Influence of controlled atmosphere storage on polyphenoloxidase activity in relation to colour changes of minimally processed 'Jonagored' apple

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Summary The effects of the storage atmosphere composition (2% O_2 + 4% CO_2; 2% O_2 + 8% CO_2; 2% O_2 + 12% CO_2) on polyphenoloxidase activity and phenolic content of the 'Jonagored' apple variety during cold storage was evaluated, and the relationship with enzymatic browning investigated. Controlled atmosphere storage inhibited the polyphenoloxidase (PPO) activity of apple cubes during storage. It seems that the higher the concentration of carbon dioxide in the storage atmosphere the higher inhibition of PPO and the lower browning achieved. At the substrate-enzyme levels investigated, the phenolic content, substrate level was the major factor determining darkening.

Keywords Apple, *Malus pumila*, phenolic content.

Introduction

Phenolic compounds undergo oxidation to brown compounds that discolour fruits, reducing their quality. A number of enzymes catalyze the biosynthesis or oxidation of phenolic compounds, among them phenylalanine ammonia lyase (PAL), polyphenoloxidase (PPO) and catechol oxidase. The activities of these enzymes, and thus their mediation of desirable or undesirable changes, are affected by controlled atmospheres (CA) (Zagory & Kader, 1989).

Inhibition of enzymatic browning can be achieved by removal of one of the two substrates (i.e., oxygen or phenol) from the reaction medium. Complete removal of oxygen is the most satisfactory way to control the phenolic oxidation catalyzed by PPO. However, it is not applicable to living tissues because of the risk of anaerobic respiration (Nicolas *et al.*, 1994). Nevertheless, by varying the levels of O_2 and CO_2 in the surrounding atmosphere, the enzyme action can be inhibited to a considerable degree. According to Burton (1982), because phenolases have a low O_2 – affinity, oxidation of phenolic substrates can be avoided or at least reduced at O_2 levels of 5% or less.

The effects of CA in minimizing enzymatic browning in minimally processed (MP) fruits have been evaluated (Murr & Morris, 1974; Buescher & Henderson, 1977; Siriphanick & Kader, 1985; Bolin & Huxsoll, 1989; McLellan *et al.*, 1990; Barrett *et al.*, 1991; Nicoli *et al.*, 1994). Various investigators have found that high levels of CO_2 reduced browning or other discoloration of cut vegetables (Buescher & Henderson, 1977; Siriphanich & Kader, 1985; Weichman, 1987). Siriphanick & Kader (1985) also reported that, in lettuce, an atmosphere with 15% CO_2 inhibited the production of polyphenolic compounds, the main substrate for the browning reaction. Powrie *et al.* (1990) patented a method for preserving fresh, ripe fruit pieces (apples, apricots, grapefruit, kiwifruit, mangoes, melons, oranges, papayas, pineapples,

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strawberries, tangerines and tomatoes), consisting of refrigerating the fruit pieces at a temperature between −1 to about 6 °C, to induce 'cold shock' and storage at −1 to 10 °C, keeping the pH lower than 4.5 and using modified atmosphere packaging (MAP), with the O_2 ranging from 5 to 50% and the N_2 between 50 and 95%; Barrett *et al.* (1991) reported significant losses in PPO activity and tendency to browning of 'Delicious' apples after 7–14 weeks in CA storage; Nicoli *et al.* (1994) achieved successful inhibition of enzymatic browning in apple slices for long storage periods by the application of MAP. Nevertheless, an enhanced browning rate was observed as the packages were opened. However, a combination of ethanol dips with MAP was effective in maintaining the original colour in apple slices (cv. Golden Delicious) during 9 days of storage, slowing down darkening after the packages were opened (Nicoli *et al.*, 1994).

Various investigators (Buescher & Henderson, 1977; Siriphanich & Kader, 1985) have found that levels of CO_2 above 5% inhibit PPO, the enzyme responsible for enzymatic browning in several commodities. Nevertheless, little has been done to evaluate the effects of CA conditions on PPO activity and the phenolic content of MP commodities. Buescher & Henderson (1977) obtained a reduction in discolouration of broken snap beans with increasing concentrations of CO_2 (0–30%) in the storage atmosphere and attributed this fact to reduction of phenolase activity and phenolic content. Molnar-Perl & Friedman (1990) reported that enzymatic browning of sliced apples was successfully minimized by the exclusion of O_2 from the package. Kaji *et al.* (1993) found that total phenols of shredded cabbage was little influenced by O_2 and CO_2. Mateos *et al.* (1993) reported that the total phenolic content of cut lettuce was reduced to under 20% CO_2-enriched atmosphere and attributed that to the observed decrease in the PAL activity. Nevertheless, the evolution of PPO activity and total phenols was not investigated.

The hypothesis that we have tested was that there could exist a storage atmosphere composition which would reduce the PPO activity and bring down enzymatic browning of minimally processed 'Jonagored' apple to an acceptable level during controlled atmosphere storage.

Materials and methods

Plant material

Apples *Malus pumila*, cv. Jonagored, were grown at Estação Regional de Fruticultura e Vitivinicultura, Quinta de Sergude, Felgueiras, Portugal. The harvest was in September. The fruits were stored in air at 4 °C for 1–3 months until used in the experiments.

Treatment and storage conditions

Apples stored under refrigeration (4 °C) were transported weekly to the laboratory in Porto in bulks of about 10 kg randomly selected from the whole bulk. They were initially washed with chlorinated water (150 ppm of active chlorine for 5 min) to prevent surface contamination (Wardowski & Brown, 1991). After peeling and cutting in cubes of ≈1.5 cm, apple samples were immersed in distilled water. Three replicates of 30 cubes randomly selected from the whole bulk available were used for each storage time of each experiment. Apple samples were stored in sealed glass jars of 1 L capacity, under the specific CA conditions (2% O_2 + 4% CO_2; 2% O_2 + 8% CO_2; 2% O_2 + 12% CO_2) at 4 °C for 7 days in the dark. The desired gas mixtures were established by the use of flowmeters assembled in a gas mixer and metered to the samples at a flow rate of 3.0 L/h after humidification by bubbling through water. Gas concentrations in the head space of the glass jars were measured by injection of 0.6 mL in a gas chromatograph (Shimadzu GC-14-A, Tokyo, Japan), equipped with a thermal conductivity detector (TCD) and connected to a 3 m × 0.32 cm column packed with 80/100 mesh Carbosieve S II (Supelco). The injector and detector temperatures were set at 120 and 210 °C, respectively. The temperature of the column oven was programmed for 40 °C for 6 min and subsequently for 15 °C/min to 170 °C and then held at 170 °C for 5 min. The flow rate of the carrier gas, helium, was 30 mL min^{-1} and the bridge-current of the TCD was 140 mA.

For the control, three replicates of cut apple were dipped in distilled water and stored in air humidified in the same way and supplied at the same flow rate as for the CA setting, parallel to

each of the experimental series. After 3 and 7 days of storage under the specified conditions, samples were removed and evaluated in terms of several physicochemical quality attributes as detailed below. Cut apple samples were also analyzed on day 0 (after peeling and cutting) as a reference. All the experiments were repeated within approximately 1.5 months.

Colour assessment

Cut apple surface colour was measured at the surface of each of the 30 apple cubes with a hand-held tristimulus reflectance colorimeter (Minolta CR–300, Minolta Corp., Ramsey, NJ, USA). Three replicates of 30 apple cubes were used for each storage time. Colour was recorded using a CIE-L*a*b* uniform colour space. Numerical values of a* and b* were converted into hue angle ($H° = \tan^{-1}$ b*/a*) and chroma [Chroma = $(a^{*2} + b^{*2})^{1/2}$] (Francis, 1980).

Assay for PPO activity

The enzyme extract was prepared by homogenizing the apple samples with 0.2 M pH 6.5 sodium phosphate buffer (extraction buffer) with a T 25 basic Ultra-Turrax (IKA Labortechnik, Staufen, Germany) in an external ice bath for 3 min at 1 min intervals. The homogenate was then centrifuged at 4 °C for 30 min at 16 500 × *g* (Sorvall RC-5C, Du Pont Company, Biomedical Products, Sorval Instruments Wilmington, DE 19898, USA, refrigerated superspeed centrifuge) and the supernatant subsequently filtered through cheesecloth and its volume determined for enzymatic activity assay. Three replicates were assayed for each determination.

Enzymatic activity was assayed by measuring the rate of increase in absorbance at 420 nm and 25 °C in a UV-1601 UV/VIS spectrophotometer (Shimadzu Corporation, Tokyo, Japan) (Galeazzi *et al.*, 1981). When a lag phase occurred, the reaction rate was measured after the lag phase. All the determinations were performed in triplicate. The unit for the enzymatic activity (U/μg protein/min) was defined as the change of 0.001 in the absorbance value under the conditions of the assay.

Total phenolic content

Total phenolic compounds were measured using the Folin–Ciocalteau reagent (Folin & Ciocalteau, 1927; Singleton & Rossi, 1965). Total phenols were expressed as μg dopamine 100 g^{-1} of fruit fresh weight.

Browning index (BI)

The absorbance of the apple juice was measured at 420 nm to determine the Browning index (BI). Higher values in absorbance correspond to higher browning of the tissue (Wrolstad, 1976).

Protein concentration

The protein concentration was determined in all preparations by the colorimetric method described by Bradford (1976). The values were obtained by graphic interpolation on a calibration standard curve with serum albumin (BSA) at 595 nm.

Polyacrylamide gel electrophoresis

The apple PPO was separated into multiple forms by a modification of the polyacrylamide gel electrophoresis (PAGE) procedure described by Davis (1964). The gels were dried and the relative mobility (RM) were calculated.

Statistical analysis

In order to be able to compare the experiments performed at different dates, differences between the controls in those experiments were taken into account. The results from different treatments were corrected by those differences: Corrected Experimental Result 2 = Experimental Result 2 * Control 1/Control 2; Experimental Result 1 was compared to Corrected Experimental Result 2 and the control used was Control 1.

The SAS statistical analysis computer system package (SAS Institute, Inc., 1982) was used for the analysis of the data. Statistical significance was assessed by two-way analyses of variance (the source of variation was storage atmosphere). Significant differences ($P = 0.05$) between treatments were detected using Duncan's multiple range test.

Correlation analysis was conducted between colour parameters, BI, PPO and phenolic content. R was used as the correlation coefficient.

Results and discussion

Colour

Two per cent O_2 + 12% CO_2 was the best CA combination for avoiding colour changes of apple cubes during 7 days of storage at 4 °C, as may be seen by the lower a*, b*, hue and chroma values (Tables 2, 3, 4 and 5). Differences between 2% O_2 + 12% CO_2 and 2% O_2 + 8% CO_2 or 4% CO_2 were not significant when considering the L* value (Table 1). Nevertheless, all of the CA conditions resulted in better coloured samples than air storage. The findings in this study are in agreement with those of a previous study (Rocha & Morais, 2001b).

Increased CO_2 was also observed to reduce browning or discolouration of cut or broken surface of Brussels sprouts (Weichman, 1987) and snap bean (Buescher & Henderson, 1977). In contrast, browning of mushrooms was increased at high CO_2 levels (above 5%) (Murr & Morris, 1974) and it was prevented only in an O_2-free atmosphere. Buescher & Henderson (1977), in their study with cut snap beans stored for 24 h at 27 °C, reported that discolouration (measured as greenness, a* value) was reduced with increasing concentrations of CO_2 (0, 10, 20 and 30%).

Kaji *et al.* (1993) studied the influence of CA storage during 10 days of storage at 5 °C on the colour of shredded cabbage and found that, under a fixed percentage of O_2, browning increased with decreasing concentration of CO_2 and that browning increased for levels of CO_2 above 5%. Their results suggested that the development of browning in shredded cabbage was suppressed as the CO_2 concentration increased (0 to 5%), with no influence of O_2 if higher than 2.5% (up to 10%).

Table 1 L* value of MP apple stored at 4 °C in air and several CA conditions[z]

Experimental Series	Storage time (days) 0	3	7
Control	79.22abc[†]	77.66b	74.14c
2% O_2 + 4% CO_2	79.60abc	78.94a	76.96ab
	80.17a	77.89b	77.67a
2% O_2 + 8% CO_2	79.22abc	77.09b	76.89ab
	78.59c	76.31b	75.57b
2% O_2 + 12% CO_2	78.99bc	78.39a	76.53ab
	79.22abc	77.37b	76.81ab

[z] Data are means of three replicates of 30 apple cubes.
† Mean separation in columns by Duncan's multiple range test, $P = 0.05$.
Numbers followed by letters in common are not significantly different.

Table 2 a* value of MP apple stored at 4 °C in air and several CA conditions[z]

Experimental series	Storage time (days) 0	3	7
Control	−6.43b[†]	−4.30bc	−3.23a
2% O_2 + 4% CO_2	−6.43b	−4.96ef	−3.36a
	−6.43b	−4.05b	−3.35a
2% O_2 + 8% CO_2	−6.43b	−4.56cde	−3.96b
	−6.43b	−5.34f	−3.95b
2% O_2 + 12% CO_2	−6.43b	−4.99f	−4.55c
	−6.43b	−4.72def	−4.58c

[z] Data are means of three replicates of 30 apple cubes.
† Mean separation in columns by Duncan's multiple range test, $P = 0.05$.
Numbers followed by letters in common are not significantly different.

Table 3 b* value of MP apple stored at 4 °C in air and several CA conditions[z]

Experimental series	Storage time (days) 0	3	7
Control	24.72a[†]	24.22b	31.30b
2% O_2 + 4% CO_2	24.72a	24.81b	27.62c
	24.28a	24.60bc	33.35a
2% O_2 + 8% CO_2	24.72a	26.46a	26.98cd
	24.72a	27.25a	30.00b
2% O_2 + 12% CO_2	24.72a	25.00ab	26.75cd
	24.92a	24.99ab	25.29d

[z] Data are means of three replicates of 30 apple cubes.
† Mean separation in columns by Duncan's multiple range test, $P = 0.05$.
Numbers followed by letters in common are not significantly different.

Table 4 Hue angle of MP apple stored at 4 °C in air and several CA conditions*

Experimental series	Storage time (days) 0	3	7
Control	104.7ab†	100.2bc	96.1e
2% O_2 + 4% CO_2	104.7ab	101.5b	99.0ab
	104.9a	99.4c	97.8d
2% O_2 + 8% CO_2	104.7ab	99.8c	98.5cd
	104.7ab	103.2a	97.7d
2% O_2 + 12% CO_2	104.7ab	103.4a	99.7ab
	104.68ab	103.66a	100.35a

* Data are means of three replicates of 30 apple cubes.
† Mean separation in columns by Duncan's multiple range test, $P = 0.05$.
Numbers followed by letters in common are not significantly different.

Table 5 Chroma value of MP apple stored at 4 °C in air and several CA conditions*

Experimental series	Storage time (days) 0	3	7
Control	25.56a†	24.61bc	31.50b
2% O_2 + 4% CO_2	25.55a	25.32ab	27.97c
	25.12a	26.94a	33.52a
2% O_2 + 8% CO_2	25.56a	26.86a	27.04cd
	25.56a	28.77a	30.28b
2% O_2 + 12% CO_2	25.55a	25.10ab	27.37cd
	25.75a	25.00ab	25.70d

* Data are means of three replicates of 30 apple cubes.
† Mean separation in columns by Duncan's multiple range test, $P = 0.05$.
Numbers followed by letters in common are not significantly different.

PPO activity

Controlled atmosphere storage inhibited the PPO activity of apple cubes during storage. After 3 days of storage at 4 °C, all samples stored in CA had lower PPO activity than the air-stored ones. Samples from 2% O_2 + 12% CO_2 showed the lowest PPO activity. No differences ($P > 0.05$) were detected between samples from other CA conditions (Table 6). After 7 days of storage at 4 °C, only samples stored in CA with concentrations of CO_2 higher than 4% had lower PPO activities than the air-stored apple cubes (Table 6).

Table 6 Polyphenoloxidase activity (U mg^{-1} prot. min^{-1}) of MP stored at 4 °C in air and several CA conditions*

Experimental series	Storage time (days) 0	3	7
Control	11.91a†	15.91a	16.78a
2% O_2 + 4% CO_2	12.08a	12.18b	16.04a
	12.73a	13.39b	13.34b
2% O_2 + 8% CO_2	11.99a	12.93b	13.88b
	12.19a	12.04b	12.49b
2% O_2 + 12% CO_2	11.38a	9.89c	11.70c
	12.82a	12.00c	11.52c

* Data are means of three replicates of 30 apple cubes.
† Mean separation in columns by Duncan's multiple range test, $P = 0.05$.
Numbers followed by letters in common are not significantly different.

It seems that the higher the concentration of CO_2 in the storage atmosphere the higher the inhibition of PPO achieved; 2% O_2 + 12% CO_2 was the best storage atmosphere, and 4% CO_2 seemed to be insufficient to inhibit the enzyme activity in spite of the low O_2 concentration. No differences ($P > 0.05$) were detected when these samples were compared to the control apple cubes (Table 6).

Chaves & Tom (1976) studied the effect of CO_2 on PPO activity (pH 6.8; catechol substrate) extracted from 'Red Delicious' apple, and they stated that PPO activity decreased with increasing CO_2 concentration (5–30%). Crude extracts and purified preparations from 'Red Delicious' apple were competitively inhibited by CO_2. Murr & Morris (1974) found that 0% O_2 reduced discolouration of mushrooms and *o*-diphenolase activity for up to 7 days. Nevertheless, levels above 0% O_2 had little or no effect in reducing discolouration and *o*-diphenolase activity. Additionally, concentrations of CO_2 higher than 5% appeared to markedly inhibit *o*-diphenolase activity while increasing surface discolouration. In a study with lettuce, Siriphanick & Kader (1985) stated that CO_2 did not have a very clear effect on PPO activity. Nevertheless, they achieved complete inhibition of lettuce browning and related this to a reduction in PPO activity.

Anyway, all these studies were performed with whole fruit and vegetables, and scarce information is available concerning the evaluation of MP fruits

during storage. Only few studies are reported in the literature: Buescher & Henderson (1977), evaluated snap beans, and found lower phenolase activity and phenolic levels when broken snap beans were stored in 20% CO_2 than when samples were stored in air. They correlated those results with the reduction of discolouration observed in CA-stored samples.

Phenolic content

A great variability in relation to the total phenolic content in storage under different CA conditions was observed. Apple cubes stored in 2% O_2 + 12% CO_2 showed the highest phenolic content from day 3 of storage which probably can be associated with the low PPO activity under this CA condition (Tables 6 and 7), because lower PPO activity may result in lower quantity of phenols degraded.

In a study with mushrooms, Murr & Morris (1974) also reported that a high concentration of CO_2 irreversibly inhibited the oxidation of monophenols by *o*-diphenolase, and they associated this to the decrease of *o*-diphenolase activity.

Siriphanick & Kader (1985) reported that the total phenolic content of lettuce tissue increased during the first 6 days of storage at 4 °C, by almost 50% compared to the original level in the air control and then remained relatively stable, and that samples under CO_2 treatment (15%) did not significantly change the phenolic content. They concluded that CO_2 suppressed the production of phenolic compounds. Barrett *et al.* (1991) studied the effects of CA storage on the phenolic content of 'Delicious' apple during 180 days at 0 °C and they stated that the concentration of total phenols was fairly stable. They concluded that the correlation of phenols to browning was weak.

Nevertheless, these studies dealt with the whole commodity. In their study with shredded cabbage, Kaji *et al.* (1993) reported that, regardless of the CO_2 concentration (0, 5 10 or 15%), the amount of phenols was nearly constant during 10 days of storage at 5 °C. Mateos *et al.* (1993) evaluated cut lettuce exposed to CO_2-enriched atmospheres and found that the effects of high CO_2 on total phenols and PAL activity were more pronounced with cut lettuce than with intact heads of lettuce. They associated these results with the wounding of the tissue, because it could induce phenolic metabolism and make lettuce become more sensitive to environmental stress. They found that the phenolic content of midribs kept in 20% CO_2 was lower than in air and this was associated with senescent browning.

Table 7 Total phenol (mg dopamine /100 g) of MP apple stored at 4 °C in air and several CA conditions*

Experimental series	Storage time (days)		
	0	3	7
Control	1.831b†	1.397c	1.243c
2% O_2 + 4% CO_2	1.893a	1.039c	1.009cd
	1.633a	1.279c	1.580c
2% O_2 + 8% CO_2	1.620b	1.395c	1.051cd
	1.451b	1.205c	0.998d
2% O_2 + 12% CO_2	1.618a	2.385a	2.361b
	1.735a	2.889a	3.064a

* Data are means of three replicates of 30 apple cubes.

† Mean separation in columns by Duncans multiple range test, $P = 0.05$.

Numbers followed by letters in common are not significantly different.

Browning index (BI)

Apple cubes stored in an atmosphere of 2% O_2 + 12% CO_2 showed the lowest BI on days 3 and 7 of storage. Nevertheless, the differences were not significant ($P > 0.05$) (data not shown), which is in agreement with the data for colour evaluation (Tables 1–5). The lowest BI corresponded to the lowest browning of the tissue (Wrolstad, 1976).

Overall, 2% O_2 + 12% CO_2 was found to be the most efficient CA condition for inhibiting PPO activity, phenolic degradation and, subsequently, preserving the colour of apple cubes, as expressed by colour parameters and the browning index.

Electrophoretic data

Controlled atmosphere storage did not affect the electrophoretic pattern of PPO extracted from 'Jonagored' apple stored for 7 days of storage at 4 °C. Only one band was detected with a RM of 0.45 towards the anode with identical results to a previous study (Rocha & Morais, 2001a).

Relationship between colour evaluation, BI, phenolic content and (PPO) activity

In order to understand how phenols and PPO activity contribute to browning and why CA storage can prevent these reactions, attempts were made to correlate the phenolic content and PPO activity with browning. Considering that 2% O_2 + 12% CO_2 was the best CA condition to preserve the quality of apple cubes, correlations were performed only for this storage atmosphere.

Moderate to high correlations were obtained when BI was plotted against each colour parameter, except for chroma (not consistent between both series) (Table 8).

When the phenolic content was plotted against the colour parameters, good and consistent correlations were only found for the apple cubes stored in 2% O_2 + 12% CO_2 for b*, hue angle and chroma values (Table 8). For L* and a* values data were not consistent between the experimental series. Moderate correlations were obtained between the phenolic content and the BI. Low correlation was obtained when PPO activity was plotted against the phenolic content (Table 8).

Table 8 Correlation coefficient (R) calculated for several parameters of MP apple after 7 days of storage under 2% O_2 + 12% CO_2

Quality parameters		Phenolic content	Browning Index	PPO activity
Colour:	L* value	0.995	0.85	0.44
		0.65	0.93	0.62
	a* value	0.825	0.995	0.14
		0.52	0.87	0.735
	b* value	0.995	0.79	0.54
		0.975	0.96	–
	Hue	0.995	0.86	0.44
		0.96	0.975	–
	Chroma	0.90	0.54	0.79
		0.97	0.96	–
Browning Index		0.85	x	–
		0.87	x	0.30
PPO activity		0.45	–	x
		0.20	0.30	x

–, no correlation found.
R, at $P = 0.05$.

Overall, the correlations between PPO activity and the colour parameters or BI of samples were poor and/or not consistent between experimental series.

No significant correlations were obtained between the PPO activity and the BI or colour parameters for apple cubes stored in 2% O_2 + 12% CO_2 (Table 8).

In summary, in apple cubes from 2%O_2 + 12% CO_2 the PPO activity decreased drastically and the substrate concentration increased, probably as a result of the reduction of the enzyme activity, and the phenolic content became the determining factor in the extent of browning that occurred.

Murr & Morris (1974) reported a correlation between the amount of discolouration and *o*-diphenolase activity of mushrooms stored in low O_2. However, for mushrooms stored in atmospheres with 5% CO_2 or more, such a correlation was found to be highly questionable.

Conclusion

Browning of apple cubes was reduced with an increasing concentration of CO_2. Two per cent O_2 plus 12% CO_2 was found to be more efficient than 8% or 4% CO_2 plus 2% O_2 in reducing the colour changes (mainly increases of a* value and decreases of hue angle) of apple cubes (cv. Jonagored).

Controlled atmosphere storage inhibited the PPO activity of apple cubes during storage. It seems that the higher the concentration of carbon dioxide in the storage atmosphere the higher inhibition of PPO achieved; 2% O_2 + 12% CO_2 was the best storage atmosphere and 4% CO_2 seemed to be insufficient to inhibit the enzyme activity in spite of the low oxygen concentration.

The amount of browning during CA storage (2% O_2 + 12% CO_2) was found to be mainly correlated to the total phenolic content, which indicates that, at the substrate-enzyme levels investigated, the substrate level was the major factor determining darkening. The correlation with PPO activity was poor.

The browning reaction in apples appears to be a complex process involving several factors: substrate levels, enzyme activity, and the presence of inhibitors or promoters. Of these, the PPO activity and its substrate concentrations appeared to be the two major factors involved.

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