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The Effect of Added L-Ascorbic Acid, and Sodium Chloride on the Change in Concentration of Some Volatiles in Pressurised Peach Homogenate during Storage

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Abstract: White peach (*Prunus persica* L cv Yamane) homogenates with sugar (20%), or with sugar (20%) and/or L-ascorbic acid (0·1%) and/or sodium chloride (0·5%), were packed in plastic bottles, pressurised (400 MPa, 20°C, 10 min), and then stored at 0 and 25°C for various periods. The headspace volatiles which were absorbed on Tenax TA using dynamic headspace sampling were heat desorbed and analysed by capillary gas chromatography mass spectrometry. Enzymatic formation of benzaldehyde during storage was observed in all samples. The homogenate with L-ascorbic acid showed the highest level of benzaldehyde formation. The flavour quality and colour of the pressurised homogenates with ascorbic acid stored at lower temperature were excellent.

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INTRODUCTION

The high hydrostatic pressure is useful for the purpose of processing, sterilisation and preservation of foods, as is the high temperature. The prominent merit of the high pressure is to avoid the destruction of covalent bondings and to keep natural aroma, taste and nutrients.

In a previous study (Sumitani *et al* 1994), the authors investigated the flavour quality of high-pressure-treated peach in comparison with that of ripe intact, crushed and heat-treated peaches. On the basis of the results, it seems that high-pressure treatment will become a useful method for the preservation of peach with good flavour quality. The processed peaches had an undesirable texture resulting from the tissue disruption during pressure processing. But, the aroma was very excellent. This suggests that the high-pressure treatment may be applied to manufacturing of peach homogenate (nectar) with good flavour quality. In the other hand, enzymic browning of high-pressurised peaches proceeded during the storage. The prevention of browning is a very important problem of the quality of the peach homogenate. Ascorbic acid (AsA) has been widely used as an additive to avoid browning of fruits and vegetables products during processing or storage. It is well known that dilute solution of sodium chloride (NaCl) prevents the enzymic browning of peeled apple. These additives can be handled easily, and are effective in preservation at relatively low concentration. The effect of two additives, AsA and NaCl, on the volatile compositions concerned in flavour quality was unknown. Furthermore, the multiplication effect of the two additives on the volatile changes during the storage is an interesting problem.

Horvat *et al* (1990) reported that hexanal, (*E*)-2-hexenal, benzaldehyde, linalool, and γ-decalactone, which were identified as the major volatiles, were

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present above their thresholds and should contribute to peach aroma. In the previous study (Sumitani *et al* 1994), major compounds identified in the high-pressure-treated peach were hexanal, (*E*)-2-hexenal, benzaldehyde, γ-decalactone, (*Z*)-3-hexenyl acetate. It seems that the volatiles play an important role in peach flavour. Thus, the volatile compounds were used as the indicators for concentration changes on the effect of the additives during storage.

The purpose of this study is to obtain basic information concerning both storage conditions and flavour quality of pressurised peach homogenates supplemented with AsA and/or NaCl.

MATERIALS AND METHODS

Materials and reagents

White peaches (*Prunus persica* L cv Yamane) were obtained from a local market. Intact ripe fruits were selected for the experiments. L-Ascorbic acid (AsA) and sodium chloride (NaCl) were obtained from Wako Pure Chemical Industries (Osaka, Japan). High-quality water was generated with a water purification system (Elgastat UHQ, Elga Ltd, Lane End, UK). High purity nitrogen (99·999% pure) in a bomb obtained from Sumitomo Seika Chemicals Co (Osaka, Japan) was used in the dynamic headspace collection. Tenax TA (60–80 mesh) was obtained from Enka NV (Arnhem, Holland). PFA bottles (maximum volume 146 ml) made of tetrafluoroethylene-perfluoroalkylvinylethyl co-polymer were purchased from Universal Co (Tokyo, Japan). Authentic standard flavour compounds were purchased from commercial sources.

Sample preparation

The intact ripe fruit was washed with water. After removal of the stone and peeling, four kinds of peach homogenate samples with added sugar as a sweetener were prepared as described below. Control samples were a mixture of eight peaches and granulated sugar at 20% of the peach weight; +AsA samples were a mixture of eight peaches, granulated sugar and AsA at 20 and 0·1% of the peach weight, respectively; +NaCl samples were a mixture of eight peaches, granulated sugar and NaCl at 20 and 0·5% of the peach weight, respectively; +AsA + NaCl samples were a mixture of eight peaches, granulated sugar, AsA and NaCl at 20, 0·1 and 0·5% of the peach weight, respectively. Each mixture was homogenised with a Waring blender at 11 000 rpm for 3 min, and the homogenate was filled into in 18 PFA bottles, which were screw-capped. Prior to pressurising, the control homogenate was immediately subjected to dynamic headspace sampling and gas chromatography mass spectrometry (GCMS) analysis. The high-pressure generator (Type MFP-7000, Mitsubishi Heavy Industries, Hiroshima, Japan) was able to process only one bottle at a time because of its small pressure vessel. Therefore, bottled samples were placed in freezer storage at −20°C until pressurisation. Prior to pressurisation, each bottled sample was removed from the freezer and thawed in a water-bath at 20°C for 1 h. Pressurisation conditions were as follows: hydrostatic pressure reached 400 MPa within 1 min and was held for 10 min; pressure vessel temperature rose from 20 to 28°C within 3 min and then gradually dropped to 22°C.

In order to study concentration changes of volatile constituents during storage, two bottled pressurised homogenates of each mixture were analysed immediately after pressurising. The remaining 16 bottled samples were placed in two storages controlled at 0 and 25°C, eight by eight. Samples were removed from the two storages at 1, 2, 4 and 9 weeks, then stored in a freezer at −20°C until analysed. Frozen bottled samples were thawed in a water-bath at 20°C for 1 h, then subjected to analysis as described below.

Dynamic headspace sampling and GCMS

All of the homogenate (*c* 130 g) in a PFA bottle was removed, and put into a tared headspace sampling bottle, then weighed. A volume of saturated NaCl solution equivalent to the sample volume was added to the bottle in order to inactivate enzymes and to prevent foaming during the headspace sampling. Analysis was done in duplicate. The headspace sampling method and the GCMS conditions used were as described previously (Tatsuka *et al* 1990; Sumitani *et al* 1994). Identification of compounds was based on computer matching of mass spectra and coincidence for MS pattern of authentic compounds as well as coincidence for Kováts retention indices (Kováts 1965). Concentrations were calculated from total ion intensity of individual components and internal standard (β-phenylethyl acetate) without response factor correction by using a data processor GCMS PAC200S (Shimadzu Corp, Kyoto, Japan) and are reported as $\mu g\ kg^{-1}$.

RESULTS

To investigate the influence of the freeze–thawing treatment on the volatiles, the following preliminary experiments were done prior the main experiments. Two bottled homogenated samples with sugar without AsA and NaCl were prepared. One bottled homogenate sample was subjected to dynamic headspace sampling and GCMS analysis, and another sample was immediately placed in freezer storage at −20°C for about 24 h and then analysed. Two data obtained from these

two different bottled homogenated samples indicated the good agreement within the experimental precision. The results indicate that the freeze–thawing treatment is an useful technique for sample preparation.

Table 1 lists the compounds identified by GCMS and shows concentrations of the volatile compounds of each |peach homogenate in duplicate samples during storage at 0 and 25°C, respectively, for 9 weeks.

It is known that γ-decalactone plays an important role as a character impact compound in peach flavour. Relatively large variation in the concentration of γ-decalactone was found through the storage period, but increasing and decreasing tendencies were not observed.

Esters detected in homogenates were mainly acetates. The major esters found were methyl acetate, ethyl acetate, hexyl acetate, (*Z*)-3-hexenyl acetate and (*E*)-2-hexenyl acetate. These compounds greatly decrease or disappear by heat-pasteurisation (Sumitani *et al* 1994). Changes in concentration of (*Z*)-3-hexenyl acetate stored at 0 and 25°C, respectively, are shown in Fig 1. The authors have reported previously that the level of esters in pressurised peach fruit samples clearly decreased with stored at 25 and 40°C (Sumitani *et al* 1994). The trends in ester levels of pressurised homogenates stored at 25°C were very similar to previous experimental results (Sumitani *et al* 1994).

High concentrations of (*E*)-2-hexenal and (*E*)-2-hexenol, as well as low concentrations of hexanal, haxanol and (*Z*)-3-hexenol were found. After disruption of the fruit, lipase converts glyceride lipids into linoleic acid and linolenic acid. Various lipoxygenase isoenzymes from fruit provide different unsaturated fatty acid hydroperoxides. Hydroperoxide lyase cleaves fatty acid hydroperoxides into two aldehyde fragments (Gardner 1989). 'Green and grassy' odour of fruit is largely due to the aldehydes, hexanal and (*E*)-2-hexenal. Horvat *et al* (1990) reported that hexanal and (*E*)-2-hexenal contribute to peach aroma. (*E*)-2-Hexenal seems important to the aroma of apples (Flath *et al* 1967) and, together with (*E*)-2-hexenol, to the aroma of blueberries (Parliment and Kolor 1975). Changes in concentration of (*E*)-2-hexenal stored at 0 and 25°C, respectively, are shown in Fig 2. The concentration of (*E*)-2-hexenal in the control sample rapidly increased immediately after pressurising. In the control sample, concentration of (*E*)-2-hexenal reached a maximum after 2 weeks storage at 0°C and 1 week storage at 25°C, respectively, and then decreased. Addition of AsA shifted the concentration maximum to 4 weeks storage at 0°C and 2 weeks storage at 25°C, respectively. In the other two samples, +NaCl and +AsA + NaCl, a clear increase or decrease in concentration was not observed. These results indicate that enzymic oxidation of unsaturated fatty acids occurs in the control and +AsA samples during storage periods.

It is known that benzaldehyde arises from the cyanogenic glycosides, amygdalin and prunasin, typical constituents of many *Prunus* species. Changes in

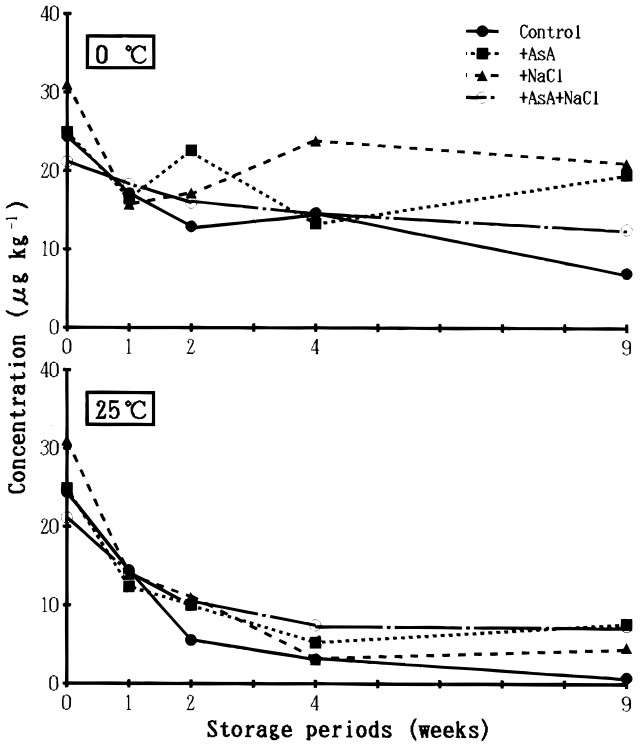

Fig 1. Concentration changes of (*Z*)-3-hexenyl acetate in peach homogenate samples stored at 0 and 25°C, respectively, for 9 weeks.

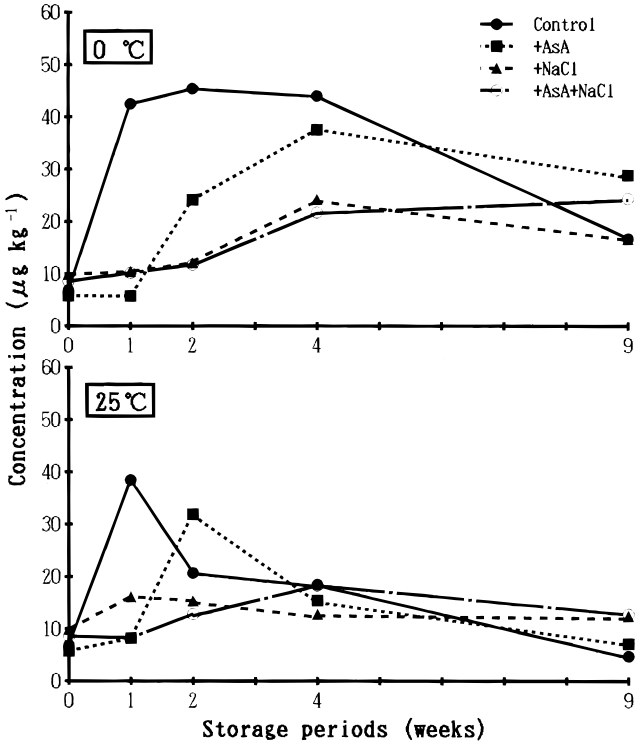

Fig 2. Concentration changes of (*E*)-2-hexenal in peach homogenate samples stored at 0 and 25°C, respectively, for 9 weeks.

TABLE 1
Approximate concentrations of volatile compounds in peach homogenate samples during storage at 0 and 25°C, respectively, for 9 weeks.

Volatile Compound[a]	*Storage (weeks)*	*Concentration (μg kg⁻¹)*							
		Control[b]		+AsA[c]		+NaCl[d]		+AsA + NaCl[e]	
		0°C	*25°C*	*0°C*	*25°C*	*0°C*	*25°C*	*0°C*	*25°C*
γ-Decalactone	before[f]	0·45		—		—		—	
	0[g]	0·55		1·22		1·38		1·20	
	1	0·50	0·83	0·85	0·80	0·54	0·90	0·66	0·76
	2	0·34	0·56	1·07	1·00	0·42	0·40	0·58	0·29
	4	0·49	0·48	0·39	0·46	0·68	0·49	0·44	0·37
	9	0·34	0·39	0·97	1·26	1·18	1·82	0·54	0·88
Hexyl acetate	before	1·87		—		—		—	
	0	4·56		5·34		7·28		6·99	
	1	2·61	2·53	4·26	2·13	3·19	2·65	4·76	5·30
	2	2·31	1·86	4·16	2·27	3·34	1·78	4·21	1·96
	4	3·41	0·39	2·42	1·48	4·67	0·49	3·94	1·57
	9	1·93	0·12	5·67	1·90	4·72	0·68	3·56	1·12
(*Z*)-3-Hexenyl acetate	before	13·76		—		—		—	
	0	24·41		24·90		31·01		21·17	
	1	17·14	14·47	16·48	12·37	15·73	13·94	18·31	14·18
	2	12·92	5·60	22·58	10·05	17·18	11·05	15·94	9·98
	4	14·64	3·14	13·28	5·27	23·81	3·10	14·57	7·45
	9	6·98	0·84	19·55	7·66	20·95	4·69	12·55	7·38
(*E*)-2-Hexenyl acetate	before	9·60		—		—		—	
	0	20·00		17·61		27·78		26·33	
	1	12·46	8·45	12·08	8·03	13·41	10·29	21·71	16·49
	2	8·73	3·16	14·37	6·12	12·96	6·83	18·40	9·39
	4	9·57	1·04	8·71	2·78	18·45	1·31	14·83	6·63
	9	4·59	0·28	13·91	4·07	14·98	2·23	14·84	5·75
(*E*)-2-Hexenal	before	15·31		—		—	—		
	0	6·84		5·76		9·89		8·52	
	1	42·49	38·43	5·73	8·23	10·46	16·13	10·16	8·25
	2	45·42	20·68	24·15	31·92	12·08	15·36	11·74	12·79
	4	44·00	18·48	37·56	15·40	24·21	12·80	21·73	18·26
	9	16·71	4·78	28·85	7·10	16·64	12·45	24·36	12·78
(*E*)-2-Hexenol	before	8·49		—		—		—	
	0	22·56		26·09		36·00		25·80	
	1	8·93	8·52	19·07	15·54	17·81	18·12	21·84	17·59
	2	2·25	0·78	20·41	5·49	19·43	13·98	14·32	9·86
	4	0·54	0·48	7·09	0·06	22·12	2·32	12·55	1·89
	9	ND[h]	0·12	0·44	0·07	22·70	4·69	4·00	0·23
(*Z*)-3-Hexenol	before	0·50		—		—		—	
	0	0·93		0·76		1·66		0·80	
	1	1·03	2·10	0·62	0·81	0·93	1·51	0·65	0·78
	2	1·01	1·51	0·81	1·11	0·95	1·41	0·60	0·69
	4	1·38	1·87	0·59	1·00	1·56	2·01	0·59	0·69
	9	0·98	1·13	0·89	1·76	2·12	3·62	0·58	1·11
Hexanal	before	0·79		—		—		—	
	0	0·55		0·73		0·80		1·91	
	1	1·24	1·89	0·73	0·45	1·36	1·88	1·60	0·98
	2	1·66	1·99	2·03	0·86	1·45	1·54	3·08	1·22
	4	2·77	5·16	2·58	1·76	2·61	0·44	2·82	1·34
	9	1·51	7·42	6·26	8·81	3·52	3·15	3·97	4·58

TABLE 1.—*Continued*

Volatile Compound[a]	*Storage (weeks)*	*Concentration (μg kg⁻¹)*							
		Control[b]		+AsA[c]		+NaCl[d]		+AsA + NaCl[e]	
		0°C	*25°C*	*0°C*	*25°C*	*0°C*	*25°C*	*0°C*	*25°C*
Hexanol	before	2·09		—		—		—	
	0	4·46		9·00		10·44		8·32	
	1	4·60	7·49	5·89	5·80	5·15	7·28	6·31	6·05
	2	4·27	4·58	7·76	6·72	5·49	6·35	5·03	4·98
	4	5·65	5·52	5·38	5·42	8·37	9·84	5·26	4·26
	9	3·41	2·70	7·07	9·16	10·24	18·64	4·54	6·33
Benzaldehyde	before	0·86		—		—		—	
	0	0·83		2·63		1·28		4·04	
	1	26·78	150·91	109·11	275·80	13·15	111·31	85·26	215·42
	2	37·10	162·99	185·93	278·76	23·36	116·33	125·38	235·35
	4	62·09	160·29	211·53	229·88	46·43	132·15	163·33	223·48
	9	64·61	94·07	125·37	209·36	73·78	166·29	134·77	216·60

[a] Isolation by dynamic headspace sampling method.
[b] Samples added 20% granulated sugar.
[c] Samples added 20% granulated sugar and 0·1% L-ascorbic acid.
[d] Samples added 20% granulated sugar and 0·5% sodium chloride.
[e] Samples added 20% granulated sugar, 0·1% L-ascorbic acid and 0·5% sodium chloride.
[f] Immediately before pressurising.
[g] Immediately after pressurising.

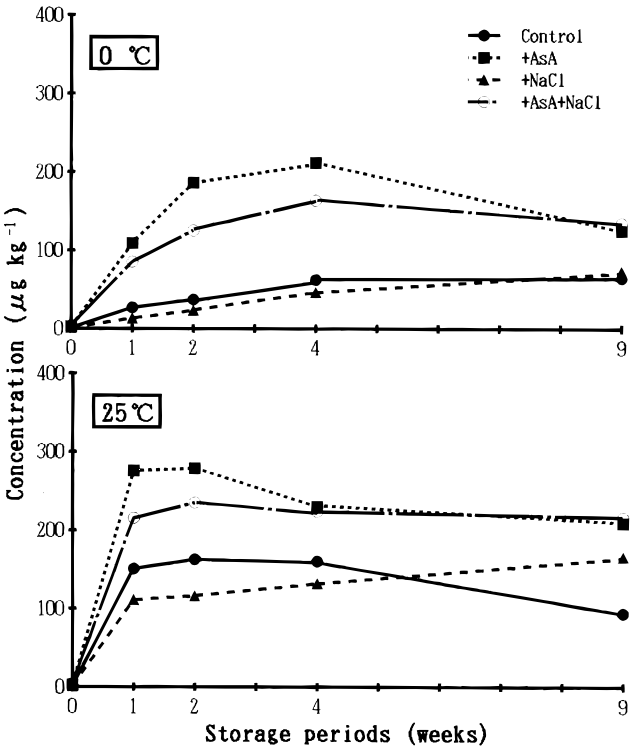

Fig 3. Concentration changes of benzaldehyde in peach homogenate samples stored at 0 and 25°C, respectively, for 9 weeks.

concentration of benzaldehyde stored at 0 and 25°C, respectively, are shown in Fig 3. The author reported previously that the increase in benzaldehyde content was probably responsible for the remaining activity of β-glucosidase and mandelonitrile lyase in high-pressure-treated peach (Sumitani *et al* 1994). The increase in concentration of benzaldehyde in samples stored at 0°C was apparently less than that stored at 25°C. It was found that the enzyme acts even at 0°C. In all samples, the concentrations of benzaldehyde rapidly increased and reached a maximum after 4 weeks storage at 0°C and 2 weeks storage at 25°C, respectively, and then decreased gradually. The increasing orders of benzaldehyde at the maximum concentration were +AsA, +AsA + NaCl, control, and +NaCl at 0 and 25°C, respectively. The concentration of benzaldehyde is higher in all cases for the 25°C samples than for the 0°C samples.

DISCUSSION

Remarkable effects by the supplemental additives (AsA and/or NaCl) were observed in the concentration changes of hexyl acetate, (*Z*)-3-hexenyl acetate, (*E*)-2-hexenyl acetate, (*E*)-2-hexenal and benzaldehyde, respectively.

The volatiles that had the increasing tendency in their concentration immediately after pressurising compare to before were hexyl acetate, (*Z*)-3-hexenyl acetate, (*E*)-2-hexenyl acetate, (*E*)-2-hexenol and hexanol. The concentration of (*E*)-2-hexenal decreased immediately after pressurising, and then increased. Other volatiles did not indicate the large difference between immediately before pressurising and immediately after pressurising. These results could not be reasonably explained. But, it could not be considered that these results were caused by the analysis technique.

At 25°C storage, the concentrations of esters rapidly decreased in the control sample, in contrast with that of the sample with AsA and/or NaCl. The behaviour of concentration change of esters in the control samples at 0°C storage is similar to that of samples of +AsA, +NaCl and +AsA + NaCl at 25°C storage. The additives were effective to maintain the concentration of esters at room temperature.

In all samples, the concentration of benzaldehyde reached maximum for 4 weeks at 0°C, and 1 week at 25°C, and then gradually decreased (Fig 3). The increasing rate of benzaldehyde is in the order of +AsA, +AsA + NaCl, control and NaCl. It seems that AsA apparently accelerates the enzyme reaction, but the mechanism of enzyme activation is not clear. Some reports have been published on the activation of myrosinase (thioglucosidase) by AsA (Nagashima and Uchiyama 1959; Björkman and Lönnerdal 1973; Ohtsuru and Hata 1979). However, as far as is known, no reports have been published on the activation of β-glucosidase by AsA. Horvat and Chapman (1990) reported that benzaldehyde would not contribute to peach aroma. The characteristic aroma of benzaldehyde was apparently perceived in pressurised-stored samples by sensory evaluation tests. The reported threshold value in water for benzaldehyde is 350 $\mu g\ kg^{-1}$ (Buttery *et al* 1969). The amount of benzaldehyde collected from pressurised-stored +AsA sample (25°C, 2 weeks) was 278·8 $\mu g\ kg^{-1}$ (Table 1). A large amount of benzaldehyde during storage in comparison with that immediately after pressurising may contribute to the enhancement of the sweet almond-like aroma of the pressurised peach homogenates.

Enzymic browning of raw fruits is caused by conversion of native phenolic substrates to quinones, which are polymerised, in turn, to produce the brown colour. Polyphenoloxidase is responsible for the browning reaction sequence. In the present study, ability of additives to inhibit browning was in the order of +AsA + NaCl, +AsA, +NaCl and control. Brown colour developed during homogenisation in the control sample. Browning changes for control samples were apparently faster and more extensive than those for samples with AsA and/or NaCl added. Many reports have been published on the prevention of enzymic browning by AsA and NaCl. Inhibition studies by reductant, carboxylic acid and halide compounds have been carried out on purified apple polyphenoloxidase (Janovitz-Klapp *et al* 1990). Electron spin resonance studies demonstrated that the Cu^{2+} of polyphenoloxidase was reduced to Cu^{+} by AsA. This is a mechanism of direct inhibition of polyphenoloxidase by AsA (Hsu *et al* 1988).

In conclusion, AsA and/or NaCl were added to homogenates before homogenisation to inhibit colour deterioration during storage after high-pressure treatment. The additives could considerably inhibit the browning during homogenisation and storage. The high concentration of benzaldehyde contributed to the enhancement of the sweet almond-like aroma of the pressurised peach homogenates. It was found that AsA has an effect to accelerate the benzaldehyde formation from the glucosides in samples. The benzaldehyde concentrations of homogenate with AsA reached the highest level of all samples. The effect of additives influenced to esters concentration was not so serious. The flavour quality and colour of the pressurised homogenates with AsA stored at lower temperature were excellent.

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