuji apples were maintained along the time without any significant decrease after a dip in a solution containing 1% N-acetylcysteine, glutathione or combination of them with ascorbic acid (Figure 4). Richard et al., (1991) indicated that both cysteine and glutathione act by reducing *o*-quinones back to their *o*-diphenols precursor, preventing the formation of pigments or reacting with *o*-quinones to yield colorless compounds. Nevertheless, the main increase of a^* values was observed in apple slices dipped in 1% ascorbic acid solution, reaching values of 1.30 after 2 week of storage and suggesting a fast darkening from the early hours of storage (Figure 4). Similar results were reported by Oms-Oliu et al., (2006) who reported that ascorbic acid treatment did not prevent fresh-cut pears from darkening. Additionally, Son et al., (2001) indicated that a treatment with 1% of ascorbic acid on Liberty apple slices revealed that reducing power of ascorbic acid was depleted and consequently, brown color developed rapidly thereafter.

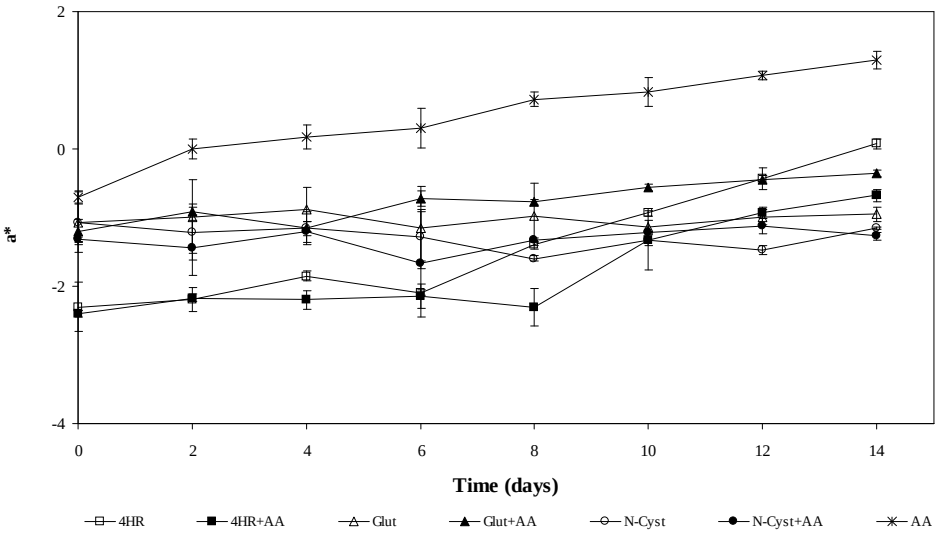

Figure 4.- Evolution of a* values in fresh-cut apples treated with several antibrowning agents with or without ascorbic acid during storage at 4ºC. Data shown are mean ± standard deviation.

It can be observed that the use of 4-hexylresorcinol (0.5% w/v) combined or not with ascorbic acid (1% w/v), caused a significant effect on a* values of fresh-cut apple slices, maintaining the samples free of darkening during the first week of storage (Figure 4). However, after the first week of storage, a suddenly increase of a* values in samples dipped with 4-hexylresorcinol solutions was observed, which could have been triggered by the high PPO activity reached after this period (figure 1). Several authors have studied the effectiveness of 4-hexylresorcinol in the control of enzymatic browning, some of them reporting that its effectiveness is better when used in combination with other compounds (Monsalve-Gonzalez et al., 1993; Luo & Barbosa-Canovas 1997; Dong et al., 2000). Monsalve-Gonzalez, Barbosa-Canovas, McEvily and Iyengar (1995) reported that a combination of ascorbic acid (0.2%) and 4-hexylresorcinol (200 ppm) preserved efficiently color of Red Delicious apple up to 32 days of storage at 25ºC.

CONCLUSIONS

This study demonstrated that 1% of N-acetylcysteine and glutathione were effective inhibitors of PPO. The individual use of ascorbic acid and 4-hexylresorcinol were not effective antioxidants for fresh-cut Fuji apples. The presence of ascorbic acid in a solution of N-acetylcysteine, glutathione or 4-hexylresorcinol did not improve the effectiveness of these compounds against PPO activity although a strong inhibition of apple POD activity was observed. The results obtained with L^* and a^* values corroborated the positive effect of N-acetylcysteine as an alternative method in the control of the enzymatic browning as compared with the more traditional antibrowning agent, ascorbic acid.

ACKNOWLEDGMENTS

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REFERENCES

Billaud, C., Brun-Mérimée, S., Louarme, L., & Nicolas, J. (2004). Effect of glutathione and Maillard reaction products prepared from glucose or fructose with glutathione on polyphenoloxidase from apple-I: Enzymatic browning and enzyme activity inhibition. *Food Chemistry, 84*, 223-233.

Brooks, R.M. & Olmo, H.P. (1997). The Brooks and Olmo Register of fruit and Nut varieties. Alexandria, VA: American Society Horticulture Science Press.

Buta, J.G. & Abbott, J.A. (2000). Browning inhibition of fresh-cut 'Anjou', 'Bartlett', and 'Bosc' pears. *Hortscience 35*(6), 1111-1113.

Cano, M.P., DeAncos, B., & Lobo, G. (1995). Peroxidase and polyphenoloxidase activities in papaya during postharvest ripening and after freezing-thawing. *Journal of Food Science, 60*(4), 815- 820.

Clemente, E. (1998). Purification and thermostability of purified isoperoxidases from oranges. *Phytochemistry, 49*, 29-36.

Dong, X., Wrolstad, R.E., & Sugar, D. (2000). Extending shelf life of fresh-cut pears. *Journal of Food Science, 65*(1), 181-186.

Eissa, H.A., Fadel, H., Ibrahim, G.E., Hassan, I.M., & Elrashid, A.A. (2006). Thiol containing compounds as controlling agents of enzymatic browning in some apple products. *Food Research International, 39*(8), 855-863.

Fils, B., Sauvage, F.X., & Nicolas, J. (1985). Tomato peroxidases purification and some properties. *Sciences des aliments, 5* (2), 217-232.

Friedman, M. & Bautista F.F. (1995). Inhibition of polyphenol oxidase by thiols in the absence and presence of potato tissue suspensions. *Journal Agricultural of Food Chemistry, 43*(1), 69-76.

Gorny, J.R., Gill, M.I., & Kader, A.A. (1998). Postharvest physiology and quality maintenance of fresh-cut pears. *Acta Horti, 464*:231-236.

Gorny, J.R., Hess-Pierce, B., Cifuentes, R.A., & Kader, A.A. (2002). Quality changes in fresh-cut pear slices as affected by controlled atmospheres and chemical preservatives. *Postharvest Biology and Technology, 24* (3), 271-278.

Hsu, A.F., Shieh, J.J., Bill, D.D. & White, K. (1988). Inhibition of mushroom polyphenoloxidase by ascorbic acid derivatives. *Journal of Food Science, 53*, 765-771.

İyidoğan, N.F. & Bayındırlı, A. (2004). Effect of L-cysteine, kojic acid and 4-hexylresorcinol combination on inhibition of enzymatic browning in Amasya apple juice. *Journal of Food Engineering, 62*(3):299-304.

Kahn, V. (1977). Latency properties of polyphenol oxidase in two avocado cultivars differing in their rate of browning. *Journal of the Science of Food and Agriculture, 28*, 233-229.

Kahn, V. & Andrawis, A. (1985). Inhibition of mushroom tyrosinase by tropolone. *Phytochemistry, 24*, 905-908.

Kim, D.M., Smith, N.L., & Lee, C.Y. (1993). Quality of minimally processed apple slices from selected cultivars. *Journal of Food Science, 58*(5):1115-1117.

Lamikanra, O. & Watson, M.A. (2001). Effects of ascorbic acid on peroxidase and polyphenoloxidase activities in fresh-cut cantaloupe melon. *Journal of Food Science, 66*, 1283-1286.

Lozano-de-González, P., Barret, D.M., Wolstad, R.E. & Durst, R.W. (1993). Enzymatic browning inhibited in fresh and dried apple rings by pineapple juice. *Journal of Food Science, 58*, 399-345.

Luo, Y. & Barbosa-Canovas, G.V. (1997). Enzymatic browning and its inhibition in new apple cultivars slices using 4-hexylresorcinol in combination with ascorbic acid. *Food Science Technology International, 3*(3), 195-201.

Mastrocola, D. & Lerici, C.R. (1991). Colorimetric measurements of enzymatic and no-enzymatic browning in apple purees. *International Journal of Food Science, 3*, 219-229.

McEvily, A.J, Iyengar, R., & Otwell, W.S. (1992). Inhibition of enzymatic browning in foods and beverages. *Critical Reviews in Food Science and Nutrition, 32*, 253-273.

Moline, H.E., Buta, J.G. & Newman, I.M. (1999). Prevention of browning of banana slices using natural products and their derivatives. *Journal of Food Quality 22*, 499-511.

Molnar-Perl, I., Friedman, M. (1990). Inhibition of browning by sulfur amino acids. 3. Apples and potatoes. *Journal Agricultural and Food Chemistry*, *38*(8), 1652-6.

Monsalve-Gonzalez, A., Barbosa-Canovas, G.V., Cavalieri, R.P., McEvily, A.J., & Iyengar, R. (1993). Control of browning during storage of apple slices preserved by combined methods. 4-hexylresorcinol as anti-browning agent. *Journal of Food Science, 58*(4),797-800.

Monsalve-Gonzalez, A., Barbosa-Canovas, G.V., McEvily, A.J., & Iyengar, R. (1995). Inhibition of enzymatic browning in apple products by 4-hexylresorcinol. *Food Technology 49*(4), 110-118.

Oms-Oliu, G., Aguiló-Aguayo, I., Martín-Belloso, O. (2006). Inhibition of browning on fresh-cut pear wedges by natural compounds. *Journal of Food Science 71*(3), S216-224.

Özoğlu, H. & Bayındırlı, A. (2002). Inhibition of enzymatic browning in cloudy apple juice with selected antibrowning agents. *Food Control 13*, 213-221.

Padiglia, A., Cruciani, E., Pazzaglia, G., Medda, R. & Floris, G. (1995). Purification and characterization of opuntia peroxidase. *Phytochemistry 38*, 137-162.

Pittia, P., Nicoli, M.C., Comi, G., & Massini, R. (1999). Shelf-life extension of fresh-like ready-to-use pear cubes. *Journal of the Science of Food and Agriculture, 79*(7), 955-60.

Pizzocaro, F., Torregiani, D. & Gilardi, G. (1993). Inhibition of apple polyphenoloxidase (PPO) by ascorbic acid, citric acid and sodium chloride. *Journal of Food Process and Preservation, 17*, 21-30.

Richard, F.C., Goupy, P.M., Nicolas, J.J., Lacombe, J.M., Pavia, A.A. (1991). Cysteine as an inhibitor of enzymatic browning. 1.-Isolation and characterization of addition compounds formed during oxidation of phenolics by apple polyphenol oxidase. *Journal Agriculture and Food Chemistry*, *39*(5), 841-7.

Richard, F.C., Goupy, P.M., & Nicolas, J.J. (1992). Cysteine as an inhibitor of enzymatic browning. 2. Kinetic studies. *Journal Agriculture and Food Chemistry, 40*, 2108-2114.

Rojas-Graü, M.A., Sobrino-López, A., Tapia, M.S., & Martín-Belloso, O. (2006). Browning inhibition in fresh-cut 'Fuji' apple slices by natural antibrowning agents. *Journal of Food Science, 71*(1) S59-S65.

Rolle, R. & Chism, G. (1987). Physiological consequences of minimally processed fruits and vegetables. *Journal of Food Quality, 10*, 157-177.

Sapers, G.M. (1993). Browning of foods: control by sulfates, antioxidants, and other means. *Food Technology, 47*, 75-84.

Senesi, E., Galvis, A., Fumagalli, G. (1999). Quality indexes and internal atmosphere of packaged fresh-cut pears (Abate fetel and Keiser varieties). *Italian Journal of Food Science, 2*, 111-120.

Soliva-Fortuny, R.C., Grigelmo-Miguel, N., Odriozola-Serrano, I., Gorinstein, S., & Martin-Belloso, O. (2001). Browning evaluation of ready-to- eat apples as affected by modified atmosphere packaging. *Journal Agriculture and Food Chemistry, 49*(8), 3685-90.

Soliva-Fortuny, R.C., Oms-Oliu, G., & Martín-Belloso, O. (2002). Effects of ripeness stages on the storage atmosphere, color, and textural properties

of minimally processed apple slices. *Journal of Food Science, 67,* 1958-1963.

Son, S., Moon, K., Lee, C. (2001). Inhibitory effects of various antibrowning agents on apple slices. *Food Chemistry 73*(1), 23-30.

Valderrama, P. & Clemente, E. (2004). Isolation and thermostability of peroxidase isoenzymes from apple cultivars Gala and Fuji. *Food Chemistry, 87*(4), 601-606.

Varoquaux, P. (1991). Ready-to-use fresh fruits and vegetables. *Rev. Gen Froid 81*, 33-43.

Yoshida, Y., Fan, X., & Patterson, M. (1995). "Fuji" apple. *Fruit Varieties Journal, 49*:194-7.

Zawistowski, J., Biliaderis, C.G., Eskin, N.A.M. (1991). Polyphenol oxidases. In Oxidative enzymes in foods; Robinson, D.S., Eskin, N.A.M., Eds., Elsevier: London, UK.

QUALITY CHANGES IN FRESH-CUT FUJI APPLE AS AFFECTED BY RIPENESS STAGE, ANTIBROWNING AGENTS AND STORAGE ATMOSPHERE

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ABSTRACT

The effect of ripening state, modified atmosphere and the use of antibrowning agents was investigated in an attempt to determine optimum ripeness and processing conditions for extending the shelf-life of fresh-cut Fuji apple. Apples were classified in three groups: mature-green, partially-ripe and ripe; after peeling and slicing fruits were treated with 1% (w/v) *N*-acetylcysteine or 1% (w/v) ascorbic acid (control), and then packed into polypropylene trays with air or a gas mixture (2.5% O_2 + 7% CO_2 + 90.5% N_2) and sealed. Trays containing the apple slices were stored in darkness at 4 ± 1 ºC and analysed periodically during 43 days. Changes in atmosphere composition, color and firmness were examined. Partially-ripe apples, based on their lower ethanol production and maintenance of their original color and firmness, were the most suitable to prepare the fresh-cut commodities. A post-cutting dip on 1% (w/v) *N*-acetylcysteine was the most effective treatment to prevent cut surface browning and preserve the initial appearance of Fuji apple slices during more than one month at 4 ºC. Low O_2 and elevated CO_2 (2.5% O_2 + 7% CO_2) atmosphere extended the shelf-life of apple slices because of a significant inhibition of ethylene production.

Key words: fresh-cut Fuji apples, shelf-life, ripening, antibrowning agents, modified atmosphere packaging.

INTRODUCTION

The quality of fresh fruits and their derivatives is governed by a great number of preharvest and postharvest factors (Kader and Barrett 1996). Aspects that affect the shelf life of fresh-cut fruits include cultivar, stage of ripeness at cutting, processing conditions and storage atmosphere (Gorny and others 1998; Gorny and others 2000). Ripeness stage is probably the most significant parameter in fresh-cut fruit processing because influences both the eating quality and the shelf-life. Fruit ripening is a sequence of biochemical events, which transform physiologically mature but inedible fruit into edible and tasty products. Major changes during ripening of fleshy fruit include loss of flesh firmness, degradation of chlorophyll and formation of pigments, flavours and aromas (Golding and others 2005).

Modified atmosphere packaging has been reported by several authors to be effective in delaying the physico-chemical changes related to fruit quality loss. Shelf-life of fresh-cut fruits may be extended by atmospheres low in O_2 and elevated in CO_2, which reduce ethylene production and respiration rates (Day and others 1994; Watada and Qi 1999; Verlinden and Nicolaï 2000; Soliva-Fortuny and others 2001). Gorny and Kader (1997) stated that low O_2 and/or elevated CO_2 environments generated by modified atmosphere packaging of fresh-cut products can extend their shelf-life by (1) slowing browning reactions at cut surfaces; (2) reducing the rates of water loss and respiration; and (3) decreasing ethylene biosynthesis and action. The effectiveness of the modified atmosphere may be enhanced by an appropriate use of antibrowning agents. Ascorbic acid, citric acid, *N*-acetylcysteine, glutathione and 4-hexylresorcinol have been reported to prevent the browning of fresh-cut apples (Baldwin and others 1996; Son and others 2001; Rocha and Morais 2003; Rojas-Graü and others 2006; Perez-Gago and others 2006). Ascorbic acid is almost indispensable in the modern minimal processing fruit industry for controlling browning and oxidative reactions, being the most frequent alternative to sulphites. Although the effectiveness of ascorbic acid in the control of enzymatic browning has been established by several authors, it has been demonstrated that its effect is temporary (Özoğlu and Bayındırlı 2002; Rojas-Graü and others 2006). Recently, cysteine and their derivates have been investigated as an alternative method to control enzymatic browning (Nicoli and others 1994; Son and others 2001; Gorny and others 2002; Rojas-Graü and others 2006) which act by reducing *o*-quinones back to their *o*-diphenols precursor, preventing the formation of pigments or reacting with *o*-quinones to yield colorless compounds (Richard and others 1991).

New apple varieties, as “Fuji”, are becoming very popular throughout Europe at the expense of traditional major commercial varieties like “Red Delicious”, “Golden Delicious” and “Granny Smith” (Varela and others 2005). According to a recent survey, most consumers indicated their preference of a specific cultivar of apple over another because of anticipated flavor and texture or mouth-feel (Doyle 2004). This behavior is usually attributed to their superior quality. Fuji apple (*Malus domestica* Borkh.), a cross between red “Delicious” and “Ralls Janet”, is a cultivar which stands out for its excellent sensory characteristics such as juicy, firm, crispy, fine-grained with a sweet, spicy flavor that has

high sugar and low acid content, as well as good storage potential (Stebbins and others 1991; Yoshida and others 1995).

In minimal processing industry, assessing the appropriate ripeness stage of commodities is of great interest in order to improve the quality and extend the shelf-life of fresh-cut fruits. This fact motivated works as those of Soliva-Fortuny and others (2002, 2004) for Golden delicious apples and Conference pears, and Gorny and others (1998, 1999, 2000, 2002) for peach, nectarine and some cultivars of pears. Nevertheless, not many studies on the influence of ripening at processing and its effect on respiratory, quality changes and shelf-life of fresh-cut Fuji apple products have been reported. The objective of our research was to determine the optimum stage of ripeness for extending the shelf-life of fresh-cut Fuji apple, evaluate the effect of air or a modified atmosphere packaging, as well as comparing the use of ascorbic acid and *N*-acetylcysteine as antibrowning agents following quality changes through refrigerated storage.

MATERIALS AND METHODS

Fruit ripeness evaluation

Fuji apples were provided by ACTEL, Lleida, Spain. Fruits were held at 4 ± 1 ºC, and following the procedure described by Soliva-Fortuny and others (2002) were ripened by exposure to temperatures of 20 ± 1 ºC for a period of 1 to 2 d until the desired ripeness stage was reached. Ripeness stage was established by assessing the flesh firmness of 10 fruit randomly taken from each lot. Whole apple firmness (penetration force) was determined with a TA-XT2 Texture Analyser (Stable Micro Systems Ltd., Surrey, UK) by measuring the force required for a 4 mm diameter probe to penetrate the flesh of a whole apple without skin. Fruits were then grouped within the following three categories according to its firmness: mature-green (79 ± 3.2 N), partially-ripe (67 ± 3.2 N) and ripe (56 ± 2.2 N).

Fruit processing and storage

Fruits were washed and sanitized by immersion into a 200 ppm sodium hypochlorite solution for 3 min, rinsed and dried prior to cutting operations. Apples were then peeled by a mechanical-peeler (Matfer, Les Lilas, France). Peeled apples were cored and cut into nine slices of 2 mm thickness using a hand operated apple corer and slicer. A maximum of 6 fruits were processed at the same time to minimize excessive exposure to ambient conditions. The apple slices were then immersed for 1 min in chilled (5 ºC) antibrowning solutions consisting of 1% (w/v) *N*-acetylcysteine or 1% (w/v) ascorbic acid, which was considered as the control due to its extended use in apple processing. Samples were drained to drop off excess solution and packed (150 g) into polypropylene trays (Mcp Performance Plastic LTC, 2005). Trays were wrapped with a plastic film of a 110 cm^3 O_2 m^{-2} bar^{-1} day^{-1} permeability to oxygen (Tecnopack S.R.L.) using a vacuum packaging machine with injection of gases (Ilpra Foodpack Basic V/G, Ilpra, Vigenovo, Italy). Trays were filled with air or with a gas mixture of 2.5% O_2 + 7% CO_2 + 90.5% N_2, heat sealed and stored in darkness at 4 ± 1 ºC. Analyses were carried out periodically during 43 days on randomly sampled pairs of trays. Replicate determinations were performed for each tray.

Headspace gases analysis

The atmosphere of each single tray was analyzed using a gas chromatograph equipped with a thermal conductivity detector (Micro-GC CP 2002 gas analyzer, Chrompack International, Middelburg, The Netherlands). The gaseous content of each tray was gently mixed prior to sampling and an adhesive septum was stuck to the film wrap. A 1.7 ml sample was automatically withdrawn from the headspace atmosphere. Portions of 0.25 and 0.33 ml were injected for O_2 and CO_2 determination, respectively. The O_2 content was analyzed with a CP-Molsieve 5Å packed column (Chrompack International, Middelburg, The Netherlands) (4 m x 0.32 mm, df = 10 mm) at 60 ºC and 100 kPa. For quantification of CO_2, ethylene (C_2H_4), and ethanol (C_2H_5OH), a Pora-PLOT Q column (Chrompack International, Middelburg, The Netherlands) (10 m x 0.32 mm, df = 10 mm), held at 70 ºC and 200 kPa, was used.

Color measurement

Cut apple surface color values were directly measured with a color meter (Minolta Chroma Meter Model CR-400, Minolta, Tokyo, Japan). The equipment was set up for illuminant

D65 and 10º observer angle and calibrated using a standard white reflector plate. Color was measured using the CIE L^*, a^*, b^* scale. Six replicates were evaluated for each tray. Hue angle (h^*) was calculated as the arctangent (b^*/a^*).

Firmness measurements

Apple firmness was determined by measuring the force required for a 4 mm diameter probe to penetrate apple slices of 2 mm height to a depth of 1 mm at a rate of 1 mm s^{-1}. Apple slices were placed perpendicular to the probe, so that penetrate in their geometric centre using a TA-XT2 Texture Analyzer (Stable Micro Systems Ltd., England, UK).

Statistical analysis

Data were analyzed by analysis of covariance using statistical procedures of the Statgraphics Plus V.5.1. (Statistical Graphics Co., Rockville, MD, USA). Specific differences were determined by least significant difference (LSD). All comparisons were made at a 5% level of significance.

RESULTS AND DISCUSSION

Changes on the package atmosphere composition

Table 1 shows that oxygen concentration in the headspace atmosphere of the trays was not significantly influenced either by the ripeness state or by the dipping treatment (ascorbic acid or *N*-acetylcysteine), while the packaging atmosphere had a significant effect ($p \leq 0.05$) on the respiratory use of oxygen.

Table 1. Analysis of covariance of the studied parameters.

	F-ratio						
	Oxygen	**Carbon dioxide**	**Ethylene**	**Ethanol**	***L****	***h****	**Firmness**
COVARIATE							
Time	283.14*	1605.54*	11.68*	95.07*	251.42*	32.16*	138.74*
MAIN EFFECTS							
A: Ripeness state	1.13NS	50.70*	104.49*	28.49*	231.38*	140.62*	1056.49*

B: Atmosphere	251.26*	11.69*	182.28*	1.03 NS	185.50*	69.98*	0.58 NS
C: Dipping treatment	8.18NS	6.55NS	163.96*	6.64NS	424.44*	2897.54*	0.34 NS
INTERACTIONS							
AB	3.36 NS	5.35NS	27.04*	2.34 NS	3.24NS	3.07 NS	1.00 NS
AC	2.84 NS	5.60 NS	7.38*	5.17*	13.53*	32.48*	3.46 NS
BC	4.06 NS	29.55 *	18.10*	0.47 NS	22.69*	16.43*	0.01 NS
ABC	2.70 NS	13.15 *	3.05 NS	1.89 NS	2.46NS	1.68 NS	0.75 NS

* $p \leq 0.05$
NS no significant

Under modified atmosphere (2.5 kPa O_2 + 7 kPa CO_2), an abrupt decrease in the initial oxygen level was observed, indicating a rapid use of the low oxygen concentration available, until values below 1 kPa at the first week of storage (Fig. 1). This effect was more pronounced from the first hours after processing in MAP packed apple slices treated with ascorbic acid than those slices treated with *N*-acetylcysteine (Fig. 1c and d). For apple slices packaged under air, the initial oxygen concentration (21 kPa O_2) declined in a gradual manner from the beginning of storage, reaching values as low as 1 and 2 kPa at the end of the storage period (Fig. 1a and b). Although the effect of dipping treatment was not significant on oxygen rate, we observed that apple slices packaged under air atmosphere and treated with ascorbic acid consumed the oxygen of the headspace much more rapidly that those samples treated with N-acetylcysteine, especially in ripe and partially-ripe apples (Fig. 1a and b). These findings are in accordance with Gil and others (1998) who compared the effect of ascorbic acid treatment on Fuji apple slices stored in air or oxygen-free atmosphere. They found that ascorbic acid dips increased the respiration rate of apple slices stored under air atmosphere, while the respiration was reduced when an atmosphere of 0% O_2 was used.

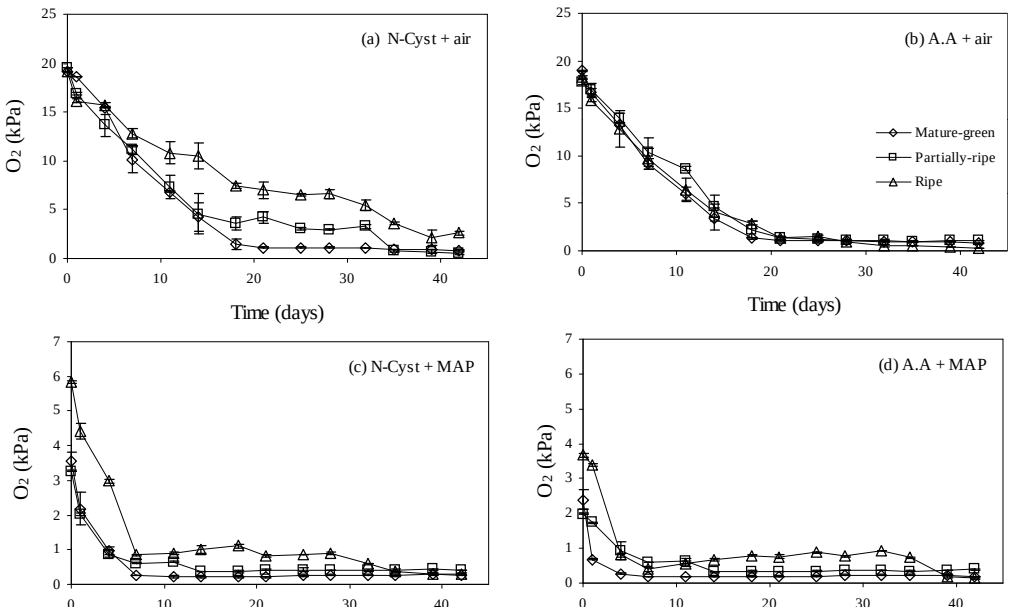

Figure 1. Oxygen concentrations in the package headspace of fresh-cut Fuji apples processed at different maturity. (a) N-Cyst + air: slices dipped in a 1% *N*-acetylcysteine solution and packaged under non-modified atmosphere; (b) A.A + air: control slices dipped in a 1% ascorbic acid solution and packaged under non-modified atmosphere; (c) N-Cyst + MAP: slices dipped in a 1% *N*-acetylcysteine solution and packaged under 2.5 kPa O_2 + 7 kPa CO_2; (d) A.A + MAP: control slices dipped in a 1% ascorbic acid solution and packaged under 2.5 kPa O_2 + 7 kPa CO_2. Data shown are the means (± standard deviation).

In this study, both, the ripeness stage and the gas composition of the packaging atmosphere had a significant effect ($p \leq 0.05$) on the CO_2 production of the apple slices, while the use of *N*-acetylcysteine or ascorbic acid as antibrowning agents did not have any significant influence (Table 1).

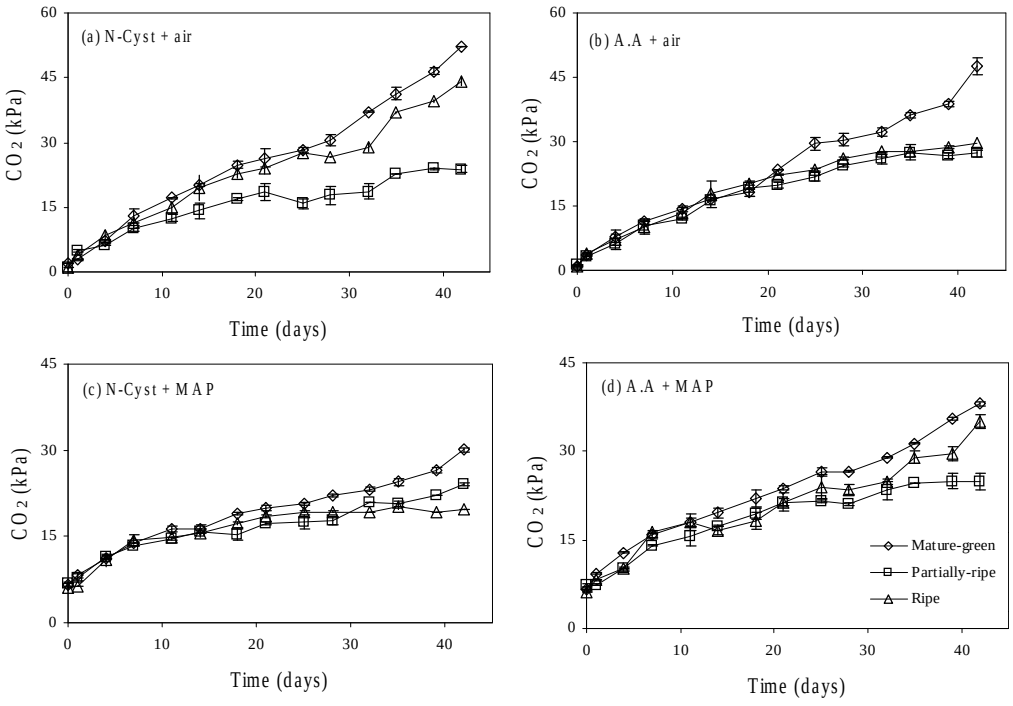

Figure 2. Carbon dioxide concentrations in the package headspace of fresh-cut Fuji apples processed at different maturity. (a) N-Cyst + air: slices dipped in a 1% *N*-acetylcysteine solution and packaged under non-modified atmosphere; (b) A.A + air: control slices dipped in a 1% ascorbic acid solution and packaged under non-modified atmosphere; (c) N-Cyst + MAP: slices dipped in a 1% *N*-acetylcysteine solution and packaged under 2.5 kPa O_2 + 7 kPa CO_2; (d) A.A + MAP: control slices dipped in a 1% ascorbic acid solution and packaged under 2.5 kPa O_2 + 7 kPa CO_2. Data shown are the means (± standard deviation).

Carbon dioxide increased continuously during storage, reaching a concentration over 20 kPa at the third week of storage, with production rates similar for all the treatments (Fig. 2). From the third week, the evolution of CO_2 was significantly influenced by the stage of maturity and storage atmosphere, reaching values over 45 kPa in mature-green samples storage under non-modified atmosphere (Fig. 2a and b). CO_2 production of fresh-cut Fuji apples was significantly lower in samples storage under a 2.5 kPa O_2 + 7 kPa CO_2 atmosphere than under air packaging atmosphere despite of that initial levels inside the trays were about 7 kPa in MAP conditions (Fig. 2). Lakakul and others (1999) pointed out the importance of maintaining O_2 levels just above the fermentation threshold and maintaining CO_2 below the range that causes injury in the package headspace. Our results show that partially-ripe fresh-cut apples evolved less CO_2 in comparison with the others ripeness stages suggesting its suitability for use in minimal processing.

Ethylene concentration in the package headspace atmosphere was significantly influenced ($p \leq 0.05$) by the ripeness state, storage atmosphere and dipping treatments (Table 1). As shown in Figure 3, mature-green apple slices produced less ethylene than those partially-ripe or ripe slices. This behavior was expected since ethylene is a hormone that is produced during maturation of climacteric plants, affecting growth, development and storage life

(Saltveit 1999). Biale and Young (1981) indicated that the rate of ethylene production is much greater in ripe than in non-ripe fruits.

On the other hand, ethylene production was noticeably higher in apples packaged under initial air atmospheres, achieving a maximum concentration of 60 ppm after 1 week of storage (Fig. 3a and b). By the contrary, apples slices packaged under 2.5 kPa O_2 + 7 kPa CO_2 produced less ethylene and reach a maximum peak of 35 ppm (Fig. 3c and d). Gil and others (1998) detected a complete inhibition of ethylene in fresh-cut Fuji apples stored under oxygen-free conditions, since O_2 is required for ethylene production. The inhibition of ethylene generation under anaerobic or low O_2 conditions has been observed by many authors, suggesting that oxygen participates in the conversion of 1-amino-cyclopropane-1-carboxylic acid (ACC) to ethylene (Yang 1981).

Furthermore, addition of antibrowning agents to preserve color had a significant effect ($p \leq 0.05$) on ethylene production of fresh-cut apples. In fact, the use of the N-acetylcysteine reduced significantly the ethylene evolution on fresh-cut apples (Fig. 3a and c) compared with the use of ascorbic acid (Fig. 3b and d). In contrast to our findings, Gil and others (1998) reported that ascorbic acid dips reduced ethylene production of Fuji apple slices packed in air atmosphere. Ethylene production is stimulated when plant tissues are injured and it can accumulate in packages of fresh-cut product, which can lead to undesirable effects (Watada and Qi, 1999). In our case, the dipping treatments prior to packaging proved to significantly reduce ethylene production, thanks to a protection effect from mechanical stresses exerted on the cut apple pieces that results in turn in less stress-induced ethylene synthesis.

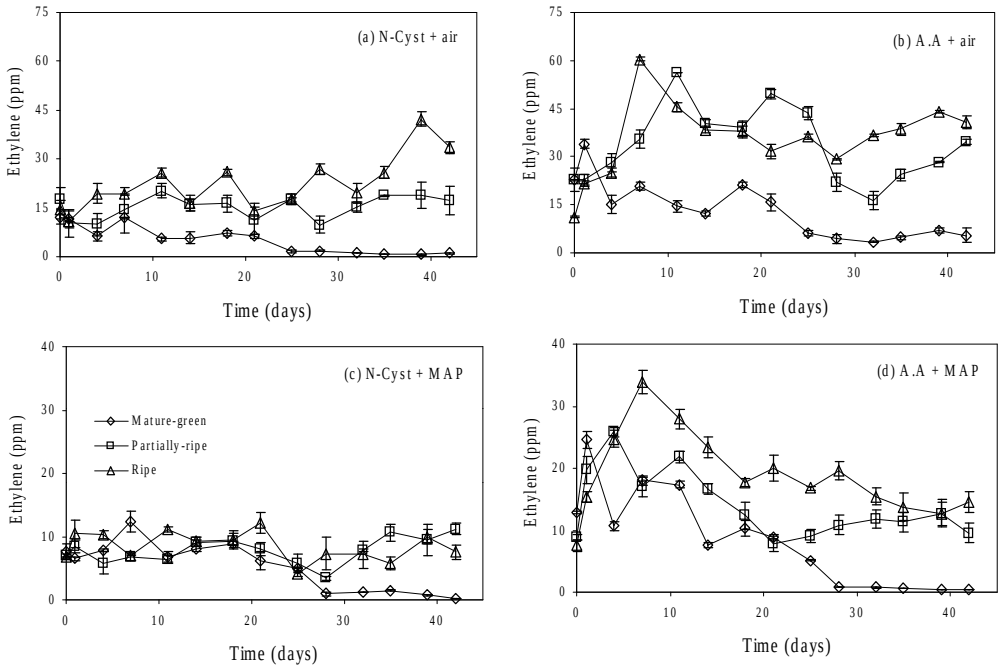

Figure 3. Ethylene concentrations in the package headspace of fresh-cut Fuji apples processed at different maturity. (a) N-Cyst + air: slices dipped in a 1% *N*-acetylcysteine solution and packaged under non-modified atmosphere; (b) A.A + air: control slices dipped in a 1% ascorbic acid solution and packaged under non-modified atmosphere; (c) N-Cyst + MAP: slices dipped in a 1% *N*-acetylcysteine solution and packaged under 2.5 kPa O_2 + 7 kPa CO_2; (d) A.A + MAP: control slices dipped in a 1% ascorbic acid solution and packaged under 2.5 kPa O_2 + 7 kPa CO_2. Data shown are the means (± standard deviation).

Table 1 also shows the effect of the ripeness on the evolution of ethanol in the package headspace, indicating a significant correlation between them ($p \leq 0.05$). A gentle but progressive accumulation of ethanol during the first 14 days of storage is observed in Figure 4. An increase was triggered in samples of ripe apple slices after the third week, regardless of the packaging atmosphere or dipping treatment, showing a sudden increase and hence suggesting anoxic pathways which may lead to production of off-flavors. Ke and others (1991) stated that the main plant fermentative metabolism products in fruits are ethanol and acetaldehyde and their accumulation correlated well with off-flavor development. Soliva-Fortuny and others (2002) reported that fermentative pathways in ripe pears were more active than in mature-green or partially-ripe pears, suggesting that post-climatic fruits are more susceptible to undergo anaerobic metabolism. Pesis (2005), indicated that the increase of anaerobic respiration in over-mature fruits is probably due to that in this tissue the mitochondrial activity is reduced (perhaps because of membrane damage) and the cells are unable to produce enough energy.

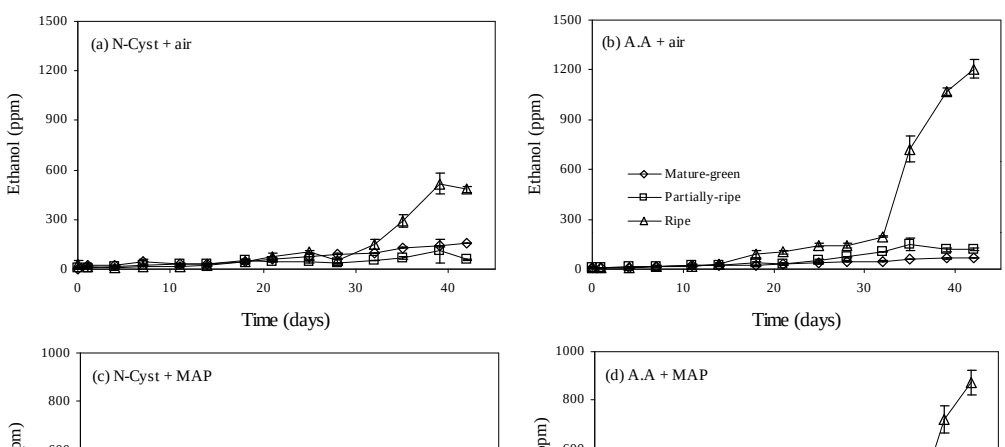

Figure 4. Ethanol concentrations in the package headspace of fresh-cut Fuji apples processed at different maturity. (a) N-Cyst + air: slices dipped in a 1% *N*-acetylcysteine solution and packaged under non-modified atmosphere; (b) A.A + air: control slices dipped in a 1% ascorbic acid solution and packaged under non-modified atmosphere; (c) N-Cyst + MAP: slices dipped in a 1% *N*-acetylcysteine solution and packaged under 2.5 kPa O_2 + 7 kPa CO_2; (d) A.A + MAP: control slices dipped in a 1% ascorbic acid solution and packaged under 2.5 kPa O_2 + 7 kPa CO_2. Data shown are the means (± standard deviation).

Neither modified atmosphere nor the dipping treatment had a significant influence on ethanol evolution. However, the interaction between ripeness stage and dipping treatment had a significant effect on ethanol production (Table 1). As Figure 4 shows, a sudden increase in ethanol concentration was observed in ripe apple slices treated with ascorbic acid after the 30 days of storage. This raise could have been triggered by the low O_2 concentrations inside of packages observed in those samples after third week of storage. Soliva-Fortuny and others (2002) indicated that low O_2 may lead to production of fermentative metabolites like ethanol, which are responsible for unpleasant off-flavors and odors.

Changes on the color of apple slices

Color changes on the pulp of fresh-cut Fuji apples were determined by changes in lightness (L^*) – L^* values are the most reliable measure of browning - and hue angle (h^*), which was calculated from values of a^* and b^* parameters. Analysis of covariance indicated that ripeness stage at processing, storage atmosphere and the type of dipping solution had a significant effect ($p \leq 0.05$) in both L^* and h^* values.

In general, lightness of partially-ripe and mature-green apple slices was more efficiently preserved that the lightness of ripe slices, which experienced greater changes throughout time (Fig. 5). In fact, ripe apple slices underwent the most important decrease in L^* values through the 43 days of storage regardless of the packaging conditions or dipping treatments (Fig. 5). Soliva-Fortuny and others (2002) indicated that in more mature apples the chloroplast begins to disintegrate, causing a solubilization of polyphenol oxidases that would be the cause of increasing browning oversensitivity.

L^* initial values of fresh-cut Fuji apples were maintained along the time without any significant decrease after a dip in a 1% N-acetylcysteine and under a 2.5 kPa O_2 + 7 kPa CO_2 atmosphere storage (Fig. 5c). By contrast, browning intensity was much higher in samples packaged under non-modified atmosphere (Fig. 5a and b). This behaviour had already been observed by Soliva-Fortuny and others (2002) in Golden Delicious apple slices packaged under initial air atmosphere, who reported a depletion of L^* values during the first weeks storage.

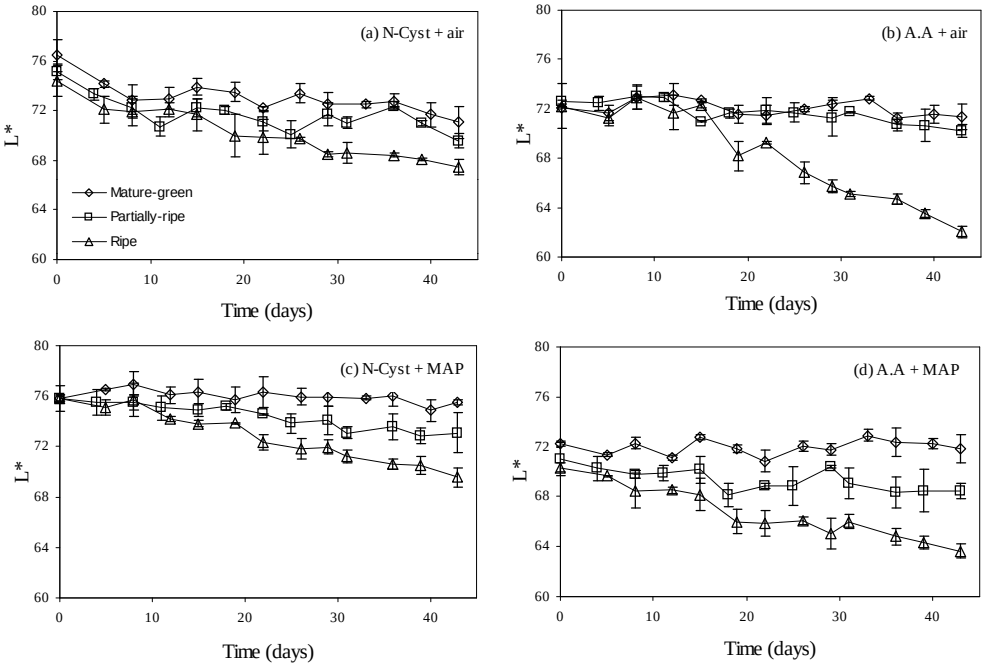

Figure 5. Lightness (L^*) of fresh-cut Fuji apples processed at different maturity. (a) N-Cyst + air: slices dipped in a 1% *N*-acetylcysteine solution and packaged under non-modified atmosphere; (b) A.A + air: control slices dipped in a 1% ascorbic acid solution and packaged under non-modified atmosphere; (c) N-Cyst + MAP: slices dipped in a 1% *N*-acetylcysteine solution and packaged under 2.5 kPa O_2 + 7 kPa CO_2; (d) A.A + MAP: control slices dipped in a 1% ascorbic acid solution and packaged under 2.5 kPa O_2 + 7 kPa CO_2. Data shown are the means (± standard deviation).

It can be observed that the type of additive used as antibrowning agent had a significant effect on absolute L^* values of fresh-cut Fuji apples. It is important to highlight that apple slices dipped in ascorbic acid showed lower initial L^* values (around 72) than samples treated with N-acetylcysteine (around 76), suggesting a fast darkening from the early hours of storage (Fig. 5). The effectiveness of applying N-acetylcysteine as antibrowning agent to preserve fresh-cut apples from quality losses is evident. The antibrowning effect of *N*-acetylcysteine was observed by Rojas-Graü and others (2006) in fresh-cut Fuji apple and by Son and others (2001) in Liberty apple slices. Even if the efficiency of ascorbic acid in the control of enzymatic browning has been demonstrated by several authors and is of extended use in the fresh-cut fruit industry, its effectiveness in this study proved to be lower than that of *N*-acetylcysteine. Sapers (1993) stated that the effectiveness of ascorbic acid is due to its ability to reduce quinones back to phenolic compounds. Nevertheless, once the ascorbic acid has been completely oxidized to dehydroascorbic acid, quinones can again accumulate and undergo browning.

In general, the greater the maturity degree, the greater the changes experienced by the hue angle, regardless of the packaging conditions (Fig. 6)**.** Nevertheless, the main depletion of h^* values was observed in ripe apples packaged under initial air atmosphere and dipped in 1% ascorbic acid solution, reaching values of 83.80 ± 0.07 after 43 days of storage (Fig. 6b). Apple slices tissue developed browning more rapidly as consequence of the operations during processing, especially in samples of advanced ripeness, where the levels of soluble catechol oxidases are greater. By contrast, mature-green and partially-ripe apple slices underwent the lowest decrease in h^* values during storage.

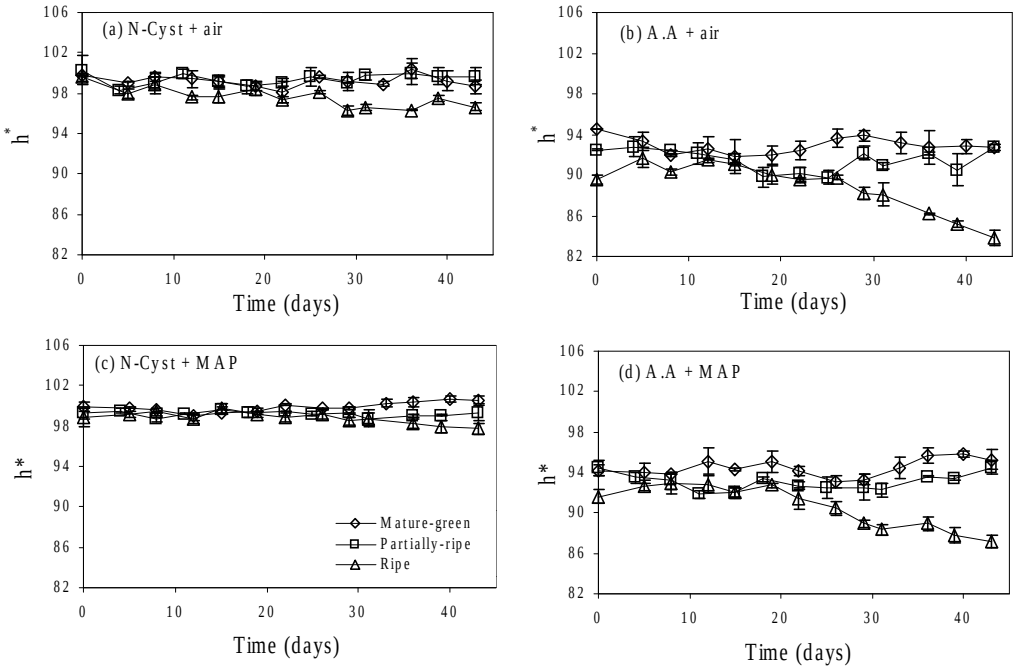

Figure 6. Hue angle (h^*) of fresh-cut Fuji apples processed at different maturity. (a) N-Cyst + air: slices dipped in a 1% *N*-acetylcysteine solution and packaged under non-modified atmosphere; (b) A.A + air: control slices dipped in a 1% ascorbic acid solution and packaged under non-modified atmosphere; (c) N-Cyst + MAP: slices dipped in a 1% *N*-acetylcysteine solution and packaged under 2.5 kPa O_2 + 7 kPa CO_2; (d) A.A + MAP: control slices dipped in a 1% ascorbic acid solution and packaged under 2.5 kPa O_2 + 7 kPa CO_2. Data shown are the means (± standard deviation).

Samples treated with 1% *N*-acetylcysteine solution maintained higher absolute h^* values (around 100) during all the storage time, regardless of the ripeness states or package atmospheres (Fig. 6a and c). On the contrary, apple slices treated with ascorbic acid showed a lower initial h^* values (below 94), underwent a strong decrease in h^* values from the third week of storage. The effectiveness of *N*-acetylcysteine on h^* values is in agreement with the results obtained by Rojas-Graü and others (2006). The positive effect of *N*-acetylcysteine as an alternative method in the control of the enzymatic browning as compared with the more traditional antibrowning agent, ascorbic acid, is corroborated with the results obtained on L^* and h^* values.

Changes on the texture of apple slices

Only ripeness stage had a significant effect ($p \leq 0.05$) on the firmness of the fresh-cut Fuji apples (Table 1). Table 2 compares the firmness values of the three studied ripeness stages and it is noted that mature-green and partially-ripe apples maintained their initial firmness better than ripe apples. Additionally, rates of firmness depletion were much higher on ripe fresh-cut apples, achieving values as low as 1.9 N at the end of storage time. Soliva-Fortuny and others (2002) found similar results in Golden Delicious apple slices, indicating that texture degradation was directly correlated to ripening processes. Fruit softening is a consequence of changes in physical and mechanical properties of the tissue based on changes in the chemical structure of the cell wall polysaccharides. In fact, pectins play a key role in fruit softening, since they are partially solubilized by endogenous pectin-degrading enzymes (polygalacturonases and pectin methyl esterases) during ripening, leading to tissue softening (Fischer and others 1991). In addition, fruit tissue softening during ripening and senescence is triggered by ethylene and has been demonstrated to be a consequence of alterations in cell wall metabolism (Gorny and others 2002).

Although the firmness was observed not to be significantly affected by the composition of the packaging atmosphere or the dipping treatment, apple texture was greatly affected by the type of antibrowning agent used. In fact, ripe apple slices treated with ascorbic acid presented lower values of firmness than those slices treated with *N*-acetylcysteine (Table 2). This effect was reported by Ponting and others (1972) who demonstrated that acid solutions as ascorbic acid significantly reduced apple slices firmness.

Table 2. Firmness values throughout storage of fresh-cut apples processed at different states of maturity.

Days	Firmness (N)											
	Mature-green				Partially-ripe				Ripe			
	Air		(2.5 kPa O_2 + 7 kPa CO_2)		Air		(2.5 kPa O_2 + 7 kPa CO_2)		Air		(2.5 kPa O_2 + 7 kPa CO_2)	
	***N*-Cyst**	**AA**	***N*-Cyst**	**AA**	***N*-Cyst**	**AA**	***N*-Cyst**	**AA**	***N*-Cyst**	**AA**	***N*-Cyst**	**AA**
0	9.5 ± 0.1	9.3 ± 0.1	9.1 ± 0.2	9.2 ± 0.4	6.7 ± 0.3	6.8 ± 0.3	7.1 ± 0.3	7.4 ± 0.3	6.2 ± 0.7	6.2 ± 0.3	6.1 ± 0.2	5.8 ± 0.6
7	9.5 ± 0.4	9.5 ± 0.3	8.9 ± 0.1	9.8 ± 0.2	6.8 ± 0.3	7.3 ± 0.8	7.7 ± 0.5	7.3 ± 0.3	5.5 ± 0.6	5.0 ± 0.5	4.8 ± 0.1	4.8 ± 0.3
14	9.4 ± 0.1	8.7 ± 0.2	8.8 ± 0.4	8.9 ± 0.6	7.2 ± 0.1	7.0 ± 0.7	7.6 ± 0.4	7.6 ± 0.7	5.5 ± 0.3	5.6 ± 0.3	3.8 ± 0.4	4.6 ± 0.3
21	9.1 ± 0.1	9.0 ± 0.1	8.7 ± 0.1	9.5 ± 0.1	7.3 ± 0.3	7.8 ± 0.1	6.7 ± 0.6	6.1 ± 0.1	3.6 ± 0.3	4.7 ± 0.4	3.9 ± 0.3	5.0 ± 0.2
28	9.0 ± 0.3	9.1 ± 0.3	9.1 ± 0.2	9.2 ± 0.1	6.8 ± 0.5	6.3 ± 0.2	7.6 ± 0.5	5.9 ± 0.2	4.2 ± 0.5	3.0 ± 0.2	4.0 ± 0.1	2.9 ± 0.1
35	8.2 ± 0.5	8.5 ± 0.2	9.1 ± 0.2	8.6 ± 0.4	7.3 ± 0.4	5.9 ± 0.2	7.6 ± 0.4	6.0 ± 0.2	3.6 ± 0.2	2.3 ± 0.2	3.9 ± 0.2	2.5 ± 0.1
43	8.9 ± 0.2	8.7 ± 0.2	8.1 ± 0.4	8.8 ± 0.2	6.2 ± 0.6	6.2 ± 0.6	6.4 ± 0.6	6.2 ± 0.6	3.8 ± 0.3	1.9 ± 0.6	3.8 ± 0.1	1.9 ± 0.1

Values are the means ± standard deviation
***N*-Cyst = *N*-acetylcysteine, AA = ascorbic acid**

CONCLUSIONS

Results showed that ripeness stage at processing has an important influence on the post-cutting life of Fuji apple slices and must be carefully controlled. The use of a low O_2 and elevated CO_2 atmosphere combined with a dip in *N*-acetylcysteine allowed the maintenance of fresh quality (color and firmness) of cut apples during more than 1 month of refrigerated storage, specially in mature-green and partially-ripe apples. On the contrary, apple in advanced ripeness state showed signs of evident physiological damage, exhibiting a substantial augment of ethanol production as a result of fermentative anaerobic processes, limiting their shelf-life to less of 3 week. Furthermore, as expected, ripe apple slices showed a considerable cut surface browning and softening of its tissues from early days of storage. The use of partially ripe fruit for the fresh cut apple industry was confirmed for Fuji apples, considered a "new" variety on which information on this particular aspect was scarce, being proper initial maturity as well as processor assessment of fruit maturity essential if commercial production is attempted.

REFERENCES

Baldwin EA, Nisperos MO, Chen X, Hagenmaier RD. 1996. Improving storage life of cut apple and potato with edible coating. Postharvest Biol Technol 9:151-163.

Biale JB, Young RE. 1981. Respiration and ripening in fruits. Retrospect and prospect. In: Friend J, Rhodes MJC, editors. Recent advances in the biochemistry of fruit and vegetables. London: Academic Press. pp 1-39.

Day BP. 1994. Modified atmosphere packaging and active packaging of fruits and vegetables. In: Ahvenainen R, Mattila-Sandholm T, Ohlsson T, editors. Minimal processing foods (VTT Symposium series 142), Majvik 14-15 April 1994.

Doyle M. 2004. Personal communication: The consumer Network, Philadelphia, Pa.

Fischer RL, Bennett AB. 1991. Role of Cell Wall Hydrolases in Fruit Ripening. Ann Rev Plant Physiol and Plant Molec Biol 42:675-703.

Gil MI, Gorny JR, Kader AA. 1998. Responses of "Fuji" apple slices to ascorbic acid treatments and low-oxygen atmospheres. Hortscience 33: 305-309.

Golding JB, Ekman JH, McGlasson WB. 2005. Regulation of fruit ripening. Stewart Posthar Rev 3(5).

Gorny JR, and Kader AA. 1997. Low oxygen and elevated carbon dioxide atmospheres inhibit ethylene biosynthesis in preclimacteric and climacteric apple fruit . J Amer Soc Hort Sci 122(4):542-546.

Gorny JR, Hess-Pierce B, Kader AA. 1998. Effects of fruit ripeness and storage temperature on the deterioration rate of fresh-cut peach and nectarine slices. HortScience 33:110-113.

Gorny JR, Hess-Pierce B, Kader AA. 1999. Quality changes in fresh-cut peach and nectarine slices as affected by cultivar, storage atmosphere and chemical treatments. J Food Sci 64: 429-432.

Gorny JR, Cifuentes RA, Hess-Pierce B, Kader AA. 2000. Quality changes in fresh-cut pear slices as affected by cultivar, ripeness stage, fruit size, and storage regime. Int J Food Sci Technol 65:541-544.

Gorny JR, Hess-Pierce B, Cifuentes RA, Kader AA. 2002. Quality changes in fresh-cut pear slices as affected by controlled atmospheres and chemical preservatives. Postharvest Biol Technol 24(3):271-8.

Kader AA, Barrett DM. 1996. Classification, composition of fruits and postharvest maintenance of quality. In: Processing Fruits: Science and Technology - Biology, Principles and Applications. Somogyi LP, Ramaswamy LP, Hui YH, editors. Lancaster, PA: Technomic Publishing Co. Inc. pp. 1-24.

Ke DY, Goldstein L, Omahony M, Kader AA. 1991. Effects of short-term exposure to low O_2 and high CO_2 atmospheres on quality attributes of strawberries. J Food Sci 56 (1): 50-54.

Lakakul R, Beaudry R, Hernandez RJ. 1999. Modeling respiration of apple slices in modified-atmosphere packages. J Food Sci 64(1):105-110.

Luo Y, Barbosa-Canovas GV. 1997. Enzymatic browning and its inhibition in new apple cultivars slices using 4-hexylresorcinol in combination with ascorbic acid. Food Sci Tech Int 3(3):195-201.

McHugh TH, Senesi E. 2000. Apple wraps: a novel method to improve the quality and extend the shelf life of fresh-cut apples. J Food Sci 65(3):480-5.

Nicoli MC, Anise M, Severinc C. 1994. Combined effects in preventing enzymatic browning reactions in minimally processed fruit. J Food Qual 17(3):221-9.

Özoğlu H, Bayındırlı A. 2002. Inhibition of enzymatic browning in cloudy apple juice with selected antibrowning agents. Food Control 13: 213-21.

Perez-Gago MB, Serra M, del Rio MA. 2006. Color change of fresh-cut apples coated with whey protein concentrate-based edible coatings. Postharvest Biol Technol 39: 84-92.

Pesis E. 2005. The role of the anaerobic metabolites, acetaldehyde and ethanol, in fruit ripening, enhancement of fruit quality and fruit deterioration. Postharvest Biol Technol 37:1-19.

Ponting JD, Jackson R, Watters G. 1972. Refrigerated apple slices: preservative effect of ascorbic acid, calcium and sulfites. J Food Sci 37: 434-436.

Richard FC, Goupy PM, Nicolas JJ, Lacombe JM, Pavia, AA. 1991. Cysteine as an inhibitor of enzymatic browning. 1.-Isolation and characterization of addition compounds formed during oxidation of phenolics by apple polyphenol oxidase. J Agric Food Chem 39(5):841-7.

Rocha AMCN, Morais AMMB. 2003. Shelf life of minimally processed apple (cv. Jonagored) determined by color changes. Food Control 14(1):13-20.

Rojas-Graü MA, Sobrino-López A, Tapia MS, Martín-Belloso O. 2006. Browning inhibition in fresh-cut 'Fuji' apple slices by natural antibrowning agents. J Food Sci 71(1): S59-S65.

Saltveit ME. 1999. Effect of ethylene on quality of fresh fruits and vegetables. Postharvest Biol Technol 15:279-92

Sapers GM. 1993. Browning of foods: control by sulfates, antioxidants, and other means. Food Technol 47: 75-84.

Soliva-Fortuny RC, Grigelmo-Miguel N, Odriozola-Serrano I, Gorinstein S, Martin-Belloso O. 2001. Browning evaluation of ready- to- eat apples as affected by modified atmosphere packaging. J Agric Food Chem 49(8):3685-90.

Soliva-Fortuny RC, Oms-Oliu G, Martín-Belloso O. 2002. Effects of ripeness stages on the storage atmosphere, color, and textural properties of minimally processed apple slices. J Food Sci 67: 1958-1963.

Soliva-Fortuny RC, Alòs-Saiz N, Espachs-Barroso A, Martín-Belloso O. 2004. Influence of maturity at processing on quality attributes of fresh-cut Conference pears. J Food Sci 69 (7): S290-S294.

Son S, Moon K, Lee C. 2001. Inhibitory effects of various antibrowning agents on apple slices. Food Chem 73(1):23-30.

Stebbins RL, Duncan A, Compton C, Duncan D. 1991. Taste ratings of new apple cultivars. Fruit Var J 45:37-44.

Varela P, Salvador A, Fiszman S. 2005. Shelf-estimation of "Fuji" apples: Sensory characteristics and consumer acceptability. Postharvest Biol Technol 38:18-24.

Verlinden BE, Nicolaï BM. 2000. Fresh-cut fruits and vegetables. Acta Hort 518:223-230.

Watada AE, Qi L. 1999. Quality of fresh-cut produce. Postharvest Biol. Technol. 15, 201-205.

Yang SF. 1981. Biosyntesis of ethylene and its regulation. In: Friend J, Rhodes MJC, editors. Recent advances in the biochemistry of fruit and vegetables. London: Academic Press. pp 89-106.

Yoshida Y, Fan X, Patterson M. 1995. "Fuji" apple. Fruit Var J 49:194-7.

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ALGINATE AND GELLAN BASED EDIBLE COATINGS AS CARRIERS OF ANTIBROWNING AGENTS APPLIED ON FRESH-CUT FUJI APPLES

M.A. Rojas-Graü, M.S. Tapia, F.J. Rodríguez, A.J. Carmona, O. Martín-Belloso

Food Hydrocolloids (2007); 21:118-127

ABSTRACT

Alginate (2% w/v) and gellan (0.5% w/v) - based edible coatings were formulated to study the effect of glycerol (G) and antibrowning agents (N-acetylcysteine and glutathione) on water vapor resistance (WVR). The ability of the coatings to carry antibrowning agents was investigated following color changes of coated fresh-cut Fuji apples. Selected formulations obtained by a response surface analysis were 1.5% G, 1% N-acetylcysteine and 0.63% G, 1% N-acetylcysteine for alginate and gellan respectively. The addition of sunflower oil with essential fatty acids (ω3 and ω6) at 0.025, 0.05, and 0.125% w/v concentrations was also investigated in an attempt to improve the barrier properties of the alginate and gellan coatings for fresh-cut apples. The WVR increased significantly from 15.70 and 14.60 s cm^{-1} to 19.2 and 27.6 s cm^{-1} for alginate and gellan coatings with sunflower oil respectively, in comparison with oil free coatings. The addition of sunflower oil in gellan was more effective than in alginate to increase the WVR of coated apples. Alginate and gellan based coatings proved to be good carriers for antibrowning agents.

Keywords: Alginate, gellan, edible coatings, antibrowning, fresh-cut apples, WVR.

INTRODUCTION

Edible coatings are gaining importance as an alternative to reduce the deleterious effects imposed by minimal processing on fresh-cut fruits. The semipermeable barrier provided by edible coatings is aimed to extend shelf life by reducing moisture and solute migration, gas exchange, respiration and oxidative reaction rates, as well as suppress physiological disorders on fresh-cut fruits (Wong, Camirand & Pavlath, 1994a; Baldwin, Nisperos, Chen & Hagenmaier, 1996; Park, 1999). Edible coatings may also serve as carriers of food additives such as antibrowning and antimicrobials agents, colorants, flavors, nutrients and spices (Pena & Torres, 1991; Wong,

Gregorski, Hudson & Pavlath, 1996; Li & Barth, 1998; Pranoto, Salokhe & Rakshit, 2005).

Polysaccharide-based coatings have been used to extend the shelf-life of fruits and vegetables by reducing respiration and gas exchange due to selective permeabilities to O_2 and CO_2 (Nísperos-Carriedo, 1994; Nussinovitch, 1997, 2000). Among them, alginate and gellan are biopolymers that could be considered for edible films and coatings because of their unique colloidal properties and their ability to form strong gels or insoluble polymers upon reaction with multivalent metal cations like calcium (King, 1983; Rhim, 2004). Alginate, a polysaccharide derived from marine brown algae (*Phaeophyceae*) and gellan, a microbial polysaccharide secreted by the bacterium *Sphingomonas elodea* (formerly referred to as *Pseudomonas elodea*) are finding increasing use in the food industry as texturizing and gelling agents (Mancini & McHugh, 2000; Yang & Paulson, 2000).

Hydrophilic films and coatings, such as those of protein and polysaccharide nature, generally provide a good barrier to oxygen and carbon dioxide transmission, but are a poor barrier to water vapor (Kester & Fennema, 1986; García, Martinó & Zaritzky, 1998). However, since a certain degree of oxygen and carbon dioxide permeability is needed for respiration of living tissues as those of fresh and fresh-cut fruits and vegetables, moderate barriers that allow a controlled respiratory exchange -avoiding anaerobic respiration- are considered more appropriate (Kester et al., 1986; Ayranci & Tunc, 2003, 2004). Additionally, the poor water vapor barrier may provide some benefit since it allows movement of water vapor across the film, thus preventing water condensation, which is a potential source of microbial spoilage (Park, Chinnan & Shewfelt, 1994). Plasticizers like glycerol are required for polysaccharide and protein-based edible films to increase film flexibility and processability by increasing the free volume or molecular mobility of polymers reducing internal hydrogen bonding between polymer

chains while increasing intermolecular spacing. Plasticizers affect the ability of the system to attract water and also generally increase film permeability to oxygen (McHugh & Krochta, 1994a, 1994b; Sothornvit & Krochta, 2000).

The incorporation of lipids, either in an emulsion or as a layer coating into the films formulations, greatly improve their water vapor barrier properties (García, Martinó & Zaritzky, 2000; Yang et al., 2000). Wong, Tillin, Hudson and Pavlath (1994b) used a polysaccharide–lipid bilayer formulation to reduce respiration of fresh-cut apples and García et al., (2000) used sunflower oil in edible starch-based films and coatings intended for strawberries. Antibrowning agents are used to protect against oxidative rancidity, degradation and enzymatic browning in fruit and vegetables. Ascorbic acid (Son, Moon & Lee, 2001; Soliva-Fortuny, Grigelmo-Miguel, Odriozola-Serrano, Gorinstein & Martin-Belloso, 2001), 4-hexylresorcinol (Monsalve-Gonzalez, Barbosa-Canovas, Cavalieri, McEvily & Iyengar, 1993; Luo & Barbosa-Canovas, 1997; Dong, Wrolstad & Sugar, 2000) and some sulfur-containing amino acids as cysteine and glutathione (Molnar-Perl & Friedman, 1990; Nicoli, Anise and Severinc, 1994; Son et al., 2001; Gorny, Hess-Pierce, Cifuentes & Kader, 2002) have been widely studied, individually or in combination, to prevent enzymatic browning in the search for sulfite substitutes and for improving shelf-life of minimally processed fruits (Pizzocaro, Toregiani & Giraldi, 1993; Rojas-Graü, Sobrino-López, Tapia & Martín-Belloso, 2005). However, little research has been done using both antibrowning agents and edible coating on fresh-cut fruits. Wong et al., (1994a), Baldwin et al., (1996), Lee, Park, Lee and Choi (2003), Perez-Gago, Serra and Del Rio (2004) studied the incorporation of

antibrowning agents into edible films in minimally processed fruits. Most of these antibrowning agents however, are hydrophilic compounds and may increase water vapor permeability (WVP) and water loss when incorporated into films and coatings (Ayrancy et al., 2004).

The objective of this work was to formulate alginate and gellan based edible coatings for fresh-cut apples studying the effect of the addition of different amounts of glycerol and antibrowning agents on WVR of the coatings, and color changes of apples. The incorporation of sunflower oil with essential fatty acids (ω3 and ω6) -functional fatty acids that claim possible additional health benefits- was investigated for improving water barrier properties.

MATERIALS AND METHODS

Materials

'Fuji' apple (*Malus domestica* Borkh) was chosen for this study because of its extensive use and the rapid browning of its tissue after cutting. The apples were provided by ACTEL, Lleida, Spain, at commercial maturity and stored at 4 ± 1 ºC prior to processing. Food grade sodium alginate (Keltone® LV, ISP, San Diego, CA., USA) and gellan gum (Kelcogel®, CPKelco, Chicago, IL., USA) were used as the carbohydrate biopolymers for coating formulations. Glycerol (Merck, Whitehouse Station, N.J., USA) was added as plasticizer. Calcium chloride (Sigma-Aldrich Chemic, Steinhein, Germany) was used to induce crosslinking reaction. N-acetylcysteine and glutathione (Sigma-Aldrich Chemic, Steinhein, Germany) were the added antibrowning agents. Sunflower oil (La Española, Spain) with the following composition: 11g monosaturated, 30g monounsaturated and polyunsaturated 57,4g; 3,5g Omega-3 and 55-60g Omega-6, was used as the lipid source when emulsion films were prepared.

Coating formulation

Coating formulations were studied in two steps. A response surface analysis was first run in order to decide the final amounts of glycerol and antibrowning agents to incorporate into the formulations (Table 1 and 2). WVR values as well as color of coated fresh-cut apples were used as the response variable. The addition of sunflower oil was investigated in a second step by emulsification of the alginate and gellan film-forming solutions for coating the apple pieces with different concentrations of sunflower oil. Both statistical designs are explained in other sections.

Preparation of the film forming solutions and dipping solutions with antibrowning agents

Film forming solutions were prepared by dissolving alginate (2% w/v) and gellan (0.5% w/v) powders in distilled water under controlled heating and stirring until the mixtures became clear. Glycerol was added as plasticizer in various concentrations (Table 1 and 2). When addition of sunflower oil was investigated, film-forming solutions were emulsified with three different concentrations of the functional oil: 0.025, 0.05, and 0.125% which was dispersed using an Ultra Turrax T25 (IKA® WERKE, Germany) with a S25N-G25G device, for 5 min at 24,500 rpm, and degassed under vacuum. Film forming solutions and emulsions were used for fruit coatings. The studied antibrowning agents were added to the calcium chloride bath (2% w/v) required for crosslinking carbohydrate polymers. Concentrations of N-acetylcysteine and glutathione were varied, according to the experimental design (Table 1 and 2).

Fruit coating

Clean apples were cut in cylinders of 1.42 cm diameter x 2.06 cm of height. The apple pieces were first dipped into the alginate or gellan film forming solutions (with and without sunflower oil) for 2 minutes. Residual solutions of each polysaccharide were allowed to drip off for 1 min, before submerging the fruits for 2 min in the corresponding calcium chloride solution containing the antibrowning agents.

Water vapor resistance determination

The method and experimental set up for determination of the WVR of coatings described by García et al., (1998) was used to determine the water loss of coated fruit pieces: Cut apple cylinders were dipped in the coating formulation and calcium chloride solution with antibrowning agents. Samples were equilibrated for 24 h in desiccators maintained at 98.9% RH with a 0.6 molal solution of NaCl at room temperature (Fruit cylinders were placed in small test cups and weighed in an analytical laboratory scale prior to be placed in sealed chambers equilibrated at 33% RH with saturated $MgCl_2.6H_2O$ (Panreac Quimica SA, Barcelona, Spain) at 25 ºC. Samples were weighed at regular time intervals. Weight was taken during 4 h periods and the slope of the curve of weight loss vs. time in g/s (rate of moisture loss) was estimated by linear regression analysis. WVR was calculated using a modified Fick's first law equation (Ben-Yehoshua, Burg & Young, 1985) as done by Avena-Bustillos, Krotcha, Salveit, Rojas-Villegas and Sauceda-Perez (1994), Avena-Bustillos, Krotcha and Salveit (1997) and Wong et al., (1994b).

$ds/dt = (A\ \Delta C/R)$ Equation 1

where,

ds/d*t* is the rate of gas exchange in g/s (slope); A is exposed area of the fruit cylinders (9.25 cm^2); *R* is the resistance of the coating to water diffusion (s/cm); Δ*C* is the concentration of gas (ml/cm^3) inside and outside the fruit piece at time t. For WPR, Δ*C*= (*P*i - *P*a)/*RcT*, where

(*P*i - *P*a) is the difference in water vapor pressure (mmHg) inside and outside the fruit tissue (*P*i = a_w of the fruit x P_0 -that is the water vapor pressure of liquid water at 25 ºC and *P*a = partial water vapor pressure in the environment with 33.3% RH at 25 ºC, in mmHg x P_0); *R*c is the gas constant (3.46 L.mmHg/ ºK.g), and *T* is temperature in degrees Kelvin.

Control tests with uncoated apples were performed to determine the resistance factor of the uncoated fruit to water vapor. Water activity of the samples was measured with an Acqualab CX-2 (Decagon Devices Inc., Pullman, WA.).

Microscopic examination of fruit coatings

A microscopic examination was performed to observe the uniformity of the coatings and adherence to the fruit surface. Cross sections of the coated fruits were obtained and

monitored using a stereomicroscope Leica (Model MZ8, Leica AG, Heerbrugg, Switzerland) coupled to a computer and a camera. To best visualize the coatings, samples were colored with a solution of toluidine blue. Thickness measures were randomly taken in different sections.

Colour measurement

Cut apple surface color was directly measured with a Minolta chroma meter (Model CR-400, Minolta, Tokyo, Japan). The equipment was set up for illuminant D_{65} and 10º observer angle and calibrated using a standard white reflector plate. Three readings were obtained for each replicate by changing the position of the apple slices to get uniform color measurements. Color loss was measured through changes in a^* and h^* values during 48h in atmospheric conditions. Changes in these parameters have previously been shown to be effective in monitoring enzymatic browning at fresh-cut apple (Baldwin et al., 1996; McHugh & Senesi, 2000). Numerical values of a^* and b^* parameters were employed to calculated hue angle (h^*):

$$h^* = \arctan b^*/a^* \qquad \text{Equation 2}$$

Experimental design and statistical analysis

Response surface methodology was used to study the simultaneous effect of glycerol and antibrowning agents on edible coating applied to fresh-cut apples. A Box-Behnken experimental design was chosen for this purpose (Box & Behnken, 1960; Myers & Montgomery, 2002). In this design 17 different experiments were completed for each type of edible coating. It was assumed that a mathematical function existed for each response variable Y_k in terms of 3 independent variables: glycerol, N-acetylcysteine and glutathione. Concentrations of glycerol varied from 1.0% to 2.0% w/v and from 0.25% to 1% w/v for alginate and gellan, respectively. By contrast, concentrations of anti-browning agents varied from 0.0% to 2.0% w/v to

both film forming solutions. Full designs are shown in Table 1 and 2. The selected responses were WVR (Avena-Bustillos et al., 1994; Wong et al., 1994b) and color of coated apples represented by a^* and hue (h^*) changes. The second-order response function for our experiments was predicted by the equation 3

$$Y = \beta_\theta + \sum \beta_i \chi_i + \sum \beta_{ii} \chi_i^2 + \sum \beta_{ij} \chi_i \chi_j, \quad \text{Equation 3}$$

where *Y* is the dependent variables, β_θ is the constant, β_i, β_{ii} and β_{ij} represent the coefficients of the linear, quadratic and interactive effects, respectively; χ_i, χ_i^2 and $\chi_i\chi_j$ represent the linear, quadratic and interactive effects of the independent variables respectively. Three-dimensional surface plots and contour plots were drawn to illustrate the interactive effects of the two factors on the dependent ones, while the other factors were kept constant. The analysis of variance (ANOVA) was used to determine significant effects of Glycerol (X_1), N-Cist (X_2) and Glut (X_3) on WVR, a^* and h^*. In order to find significant differences, *F*-values at probability levels of 0.001 and 0.05 were studied. Design-Expert 6.0.1 software package (State-Ease, Inc., Minneapolis, USA) was used to generate designs to fit the response surface model to the experimental data and drawn response surface plots.

The addition of sunflower oil in the following concentrations: 0.025, 0.05, and 0.125% was investigated next, by emulsifying the formulations -selected from the response surface model- of alginate and gellan coatings that presented the highest values of water resistance, in an attempt to improve its resistance. Analysis of variance (ANOVA) and Fisher LSD

means comparison were applied at a significance level of 0.05 using Statgraphics Plus Version 5.1.

Table 1. Box-Behnken experimental design used for investigating different amounts of glycerol as plasticizer incorporated into an edible alginate (2% w/v) film forming solution in combination with antibrowning agents, and responses.

Run	Variables						Responses[b]		
	Coded			Uncoded[a]					
	X_1	X_2	X_3	(Gly)	(N-Cyst)	(Glut)	WVR	a*	h*
1	-1	-1	0	1.00	0.00	1.00	13.50	-1.30	93.35
2	1	-1	0	2.00	0.00	1.00	14.80	-2.02	95.32
3	-1	1	0	1.00	2.00	1.00	14.30	-3.14	99.51
4	1	1	0	2.00	2.00	1.00	14.40	-2.91	99.48
5	-1	0	-1	1.00	1.00	0.00	13.60	-2.79	97.86
6	1	0	-1	2.00	1.00	0.00	15.30	-2.44	96.76
7	-1	0	1	1.00	1.00	2.00	14.40	-2.71	98.80
8	1	0	1	2.00	1.00	2.00	14.90	-2.60	98.39
9	0	-1	-1	1.50	0.00	0.00	15.30	0.07	91.78
10	0	1	-1	1.50	2.00	0.00	15.50	-2.72	98.05
11	0	-1	1	1.50	0.00	2.00	15.10	-2.88	98.61
12	0	1	1	1.50	2.00	2.00	15.70	-2.52	98.35
13	0	0	0	1.50	1.00	1.00	15.00	-2.16	96.62
14	0	0	0	1.50	1.00	1.00	15.20	-2.34	97.57
15	0	0	0	1.50	1.00	1.00	15.20	-2.96	98.61
16	0	0	0	1.50	1.00	1.00	15.00	-2.55	99.60
17	0	0	0	1.50	1.00	1.00	15.20	-2.54	97.67

[a] Uncoded variables: concentrations of plasticizer and antibrowning agents in % w/v. Gly = Glycerol, N-Cyst = N-acetylcysteine, Glut = Glutathione.
[b] Responses : WVR= Water vapor resistance (s/cm), a* = colorimetric coordinate, h*= hue angle. WVR of uncoated apples = 12.50 s/cm

Table 2. Box-Behnken experimental design used for investigating different amounts of glycerol as plasticizer incorporated into an edible gellan (0.5% w/v) film forming solution in combination with antibrowning agents, and responses.

Run	Variables						Responses[b]		
	Coded			Uncoded[a]					
	X_1	X_2	X_3	(Gly)	(N-Cyst)	(Glut)	WVR	a^*	h^*
1	-1	-1	0	0.25	0.00	1.00	13.80	-1.94	95.51
2	1	-1	0	1.00	0.00	1.00	13.60	-2.86	97.66
3	-1	1	0	0.25	2.00	1.00	13.30	-2.65	100.48
4	1	1	0	1.00	2.00	1.00	13.10	-3.26	100.86
5	-1	0	-1	0.25	1.00	0.00	13.10	-2.78	97.92
6	1	0	-1	1.00	1.00	0.00	13.50	-2.72	97.99
7	-1	0	1	0.25	1.00	2.00	13.20	-2.98	99.16
8	1	0	1	1.00	1.00	2.00	13.00	-3.24	100.75
9	0	-1	-1	0.63	0.00	0.00	13.90	-1.10	92.11
10	0	1	-1	0.63	2.00	0.00	14.10	-2.81	101.01
11	0	-1	1	0.63	0.00	2.00	14.60	-2.44	98.13
12	0	1	1	0.63	2.00	2.00	13.80	-2.79	99.01
13	0	0	0	0.63	1.00	1.00	14.40	-2.75	99.07
14	0	0	0	0.63	1.00	1.00	14.50	-2.96	98.92
15	0	0	0	0.63	1.00	1.00	14.20	-2.95	99.14
16	0	0	0	0.63	1.00	1.00	14.00	-2.86	99.45
17	0	0	0	0.63	1.00	1.00	14.00	-2.49	96.54

[a] Uncoded variables: concentrations of plasticizer and antibrowning agents in % w/v. Gly = Glycerol, N-Cyst = N-acetylcysteine, Glut = Glutathione.
[b] Responses : WVR= Water vapor resistance (s/cm), a^* = colorimetric coordinate, h^*= hue angle. WVR of uncoated apples = 12.50 s/cm

RESULTS AND DISCUSSION

Results of the analysis of variance (*F*-test) for each dependent variable and their corresponding coefficients of determination (R^2) obtained by fitting the experimental data to the second order response model are shown in Table 3. The developed models appeared to be adequate ($p>0.05$) for all variables, showing no significant lack of fit ($p>0.05$). The regression coefficients for the quadratic models for WVR, a^* and h^* are listed in Table 4.

Table 3. Analysis of variance (ANOVA) for regression equation.

Source	*F*-values							
	Alginate				Gellan			
	D.F.	WVP	a^*	h^*	D.F.	WVP	a^*	h^*
Model	9	27.57**	4.50*	4.25*	9	8.42	5.23	9.38
X_1 (Gly)	1	67.50**	0.0004	0.013	1	0.099	4.90	2.56
X_2 (N-Cyst)	1	7.50*	16.98*	20.27*	1	6.35*	16.32*	47.04*
X_3 (Glut)	1	0.83	5.07	7.14*	1	0.000	6.74*	9.37*
X_1^2	1	129.75**	1.14	0.017	1	58.21*	3.74	2.37
X_2^2	1	0.018	3.39	3.55	1	0.35	8.16*	2.36
X_3^2	1	14.75**	0.23	0.048	1	2.86	0.98	0.65
X_1X_2	1	15.00**	1.14	0.61	1	0.000	0.31	0.90
X_1 X_3	1	15.00**	0.070	0.072	1	1.78	0.34	0.66
X_2 X_3	1	1.67	12.66*	6.46*	1	4.96	6.05*	18.75*
Residual	7				7			
Lack of fit	3	3.33	3.79	1.66	3	0.93	3.37	0.100
Pure error	4				4			
R^2	-	0.9726	0.8527	0.8452	-	0.9155	0.8706	0.9234

Gly = Glycerol, N-Cyst = N-acetylcysteine, Glut = Glutathione
WPR= Water vapor resistance (s/cm), a^* = colorimetric coordinate, h^*= hue angle
D.F.: degrees of freedom
* significant at $p\leq0.05$
** significant at $p\leq0.001$

Water vapor resistance (WVR)

WVR is an important property of edible films for applications in high water activity food protection, where water activity is high, or when the film must be in contact with water during processing of the coated food, to avoid exudation of fresh or frozen products (Tanada-Palmu & Grosso, 2003). Tables 1 and 2 show the water vapor resistance values obtained with the alginate and the gellan coatings in fresh-cut Fuji apples. Untreated cut apple pieces had a low WVR of 12.50s/cm. In contrast, the alginate coating had a WVR between 13.50 to 15.70s/cm (Table 1) and the gellan coating from 13.10 to 14.60s/cm (Table 2). Among the linear terms for the alginate coating, glycerol had the most pronounced effect on WVR (F=67.50 ; $p \leq 0.001$) (Table 3). The positive linear coefficient value of glycerol (+12.420) indicated that WVR in the alginate coating increased when glycerol concentration increased (Table 4). On the other hand, the quadratic coefficient value of glycerol for the same coating (F=129.75 ; $p \leq 0.001$) indicated that WVR increased when this compound concentration was increased up to 1.75% v/v, but higher concentrations led to a decrease in WVR. Similar results were observed in apples pieces coated with gellan. In this case, the quadratic coefficient value of glycerol (F=58.21; $p \leq 0.05$) indicated that the maximum value of WVR was obtained when the concentration of glycerol was around 0.63% v/v. Higher concentrations led to an decrease in the resistance of the gellan coating. The effect of plasticizer on WVP as well as on gas permeability is controversial. The addition of plasticizers to films and coatings is required to reduce brittleness, which is caused by extensive intermolecular bonding. Plasticizers reduce these forces and increase the mobility of polymers chains improving flexibility. Also, small molecules such as glycerol may fill vacancies within the polymer matrix (McHugh et al., 1994a, 1994b; Banker,

1966; Gontard, Guilbert & Cuq, 1993); but the addition of a plasticizer decreases the tensile strength of the films and increases the permeability of gas, solute and water vapor due to its hydrophilic nature. Suyatma, Tighzert and Copinet (2005) plasticized chitosan films with hydrophilic compounds like glycerol, ethylene glycol, polyethyleneglycol, and propyleneglycol and the surface properties, analyzed by contact angle measurement, revealed that plasticization increases film hydrophilicity. García et al., (1998) found that increasing plasticizer concentration, either glycerol or sorbitol, decreased WVP of starch-based coatings, as also reported by McHugh et al., (1994a) who found similar trends for whey protein edible films.

As for the antibrowning agents, N-acetylcysteine had a positive significant effect on WVR in the alginate coating (F=7.50; p≤0.05), indicating that the resistance to water diffusion increased when the N-acetylcysteine concentration in the alginate solution increased. On the contrary, N-acetylcysteine had a negative significant effect on WVR of the gellan-based formulation (F=6.35; p≤0.05), indicating that the resistance to water diffusion decreased when the N-acetylcysteine concentration increased. Additionally, quadratic term of glutathione (F=14.75; p≤0.001) had a significant effect en WVP of fresh-cut apple coated with the alginate coating, indicating that high concentrations of this compound are needed to obtain a higher resistance value. Since oxygen is involved in many oxidative reactions of foods, like enzymatic browning and vitamin loss, the incorporation of antibrowning agents as additives in the film composition is expected to exert some control on the oxygen permeability of the films. Ayranci et al., (2004) used limited amounts of ascorbic acid (AA) and citric acid (CA) in methylcellulose (MC) films plasticized with polyethylene

glycol, to lowering the oxygen permeability and thus lowering the vitamin C loss, since amounts of AA or CA greater than 0.5 / 5 g MC caused an increase in WVP since both compounds are hydrophilic.

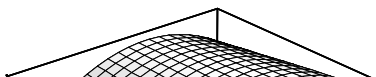
Table 4. Regression coefficients of the second order polynomial equations.

	Coefficients					
	Alginate			Gellan		
Model term	WVR	a*	h*	WVR	a*	h*
X_1 (Gly)	+12.420**	+2.395	-0.209	+7.755	+1.747	-4.608
X_2 (N-Cyst)	+0.970*	-2.933*	+7.533*	-0.080*	-1.634*	+6.368*
X_3 (Glut)	+0.270	-1.172	+2.604*	+0.870	-0.727*	+3.106*
X_1^2	-3.440**	-0.919	+0.323	-5.937*	-1.853	+4.940
X_2^2	-0.100	+0.397	-1.178	+0.065	+0.384*	-0.693
X_3^2	+0.290**	+0.104	-0.137	-0.185	+0.133	-0.364
X_1X_2	-0.600**	+0.472	-1.000	-0.118	+0.206	-1.174
X_1 X_3	-0.600**	-0.116	+0.343	-0.400	-0.214	+1.003
X_2 X_3	+0.100	+0.786*	-1.631*	-0.250	+0.340*	-2.004*

Gly = Glycerol, N-Cyst = N-acetylcysteine, Glut = Glutathione
WPR= Water vapor resistance (s/cm), a* = colorimetric coordinate, h*= hue angle
* significant at p≤0.05
** significant at p≤0.001

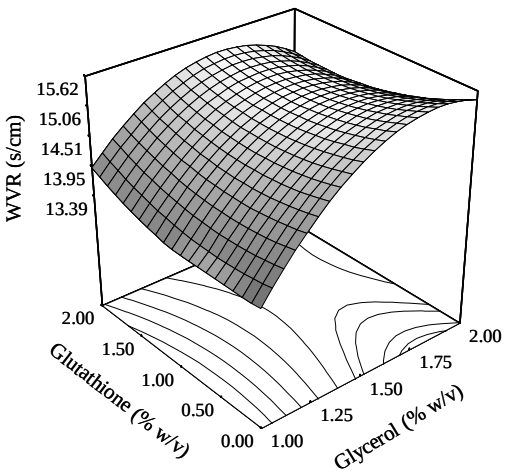
The interaction between glycerol and both N-acetylcysteine (F=15.00 ; p≤0.001) and glutathione (F=15.00; p≤0.001) had significant effect in the WVR of the apple pieces coated with alginate film (Table 3). The effects of the interaction of both antibrowning agents with glycerol are shown in Fig. 1a and 1b. The maximum values of WVR in alginate coating were obtained by combining glycerol in concentrations around 1.75% v/v with N-acetylcysteine or glutathione in concentrations lower than 1% w/v (Fig. 1a). No significant interactions between glycerol and antibrowning agents in gellan coating were observed (Table 3).

As stated by Kester et al., (1986), due to the hydrophilic nature of alginate and gellan, minimal water vapor barrier properties could be obtained in films and coatings based on these polymers. Additionally, the antibrowning



agents employed: N-acetylcysteine and glutathione and the plasticizer: glycerol, are also hydrophilic, and a decrease in WVR and an increase in water loss could be expected when they are incorporated into coatings. Maximum WVR values are obtained with given amounts of glycerol and N-acetylcysteine or glutathione -and increasing concentrations of the plasticizer and the antibrowning agents result in decrease of the water vapor resistance- however this parameter is still low when compared to uncoated apples, suggesting that water vapor barriers should be improved by addition of lipids.

(a)



(b)

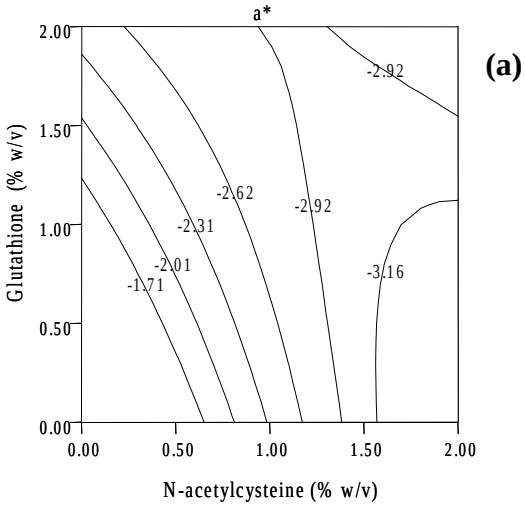
Figure 1.- Response surface for the effect of (a) glycerol-N-acetylcysteine (% w/v) and (b) glycerol-glutathione (% w/v) on water vapor resistance (WVR) of alginate based edible coating on cut apples.

Color evolution

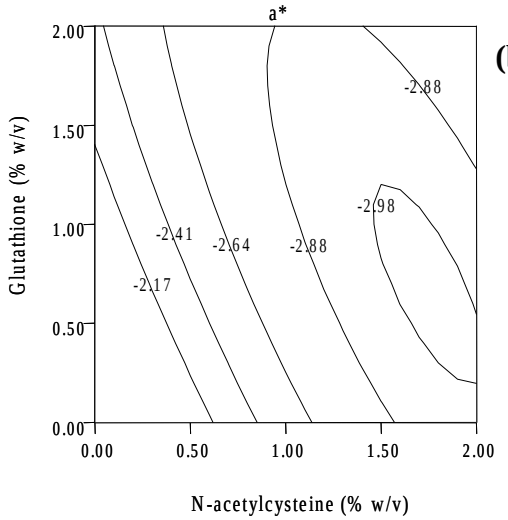
Color parameters values obtained with gellan and alginate coating on fresh-cut Fuji apples are show in tables 1 and 2. ANOVA and regression coefficients for these responses are presented in Tables 3 and 4. In our study, increasing in a^* or decreasing in h^* values were indicative of browning in fresh-cut apples. Analysis of variance indicated that antibrowning agents had only a significant effect in a^* value (Table 3). The analysis of data set indicated that N-acetylcysteine showed the most pronounced individual effect on response when using both alginate (F=16.98 ; p≤0.05) and gellan (F=16.32 ; p≤0.05) coating after 48 h of storage at atmospheric conditions. The negative values of regression coefficients of N-acetylcysteine in alginate (-2.933) and gellan (-1.634) in table 4 indicated that an increase in the concentration of this compound lead to a decrease in a^* values (absence of browning), demonstrating that N-acetylcysteine is an effective antibrowning agent to be incorporated in the formulation of edible coatings. On the other hand, linear (F= 6.74 ; p≤0.05) and quadratic (F=8.16 ; p≤0.05) terms of glutathione had a significant effect on a^* values in fresh-cut apple coated with gellan solution, indicating that addition of this agent to gellan coating resulted in significant reductions in product browning, as evidenced by lower a^* values.

The interaction between N-acetylcysteine and glutathione had a significant influence on a^* values of apples pieces coated with alginate (F=12.66 ; p≤0.05) and gellan (F=6.05 ; p≤0.05) which contained these combinations of antibrowning agents (Table 3). The effect of the interaction of both compounds on a^* values are shown in Fig. 2a and 2b, corresponding to

alginate and gellan coating respectively, after 48h storage. In both cases, concentrations around 1% w/v of each antibrowning agents were necessary to maintain a^* values near the initial one (-2.59 ± 0.27), independently of the type of coating employed. Molnar-Perl et al., (1990) showed that the combination of N-acetylcysteine and glutathione, at concentrations of 25 or 50 m*M*, prevented browning of Golden and Red Delicious apple slices.



(a)



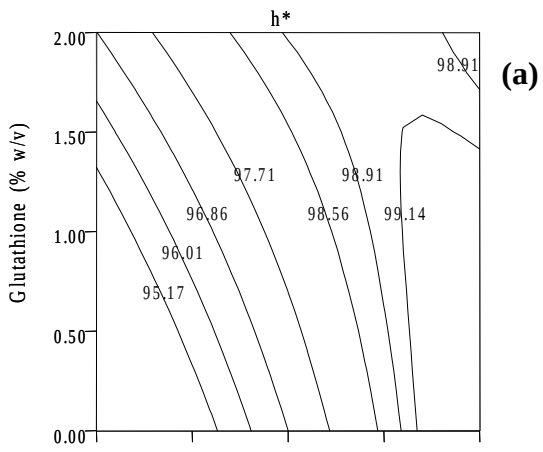
(b)

Figure 2.- Contour plots for the effect of N-acetylcysteine-glutathione (% w/v) on a^* values of alginate (a) and gellan (b) based edible coating on cut apples after 48 hours storage.

The h^* is an index frequently applied to represent changes in color of fruits over time and effective to visualize color appearance of food products (McGuire, 1992). Analysis of variance and regression coefficients for this response are presented in Table 3 and 4. The h^* values of apple pieces without treatment (control) decreased from 97.82 ± 0.21 to 89.87 ± 0.31 during 48h storage, showing an substantial enzymatic browning. The positive values of linear terms indicated that h^* values in apple pieces coated with alginate increased when N-acetylcysteine (F=20.27; p≤0.05) or glutathione (F=7.14; p≤0.05) concentrations increased. The same pattern was observed in fresh-cut apples coated with gellan, as the h^* values increased when the concentrations of N-acetylcysteine (F=47.04; p≤0.05) or glutathione (F=9.37; p≤0.05) increased (Table 3). The effectiveness of N-acetylcysteine and glutathione on h^* values is in agreement with the results obtained on a^* values. Samples coated with alginate containing N-acetylcysteine/glutathione combination (F=6.46; p≤0.05) in concentrations

around 1% w/v maintained h^* values similar to initial values (Figure 3a). The same behaviour was observed in apple pieces coated with gellan containing N-acetylcysteine/glutathione combination (F=18.75; p≤0.05) (Figure 3b). Glycerol concentration had no significant effect in the color parameters of fresh-cut apples coated with both alginate and gellan edible coating (Table 3).

Our results show that alginate and gellan based coatings are good carriers for antibrowning agents since browning is prevented in all cases. As reported by Reyes (2000), gel coating, antibrowning agents and other optional food additives, may be applied to cut and segmented fruits sequentially or separately by any suitable technique. The edible coating is generally applied before the antibrowning agents so that the gel coating can adhere to the fruit and the antibrowning agents are incorporated in the dipping solution containing calcium for crosslinking and instant gelling of the coating (Lee et al., 2003; Wong et al., 1994a; Reyes, 2000).



(a)

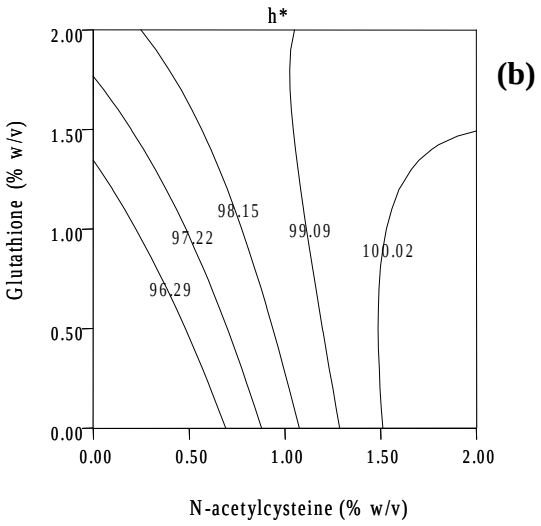


Figure 3.- Contour plots for the effect of N-acetylcysteine-glutathione (% w/v) on h^* values of alginate (a) and gellan (b) based edible coating on cut apples after 48 hours storage.

Lipid addition to improve barrier properties of coatings

Alginate and gellan coating employed in fresh-cut apples showed resistance values that are low when compared to uncoated apples. The resistance factor to water vapor of uncoated apple cylinders was determined experimentally yielding values of 12.15 ± 0.2 s/cm. This is understandable since alginate and gellan are poor water vapor barriers. As lipids are known to improve the water barrier properties of edible films, it was decided then to select from the response surface design run in the initial formulation step, those formulations with the concentrations of glycerol and antibrowning agents that exhibited the highest values of WVR as well as browning inhibition capacity: 1.5% (v/v) of glycerol and 1% w/v of N-acetylcysteine in a film forming solution of alginate 2% (w/v), and 0.63% (v/v) of glycerol, and 1% (w/v) of N-acetylcysteine in a film forming solution of gellan 0.5% (w/v), and incorporate sunflower oil in an attempt to improve their water barrier properties. In our study, N-acetylcysteine showed the most pronounced individual effect in controlling enzymatic browning of fresh-cut fuji apple coated with both alginate and gellan coatings, being the reason for choosing this compound for the final formulation.

The concentrations of sunflower oil were selected from preliminary trials performed in the lab after investigating several concentration ratios of polymeric matrix/glycerol/sunflower oil, as done by García et al., (2000). The effect of sunflower oil on WVR values of the coatings is observed in Figure 4. After addition of sunflower oil, water vapor resistance increased significantly in comparison with coatings without oil: 15.70 and 14.60s/cm to 19.2 and 27.6s/cm for alginate and gellan coatings with sunflower oil respectively. The addition of sunflower oil into gellan had a more pronounced effect in terms of increasing the WVR of the coating, than the effect obtained when the oil was added to the alginate coatings (Figure 4).

It is obvious that structural matrices of alginate and gellan do not provide effective protection against water loss (Ben-Yoshua et al., 1985; Kester et al., 1986; Wong et al., 1994a). Wong et al. (1994b) determined water vapor resistance of cut apples pieces coated with AVICEL, carrageenan, pectin and alginate. A bilayer coating of monoglyceride acetylated (AMG) was added to all coatings to improve their barrier properties. Resistance values with lipid addition varied from 38.04 s cm^{-1} for pectin and 46.06 s cm^{-1} for AVICEL. The alginate coating (0.5% w/v) showed resistance values of 44.59 s cm^{-1}. The authors do not report values for coating without lipids, but they do for uncoated apples: 3.03 s cm^{-1}.

Sunflower oil used in this study contained essential fatty acids (ω3 and ω6) that could provide an additional benefit attributed to the edible coating related to the functional properties of these fatty acids. Polyunsaturated fatty acids (PUFA) are not synthesized in the body and must be acquired through dietary sources. Linoleic acid (LA, C18:2ω6) and linolenic acid (LNA, C18:3ω3) belong to this group (Shahidi, 2004). Enzymes in our body convert both groups of PUFA through a series of desaturation and elongation steps to C20 and C22 products, some of which are quite important for health and general well-being. The C20 compounds may subsequently produce a series of hormone-like molecules known as eicosanoids, which are essential for health maintenance (Shahidi, 2004).

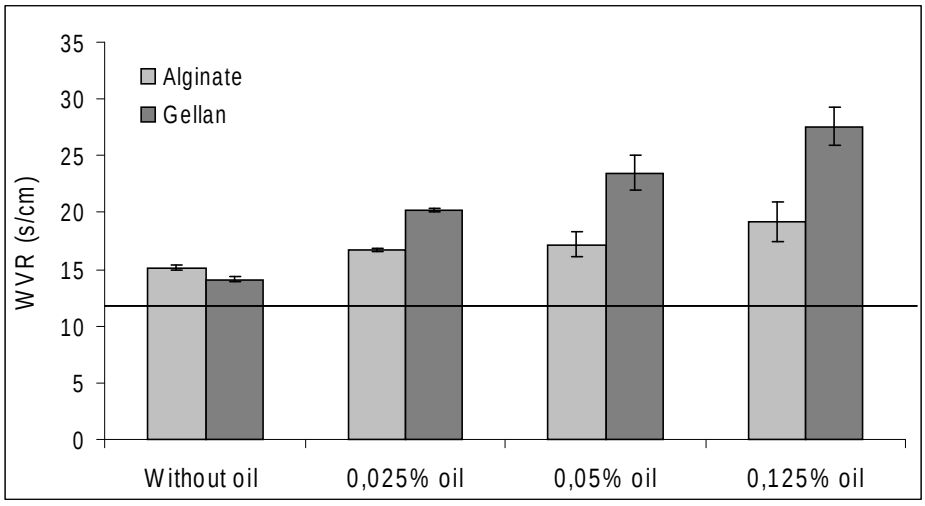
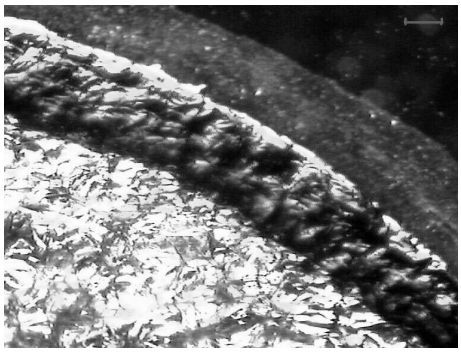


Figure 4.- Effect of addition of sunflower oil to 2% alginate-based coatings 1.5% glycerol, 1% N-acetylcysteine, and 0.5 % gellan-based coatings 0.6 % glycerol, 1% N-acetylcysteine on the resistance of fresh-cut apple to water vapor.
(—)WVR of uncoated apples 12.15 ± 1.5 s/cm

Microscopic examination of fruit coatings

Micrographs of cross-section obtained with the stereomicroscope are shown in Figures 5a and 5b. Alginate or gellan coatings containing the selected formulations were both homogeneous, covering the whole surface of apple pieces and showing good adherence. Coating thickness was also determined from the micrographs, obtaining values of 132.45 ± 20.48μm for alginate and 155.75 ± 13.30μm for gellan. Garcia et al., (1998) report coating thickness of plasticized starch-based coatings in the range of 40 to 50μm. Rodríguez (2004) obtained a thickness of 116.3μm for gellan coatings (2% w/v) on cut melon pieces, which agreed with our results.



(a)

(b)

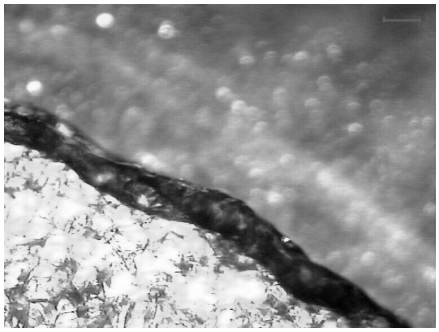


Figure 5.- Micrograph of an apple (cross-section) coated with gellan (0.5% w/v), glycerol (0.6% v/v), N-acetylcysteine (1% w/v) formulation (a), and apple (cross-section) coated with alginate (2% w/v), glycerol (1.5% v/v), N-acetylcysteine (1% w/v) formulation (b). The scale bar represents 100 μm.

CONCLUSIONS

Glycerol as plasticizer of alginate and gellan-based coatings affect WVR as do hydrophilic antibrowning agents like N-acetylcysteine and glutathione that can be carried in the coatings exerting its antibrowning effect on fresh-cut apples. Addition of lipids, such as sunflower oil is needed to improve water vapor barrier properties. Gellan/sunflower oil composite coatings were more effective in increasing WVR than alginate/sunflower oil coatings.

ACKNOWLEDGEMENTS

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REFERENCES

Avena-Bustillos, R., Krotcha, J., Salveit, M.E., Rojas-Villegas, R., & Sauceda-Pérez, J.A. (1994). Optimization of edible coating formulations on zuchini to reduce water loss. *Journal of Food Engineering*, 21, 197-214.

Avena-Bustillos, R., Krotcha, J., & Saltveit, M.E. (1997). Water vapor resistance of red delicious apples and celery sticks coated with edible caseinate-acetylated monoglyceride films. *Journal of Food Science*, 62, 351-354.

Ayranci, E., & Tunc, S. (2003). A method for the measurement of the oxygen permeability and the development of edible films to reduce the rate of oxidative reactions in fresh foods. *Food Chemistry*, 80(3), 423-431.

Ayranci, E., & Tunc, S. (2004). The effect of edible coatings on water and vitamin C loss of appricots (Armeniaca vulgaris Lam.) and green peppers (Capsicum annuum L.). *Food Chemistry*, 87(3), 339-342.

Baldwin, E. A., Nísperos-Carriedo, M.O., & Baker, R.A. (1995). Use of edible coatings to preserve quality of lightly (and slightly) processed products. *Critical Review in Food Science and Nutrition*, 35, 509-524.

Baldwin, E.A., Nisperos, M.O., Chen, X., & Hagenmaier, R.D. (1996). Improving storage life of cut apple and potato with edible coating. *Postharvest Biology and Technology*, 9,151-163.

Banker, G.S. (1966). Film coating theory and practice. *Journal Pharmacy Science*, 55, 81-89.

Ben-Yehoshua, S., Burg, S.P., & Young, R. (1985). Resistance of citrus fruit to mass transport of water vapor and other gases. *Plant Physiology*, 79, 1048.

Box, G.E.P., & Behnken, D.W. (1960). Some new three level designs for the study of quantitative variables. *Technometrics*, 2, 455-475.

Dong, X., Wrolstad, R.E., & Sugar, D. (2000). Extending shelf life of fresh-cut pears. *Journal of Food Science*, 65(1),181-6.

García, M.A., Martinó, M.N., & Zaritzky, N.E. (1998). Plasticized starch-based coatings to improve strawberry (*Fragaria ananassa*) quality and stability. *Journal of Agricultural and Food Chemistry*, 46, 3758-3767.

García, M.A., Martinó, M.N., & Zaritzky, N.E. (2000). Lipid addition to improve barrier properties of edible starch-based films and coatings. *Journal of Food Science*, 65(6), 941-947.

Gontard, N., Guilbert, S., & Cuq, J.L. (1993). Water and glycerol as plasticizer affect mechanical and water vapor barrier properties of an edible wheat gluten film. *Journal of Food Science*, 58 (1), 206-211.

Gorny, J.R., Hess-Pierce, B., Cifuentes, R. A., & Kader, A. A. (2002). Quality changes in fresh-cut pear slices as affected by controlled atmospheres and chemical preservatives. *Postharvest Biology and. Technology*, 24, 271-278.

İyidoğan, N.F., & Bayındırlı, A. (2004). Effect of L-cysteine, kojic acid and 4-hexylresorcinol combination on inhibition of enzymatic browning in Amasya apple juice. *Journal of Food Engineering*, 62(3), 299-304.

Kester, J.J., & Fennema, O. (1986). Edible Films and Coatings: a review. *Food Technology*, 40, 47-59.

King, A.H. (1983). Brown seed extracts (Alginates). Food Hydrocolloids, 2, 115-188.

Lee, J.Y., Park, H.J.; Lee, C.Y., & Choi, W.Y. (2003). Extending shelf-life of minimally processed apples with edible coatings and antibrowning agents. *Lebensmittel-Wissenschaft und-Technologie*, 36, 323-329.

Li, P., & Barth, M. (1998). Impact of edible coatings on nutritional and physiological changes in lightly-processed carrots. *Postharvest Biology and Technology*, 14, 51-60.

Luo, Y. & Barbosa-Canovas, G.V. (1997). Enzymatic browning and its inhibition in new apple cultivars slices using 4-hexylresorcinol in combination with ascorbic acid. *Food Science and Technology International*, 3(3), 195-201.

Mancini, F., & McHugh, T.H. (2000). Fruit-alginate interactions in novel restructured products. *Nahrung*, 44(3), 152-157.

McGuire, R.G. (1992). Reporting of objective color measurements. *HortScience*, 27(12), 1254-55.

McHugh, T., & Krochta, J.M. (1994a). Sorbitol-vs glycerol –plasticized whey protein edible films: Integrated oxygen permeability and tensile property evaluation. *Journal of Agricultural and Food Chemistry*, 42(4), 841-845.

McHugh, T. H., & Krochta, J. M. (1994b). Milk-protein-based edible films and coatings. *Food Technology*, 48 (1), 97-103.

McHugh, T.H., & Senesi, E. (2000). Apple wraps: a novel method to improve the quality and extend the shelf life of fresh-cut apples. *Journal of Food Science*, 65(3), 480-5.

Molnar-Perl, I., & Friedman, M. (1990). Inhibition of browning by sulfur amino acids. 3. Apples and potatoes. *Journal of Agricultural of Food Chemistry*, 38(8), 1652-6.

Monsalve-Gonzalez, A., Barbosa-Canovas, G.V., Cavalieri, R.P., McEvily, A.J. & Iyengar, R. (1993). Control of browning during storage of apple slices preserved by combined methods. 4-hexylresorcinol as anti-browning agent. *Journal of Food Science*, 58(4), 797-800.

Myers, R.H., & Montgomery, C. (2002). *Response Surface Methodology: process and product optimization using designed experiments*. New York: John Wiley and Sons.

Nicoli, M.C., Anise, M., & Severinc, C. (1994). Combined effects in preventing enzymatic browning reactions in minimally processed fruit. *Journal of Food Quality*, 17(3), 221-9.

Nísperos-Carriedo, M.O. (1994). Edible coatings and films based on polysaccharides. In J.M. Krochta, E.A. Baldwin & M.O. Nisperos-Carriedo, *Edible Coatings and Films to Improve Food Quality* (pp. 305-335). Switzerland:Technomic Publishing Co.

Nussinovitch, A. (1997). Agricultural uses of hydrocolloids. In Hydrocolloid applications: *Gum technology in the food and other industries* (pp. 169-189). London: Blackie Academic and Professional.

Nussinovitch, A. (2000). Gums for coatings and adhesives. In G. Phillips & P. Williams, *Handbook of hydrocolloids*. Cambridge, England: CRC Woodhead Publishing Limited.

Park, H.J., Chinnan, M.S., & Shewfelt, R.L. (1994). Edible coating effects on storage life and quality of tomatoes. *Journal of Food Science*, 59(3), 568-570.

Park, H.J. (1999). Development of advanced edible coatings for fruits. *Trends in Food Science and Technology*, 10, 254-260.

Pena, D. C. R., & Torres, J. A. (1991). Sorbic acid and potassium sorbate permeability of an edible methylcellulose-palmitic acid films: water activity and pH effects. *Journal of Food Science*, 56(2), 497–499.

Perez-Gago, M.B., Serra, M. & Del Río, M.A. (2004). Efecto de recubrimientos comestibles a base de proteína de suero lácteo y antioxidantes en la vida útil de manzanas cortadas en fresco. Livro de Actas. IV Simposio Ibérico, I Nacional, VII Español de Maturação e Pós-Colheita. Frutos e hortícolas. Oeiras, 6-9 Outubro, Portugal, pp:237-241.

Pizzocaro, F., Toregiani, D., & Gilardi, G. (1993). Inhibition of apple polyphenol oxidase (PPO) by ascorbic acid, citric acid and sodium chloride. *Journal of Food Processing and Preservation*, 17, 21-30.

Pranoto, Y., Salokhe, V., & Rakshit, K.S. (2005). Physical and antibacterial properties of alginate-based edible film incorporated with garlic oil. *Food Research International*, 38, 267–272.

Reyes, V.G. (2000). Preservation of exposed cut fresh fruit. United States Patent: 6,159,512.

Rhim, J. W. (2004). Physical and mechanical properties of water resistant sodium alginate films. *Lebensmittel-Wissenschaft und-Technologie*, 37, 323-330.

Rodríguez, F. J. (2004). Desarrollo de una cobertura comestible para cilindros de melón (*Cucumis melo* L.) con mínimo proceso. Licentiate Thesis. Facultad de Ciencias Universidad Central de Venezuela, Caracas.

Rojas-Graü, M.A., Sobrino-López, A., Tapia, M.S. & Martín-Belloso, O. (2006). Browning inhibition in fresh-cut 'Fuji' apple slices by natural antibrowning agents. *Journal of Food Science*, 71(1): S59-S65.

Shahidi, F. (2004). Quality characteristics of edible oils. In F. Shahidi, A.M. Spanier, Ho, Chi-Tang & T. Braggins, *Quality of fresh and processed foods*. New York: Kluwer Academic.

Soliva-Fortuny, R. C., Grigelmo-Miguel, N., Odriozola-Serrano, I., Gorinstein, S., & Martin-Belloso, O. (2001). Browning evaluation of ready-to- eat apples as affected by modified atmosphere packaging. *Journal of Agricultural of Food Chemistry*, 49(8), 3685-90.

Son, S.M., Moon, K.D., & Lee, C.Y. (2001). Inhibitory effects of various antibrowning agents on apple slices. *Food Chemistry*, 73, 23-30.

Sothornvit, R., & Krochta, J.M. (2000). Plasticizer effect on oxygen permeability of B-lactoglobulin films. *Journal of Agricultural of Food Chemistry*, 48, 6298-6302.

Suyatma, N.E., Tighzert, L., & Copinet, A. (2005). Effects of hydrophilic plasticizers on mechanical, thermal, and surface properties of chitosan films. *Journal of Agricultural and Food Chemistry*, 53(10), 3950-3957.

Tanada-Palmu, P.S. & Grosso, C. R. F. (2003). Development and characterization of edible films based on gluten from semi-hard and soft Brazilian wheat flours (development of films based on gluten from wheat flours). *Ciência e Tecnologia de Alimentos*, 23(2), 264-269.

Wong, D.W.S., Camirand, W.P, & Pavlath, A.E. (1994a). Development of edible coatings for minimally processed fruit and vegetables. In J.M. Krochta, E.A. Baldwin & M.O.

Nisperos-Carriedo, *Edible Coatings and Films to Improve Food Quality* (pp. 65-88). Switzerland:Technomic Publishing Co.

Wong, W.S., Tillin, S.J., Hudson, J.S., & Pavlath, A.E. (1994b). Gas exchange in cut apples with bilayer coatings. *Journal of Agricultural and Food Chemistry*, 42, 2278-2285.

Wong, D.W., Gregorski, K.S., Hudson, J.S., & Pavlath, A.E. (1996). Calcium Alginate Films: Thermal Properties and Permeability to Sorbate and Ascorbate. *Journal of Food Science*, 61(2), 337-341.

Yang, L., & Paulson, A.T. (2000). Effects of lipids on mechanical and moisture barrier properties of edible gellan film. *Food Research International*, 33, 571-578.