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ORIGINAL PAPER

Characterisation of Phenolic Compounds in Oils Produced from Frosted Olives

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Abstract Frost is one of the most important weather related hazards for the Australian olive industry and it has caused significant economic losses during the last decade. Its impact on oil quality was significant in 2006 with more than 20% of Australian oil of that year being affected to some degree. Early frosts will normally affect the fruit leading to significant changes in the chemical and organoleptic characteristics of the oils. The aim of this work was to study the effect of freeze damage on the phenolic composition and quality parameters of oils from three different varieties: Frantoio, Barnea and Picual. Quality chemical parameters showed significant differences in oils produced from fruit that was frozen for 2 and 4 weeks. Those chemical parameters were not significantly different in the oil produced from fruit immediately after being frosted. Nonetheless, the sensorial profile and the polyphenols showed significant changes even with oils produced within a short time after the freezing event. Those changes became more evident with the oils produced at increasing time from the moment of fruit freezing.

Keywords Lipid chemistry · Lipid analysis

Introduction

Frost is one of the most important weather related hazards for the Australian olive industry and it has caused significant economic losses during the last decade. Its impact on oil quality was significant in 2006 with more than 20% of Australian oil of that year being affected to some degree [1]. Early frosts will normally affect the fruit leading to significant changes in the chemical and organoleptic characteristics of the oils [2]. Depending on the characteristics of the frost, the damaged fruit could turn a brownish colour and remain with an aqueous consistency or they could dehydrate remaining shrivelled until harvested. Oils produced from frosted fruit develop organoleptic defects in the first instance while basic chemical quality parameters are affected significantly later after the frost event. Volatile and phenolic compounds are the substances mainly responsible for the flavour of virgin olive oils and therefore will affect the consumers' acceptance of this product [3, 4]. Studying the volatile and phenolic profile of frosted oils should enable characterisation of these oils and manage their blends more efficiently.

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Objectives

The purpose of this project was to evaluate the evolution of the chemical parameters and polyphenols profile together with sensorial analysis in the most important varieties for the Australian olive industry—Frantoio, Barnea and Picual—as a consequence of a freezing event. The study considered four treatments: before frost damage, immediately after frost damage, 2 weeks after frost damage and 4 weeks after frost damage.

This will allow description of the evolution in time before and after crushing of the most important quality parameters in (a) olive oils obtained from frosted fruit and (b) characterisation of the phenolic profile associated with those oils in order to develop an analytical system that assists both growers and trading companies to deal with this quality issue.

Methodology

The evaluation of the frost effect on oil quality as well as on chemical and organoleptic characteristics was undertaken with fruit from a commercial olive grove at Boort, Victoria (36.12°S; 143.72°E), in low areas of the property where frost damaged fruits had been observed in past years [1] Fruits from three different varieties (Picual, Barnea and Frantoio) with clearly different fatty acid, phenolic and organoleptic profiles [5] were processed in an experimental olive oil mill (Abencor®) before a frost damaging event, immediately after frost, 2 and 4 weeks after the frost occurred. All oil samples obtained were evaluated based on chemical quality parameters, organoleptic parameters and phenolic profile. Each treatment consisted of three repetitions and each sample from those repetitions was analysed in duplicate. Total samples analysed considering 3 varieties × 4 treatments × 3 repetitions × 2 analysis: 72.

Basic Quality Parameters

Determination of free fatty acids (AOCS Ca 5a-40), peroxide value (AOCS Cd 8-53), UV coefficients: K232 and K270 (AOCS Ch 5-91) were carried out (AOCS, 2003). Results were expressed as percentage of oleic acid, meq O_2/kg oil, and extinction at 232 and 270 nm, respectively.

Induction Time

Potential shelf life is expressed as induction time. This parameter was measured with a 743 Rancimat (Metrohm & Co), using an oil sample of 2.5 g warmed at 110 °C and 20 l/h air flow. The results were expressed in hours.

Total Polyphenols Content

The phenol extract was isolated using an SPE Diol column 6 ml/500 mg (Chromabond Macherey-Nagel GmbH & Co) and using an elution solution of 1:1 methanol:water. The Folin–Ciocalteu method was used to evaluate the concentration of total polyphenols in the samples at 725 nm. The results were expressed as mg/kg of caffeic acid.

Bitterness

The bitter compounds were isolated by SPE C18 column 6 ml/500 mg (Chromabond Macherey-Nagel GmbH & Co) using an elution solution of methanol:water. The extract obtained was measured at 225 nm of absorbance against methanol:water (1:1) as blank in a 1-cm quartz cuvette [6]. The results were expressed as extinction at 225 nm.

Organoleptic Analysis

Sensory analysis of the samples was carried out by trained panellists according to the method described in International Olive Council (IOC/T.20/N°15-Rev.2) [7]. The method involves, as a measuring instrument, a group of 8–12 persons suitably selected and trained to identify and evaluate the intensities of positive and negative sensory perceptions [3]. Samples were randomly presented and tasters were requested to mark their perceptions on a profile sheet and to evaluate their intensity on an unstructured scale ranked from 0 to 10. The procedure was repeated three times in different orders to minimise the error. The panellists are well trained to identify and quantify the typical organoleptic defects associated to oils produced from frosted fruit: Stewed fruit, wet hay or woody. Data provided by tasters were statistically processed to verify the reliability of the test. The median values of the defect and attributes perceived were utilised and used to identify the oil category.

HPLC Analysis of Phenolic Compounds

The phenol extract was isolated by SPE Diol column 6 ml/500 mg (Chromabond Macherey-Nagel GmbH & Co) using methanol as elution reagent. The resulting solution was evaporated under vacuum and the residue dissolved in 0.5 ml of methanol:water (1:1). The clear solution is maintained at room temperature for 4 h before being analysed by HPLC.

The HPLC system consisted of an Agilent 1100 Series Isocratic and Quaternary gradient Pump, Agilent 1100 Series Autosampler, Agilent 1100 Series Thermostated Column compartment and Agilent 1200 Series Diode Array and Multiple Wavelength Detector managed by Agilent ChemStation. The analytical column was a LiChrospher 100 RP 18, particle size of 5 μm, length of 250 mm and internal diameter of 4 mm (Macherey-Nagel GmbH & Co). HPLC analysis was performed as follow: Injection volume 20 μl, temperature of the column 30 °C, flow rate of 1 ml/min. The total run time was 1 h. The gradient used was:

Time	Solution A (%)
0	95
25	70
35	62
45	55
50	47.5
55	0
70	95

Solution A: water:phosphoric Acid (99.5:0.5)

Solution B: methanol:acetonitrile (50:50)

Chromatograms were obtained at 240, 280 and 335 nm. The internal standards were 4-hydroxyphenylacetic and *o*-coumaric acid (Sigma-Aldrich). Other reference compounds used for peak identification were vanillin, cinnamic acid, vanillic acid, mandelic acid and tyrosol (Sigma-Aldrich) and caffeic acid (TCI—Tokyo Kasei Kogyo Co). Individuals phenols were identify by comparison with commercial standards supplied and references chromatograms [8, 9]. The quantification was carried out at 280 nm.

The data was subjected to a statistical analysis of variance using the SAS version 8.02 (SAS Institute Inc., Cary, NC, USA).

Results and Discussion

Quality and Other Chemical Parameters

As shown in Table 1, quality chemical parameters (FFA, PV, K232 and K270) are not significantly different between oils obtained before and immediately after the frost event. This is in line with other similar research work [10]. Nonetheless, chemical parameter values increase in those oils processed 2 and 4 weeks from the frost event. Free fatty acid was the parameter most significantly affected by frost damage (F value of 9.098 and $P < 0.001$), which is in disagreement with other research [11].

Induction time variation is not significant immediately after the frost event but shows important variations 2 and 4 weeks later, decreasing the shelf life of the oil by almost 30%. These results are in accordance with data presented in other studies [10, 11].

Significant differences ($P < 0.001$) were found in the total polyphenols content in oil obtained 2 and 4 weeks after the frost event showing lower concentrations than in oils produced before or immediately after frost.

Similar behaviour was observed with the bitterness index ($P < 0.005$). Some studies have found that the aldehydic form of oleuropein aglycone (AOA) is responsible for the bitter attribute with high levels of correlation between the level of secoiridoid derivatives and sensory bitterness [12]. A similar level of correlation was also found in our work.

Organoleptic Analysis

Sensorial analysis was a critical parameter in this project as it was our goal to determine the influence of freeze conditions on virgin olive oil sensory attributes and relate them to their chemical composition.

It was possible to confirm that the perception of the organoleptic defects associated to oils produced from frosted olives (wet hay and/or stewed fruit) was clear (level of perception >2) even with the oils obtained immediately after the frost event. As shown in Table 1, the intensity of defect increases according to the time between the frost event and the processing of the oil, showing a high degree of significance (F value of 102.4 and $P < 0.001$).

It is important to highlight that the immediate perception of a defect after a frost event would determine a lowering of the commercial category for those oils.

As we can see in Tables 2 and 3 the correlation between the chemical parameters analysed and sensorial defect (wet hay and/or stewed fruit) was remarkable in all the varieties. The global quality index (GQI) is strongly affected by the defect with a correlation (r) of −0.99. The GQI is a complete and useful parameter to evaluate the global quality of the oils proposed by the IOC. Extra virgin olive oils have to reach values ≥6.5. Nonetheless, it is expected that fresh and well-processed oils should show a QI above 8.00 points. The quality index includes free fatty acid, peroxide value, K270 and panel test according to the following formula:

$$\mathrm{GQI} = 2.55 + 0.91 \times \mathrm{PT} - 0.78 \times \mathrm{FFA} - 7.35 \times \mathrm{K270} - 0066 \times \mathrm{PV}$$

Total polyphenols, bitterness and induction time also have a high negative correlation as the defect intensity increases.

Polyphenols Profile

Olive oil polar phenol fraction, commonly known as "polyphenols" is in fact a complex mixture of compounds with different chemical structures obtained from the oil by extraction with methanol–water [3]. The major phenolic compounds identified and quantified in olive oil belong to three different classes: simple phenols (hydroxytyrosol, tyrosol); secoiridoids derivatives (oleuropein, the aglycone of ligstroside, and their respective decarboxylated dialdehyde derivatives); and the lignans [(+)-1acetoxypinoresinol and pinoresinol]. All these three classes have potent antioxidant properties. Other important groups such as benzoic acid derivatives (vanillin and vanillic acid), flavones (luteolin and apigenin) and cinnamic acid derivatives (*p*-coumaric acid, ferulic acid, caffeic acid) have been included, too [3].

Phenolic components are related to the stability of the oil and the beneficial role in human health, due their antioxidant properties, but also have a strong relationship with the organoleptic characteristics of the oils (flavour, aroma, bitterness and pungency), which differentiates between natural or virgin oils and refined oils [3].

In Fig. 1, the chromatograms clearly show the differences between an oil produced before the frost event and

4 weeks after frost. The concentrations of most olive oil phenolic compounds were affected by the frost damage.

The main group of phenols that remarkably decreases immediately after the freeze injuries is the secoiridoid derivatives (peaks 7, 8, 10, 14 and 16). This data is in accordance with other previous research [10]. This significant decrease in the concentration of this group of phenols continues when the oil is processed 2 and 4 weeks after the frost event. The reduction of the bitterness and the shelf life of the oil are associated with this decrease as those compounds are strongly linked to those parameters. The mesocarp and epicarp tissue destruction caused by the ice crystals developing inside fruit cells would be the main factor triggering the oxidative degradation of those phenolic compounds listed above.

The simple phenols such as hydroxytyrosol and tyrosol (peaks 1 and 2, respectively), which normally increase their concentration with time, show an opposite behaviour when the oil is produced from fruits affected by frost. A significant decrease in their concentration was observed as indicated in Tables 4 and 5. Physical damage of fruits by frost is known to lead to cellular destruction which would allow phenolic substrates to mix with PPO. Consequently, the decline in the total levels of these phenolic substances could be a result of an enzymatic oxidation that is visually perceived with the browning of the olives.

Benzoic acid derivatives, particularly vanillin, were the only components that significantly increased ($P < 0.005$) as a percentage of total phenols with the frost damage. As shown in the Fig. 1 and Table 6 the vanillin concentration increases gradually according to the time between the freeze injuries and the oil processing. The biosynthesis of vanillin and vanillic acid has been extensively studied but there are some contradictions between the results of these studies, and consequently several questions about the biosynthetic pathway remain unanswered [13]. Vanillic acid and vanillin could be derived from lignan precursors or from the cinnamic acid and coumaric acid types [13]. It is important to highlight that both groups show a reduction in their content after the frost event while the vanillin and vanillic acid increase or remain stable.

Due to the significant variations found in these complex compounds between varieties, it was decided to evaluate them as groups trying to find more consistent correlations between the groups and incidence of organoleptic defects attributed to frost damage.

Table 2 also shows the correlation between the intensity of the defect and the different phenolic groups in the three varieties analysed. While secoiridoid derivatives (SD) decrease as the perception of the defect becomes stronger, benzoic acid derivatives levels increase with higher intensities of frost defect perception. These results are in agreement with other research [10]. It was possible to observe also that the lignans group have both strong correlations with the defect and with the variety. Due to these observations, the ratio between those groups SD + L/B (secoiridoids derivatives + lignans/benzoic acid derivatives, L/B) have been determined and correlated with the intensity of frost defect. The high correlation values obtained (0.85–0.96), independently of the variety, indicates that this ratio could be used to determine the degree of frost damage defect confirming the sensorial perception (Fig. 2). Table 5 shows the statistical significance of both ratios (SD/B and SD + L/B) with F values of 12.5 ($P < 0.001$) and 7.5 ($P = 0.001$), respectively.

As a consequence of this work it would be advisable to educate growers and processors focussing on the fact that even when basic chemical parameters are not immediately affected by frost, the oils can display organoleptic defects which force them into a lower category. Education should also focus on the typical evolution of such defects and chemical characteristics.

There should be also communication to processors and trading companies that the analysis of the phenolic profile of particular oils could confirm an organoleptic defect of frosted oil in order to solve a potential commercial dispute when the panel test results by themselves could be considered rather subjective.

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Appendix

See Tables 1, 2, 3, 4, 5 and 6 and Figs. 1 and 2.

Table 1 Quality and organoleptic parameters obtained from oil before, immediately after, 2 and 4 weeks after frost damage processed

	BF	AF	2 Weeks	4 Weeks	Std. error	F^{a}	Significance
FFA	0.25 a	0.29 a	0.47 b	0.72 c	0.047	9.098	0.000
PV	12.50 a	12.91 a	15.63b	18.76 c	0.822	3.586	0.018
K232	1.42 a	1.40 a	1.61 b	1.65 b	0.053	1.617	0.200
K270	0.10 a	0.09 a	0.10 a	0.12b	0.005	2.752	0.059
IND	22.09b	20.94 b	13.97a	14.68 a	1.201	3.763	0.020
PPH	83.00b	73.44 b	34.22 a	35.78 a	4.927	14.540	0.000
BIT	0.07 c	0.07 c	0.05 b	0.04 a	0.004	5.900	0.003
Defects	0.00 a	2.08 b	3.14 c	4.08 c	0.270	102.400	0.000
P.TEST	7.17 d	5.25 c	4.11 b	3.44 a	0.250	107.000	0.000
GQI	7.34 d	5.59 c	4.14 b	2.99 a	0.300	56.430	0.000

Mean sanple size = 36. Mean followed by the same roman letter within each row do not present significant differences (Duncan's multiple range test $\alpha = 0.05$)

[a] *F* tests the effect of time between frost damage and processing

Table 2 Correlations between analysis and defects per variety

	Picual	Frantoio	Barnea	Average
Parameter				
FFA	0.89	0.83	0.94	0.89
PV	0.44	0.85	0.87	0.72
K232	0.40	0.72	0.78	0.63
K270	0. 81	0.45	0.37	0.54
DK	0.55	0.06	0.54	0.38
IND	−0.89	−0.74	−0.83	−0.82
PPH	−0.96	−0.84	−0.77	−0.86
BIT	−0.92	−0.86	−0.75	−0.84
Defects	100	100	100	100
P.TEST	−0.99	−1.00	−1.00	−1.00
GQI	−0.99	−0.99	−0.99	−0.99
Groups (in ppm)				
Simple Phenols	−0.99	−0.35	−0.32	−0.56
Secoiridoid derivates	−0.89	−0.78	−0.93	−0.87
Lignans	−0.69	0.76	−0.68	−0.20
Benzoic Acid Derivates	−0.61	0.33	−0.69	−0.32
Ratio (in ppm)				
SD/B	−0.89	−0.93	−0.93	−0.92
SD/SP	0.57	−0.86	−0.65	−0.31
SD + L/B	−0.93	−0.94	−0.92	−0.93
Groups (in %)				
Simple phenols	−0.75	−0.14	−0.10	−0.33
Secoiridoid derivates	−0.77	−0.94	−0.63	−0.80
Lignans	0.91	0.72	0.76	0.80
Benzoic acid derivates	0.99	0.80	0.83	0.87
Ratio (in %)				
SD/B	−0.94	−0.91	−0.84	−0.90
SD/SP	0.58	−0.86	−0.51	−0.26
SD + L/B	−0.96	−0.93	−0.85	−0.91

SD/B Secoriridoids/benzoic derivatives, *SD/SP* secoiridoids/simple phenols, *SD + L/B* secoiridoids + lignans/benzoic derivatives

Table 3 Correlations between analysis and defects per variety

	Picual	Frantoio	Barnea	Average
Polyphenols (in ppm)				
Hydroxytyrosol	−0.99	−0.61	−0.21	−0.60
Tyrosol	−0.96	−0.20	−0.40	−0.52
Vanillic acid	0.40	−0.36	−0.95	−0.30
Vanillin	−0.88	0.96	0.97	0.35
p-Coumaric	−0.93	−0.86	−0.82	−0.87
HtyAc	−0.96	−0.89	−0.99	−0.95
DDOA	−0.88	−0.85	−0.93	−0.89
DAO	−0.93	−0.94	−0.85	−0.91
DDLA	−0.45	−0.68	−0.72	−0.61
Pinoresinol	−0.84	−0.40	−0.75	−0.66
Cinnamic acid	−0.78	−0.20	−0.82	−0.60
1-Acetoxypinoresinol	−0.23	0.78	−0.53	0.01
Luteonin	−0.93	−0.35	−0.57	−0.62
AOA	−0.94	−0.77	−0.78	−0.83
Apigenin	−0.83	−0.31	−0.92	−0.69
ALA	−0.89	−0.33	−0.96	−0.72
Totals	−0.96	−0.67	−0.90	−0.84
Polyphenols (in %)				
Hydroxytyrosol	−0.95	−0.79	−0.11	−0.62
Tyrosol	−0.28	0.44	−0.10	0.02
Vanillic acid	0.96	−0.24	0.68	0.47
Vanillin	0.67	0.77	0.86	0.77
p-Coumaric	0.31	−0.97	−0.52	−0.39
HtyAc	−1.00	−0.92	−0.72	−0.88
DDOA	−0.90	−0.94	−0.91	−0.92
DAO	−0.18	−1.00	−0.50	−0.56
DDLA	0.63	−0.84	0.25	0.01
Pinoresinol	0.90	0.22	0.76	0.63
Cinnamic acid	0.46	0.96	0.14	0.52
1-Acetoxypinoresinol	0.93	0.74	0.00	0.56
Luteonin	0.91	−0.24	−0.87	−0.07
AOA	−0.94	−0.90	−0.58	−0.81
Apigenin	0.90	−0.11	−0.01	0.26

DDOA Dialdehydic form of decarboxymethyl oleuropein aglycone, *DOA* dialdehydic form of oleuropein aglycone, *DDLA* dialdehydic form of decarboxymethyl ligstroside aglycone, *AOA* aldehydic form of oleuropein aglycone, *ALA* aldehydic form of ligstroside aglycone

Table 4 Polyphenols (in ppm) obtained from oil processed before, immediately after, 2 and 4 weeks after frost damage

	BF	AF	2 Weeks	4 Weeks	Std. error	F^a	Significance
Hydroxytyrosol	8.94 b	10.84 b	3.05 a	1.22 a	1.065	7.125	0.001
Tyrosol	16.69 c	23.48 b	10.30 a	7.32 a	1.578	8.512	0.000
Vanillic acid	1.09 b	1.04 b	1.02 b	0.76 a	0.079	0.855	0.470
Vanillin	0.72 a	0.79 b	0.93 c	0.81 d	0.049	0.776	0.520
p-Coumaric	0.68 c	0.61 c	0.43 b	0.19 a	0.054	6.023	0.002
HtyAc	22.98 c	13.29 b	3.58 a	1.58 a	2.218	7.804	0.001
DDOA	39.18c	22.48 b	3.63 a	1.62 a	3.472	13.310	0.000
DAO	0.76 d	0.46 c	0.29 b	0.18 a	0.057	7.800	0.001
DDLA	40.69 c	26.97 b	15.04 a	22.67 a	4.116	1.831	0.160
Pinoresinol	9.86 b	9.02 b	7.09 a	5.67 a	0.806	1.423	0.250
Cinnamic acid	8.51 a	10.94 a	8.04 a	5.26 a	1.399	0.674	0.570
1-Acetoxypinoresinol	2.36 a	4.64 b	2.67 a	3.33 a	0.879	0.310	0.820
Luteonin	27.22 c	34.32 d	20.33 b	9.99 a	2.650	5.198	0.005
AOA	41.31 c	33.91 b	10.63 a	5.22 a	3.168	20.420	0.000
Apigenin	18.03 c	17.04 c	12.46 b	8.13a	1.252	4.222	0.013
ALA	43.87 b	17.84 a	15.48 a	5.75 a	6.563	1.622	0.200
TOTAL	282.91 c	227.66 b	114.99 a	79.69 a	17.966	15.930	0.000

Mean sample size = 36. Means followed by the same roman letter within each row do not present significant differences (Duncan's multiple range test $\alpha = 0.05$)

HtyAc Hydroxytyrosol Acetate, *DDOA* dialdehydic form of decarboxymethyl oleuropein aglycone, *DOA* dialdehydic form of oleuropein aglycone, *DDLA* dialdehydic form of decarboxymethyl ligstroside aglycone, *AOA* aldehydic form of oleuropein aglycone, *ALA* aldehydic form of ligstroside aglycone

[a] *F* tests the effect of time between frost damage and processing

Table 5 Polyphenols groups and ratios obtained from oils processed before, immediately after, 2 and 4 weeks after frost damage

	BF	AF	2 Weeks	4 Weeks	Std. error	F^a	Significance
Groups(in ppm)							
Secoiridoid derivates	165.82c	101.66b	45.08a	35.43a	12.021	12.320	0.000
Simple phenols	25.64b	34.32c	13.35a	8.54a	2.517	9.232	0.000
Benzoic acid derivates	1.81b	1.84b	1.95b	1.57a	0.088	0.814	0.500
Lignans	12.22b	13.66b	9.77a	9.00a	1.211	0.777	0.520
Groups (in %)							
Secoiridoid derivates	29.44c	25.06b	17.30a	18.33a	1.841	2.814	0.055
Simple phenols	7.75a	10.92b	6.67a	5.50a	0.713	3.156	0.038
Benzoic acid derivates	2.57a	3.13a	5.27b	5.90b	0.430	4.597	0.009
Lignans	11.87a	12.59a	17.73b	20.04b	1.530	1.796	0.170
Ratios (%/%)							
SD/B	12.91c	9.71b	3.85a	3.08a	0.942	12.470	0.000
SD/SP	4.99b	2.53a	2.84a	3.32a	0.365	0.931	0.440
SD + L/B	17.90c	14.12b	7.55a	6.65a	1.225	7.531	0.001

Mean samples size = 36. Means followed by the same roman letter within each row do not present significant differences (Duncan's multiple range test $\alpha = 0.05$)

SD/B Secoriridoids/benzoic derivatives, *SD/SP* secoiridoids/simple phenols, *SD + L/B* secoiridoids + lignans/benzoic derivates

[a] *F* tests the effect of time between frost damage and processing

Table 6 Polyphenols (as % of total polyphenols) obtained from oil processed before, immediately after, 2 and 4 weeks after frost damage

	BF	AF	2 Weeks	4 Weeks	Std. error	F^a	Significance
Hydroxytyrosol	3.14 c	4.06 d	1.79 b	0.82 a	0.401	4.018	0.016
Tyrosol	4.60 a	6.86 b	4.88 a	4.68 a	0.426	1.694	0.190
Vanillic acid	1.20 a	1.25 a	2.13 b	2.52 b	0.248	1.854	0.160
Vanillin	1.37 a	1.89 b	3.14 c	3.38 c	0.244	5.494	0.004
p-Coumaric	1.46 b	1.61 c	1.62 c	1.22 a	0.192	0.228	0.880
HtyAc	6.46 c	4.21 b	1.81 a	0.70 a	0.664	5.048	0.006
DDOA	6.44 c	3.55 b	0.92 a	0.65 a	0.485	21.310	0.000
DAO	1.66 b	1.13 a	0.99 a	1.17a	0.163	0.776	0.520
DDLA	4.76 a	3.28 a	3.25 a	7.85 b	0.974	1.257	0.310
Pinoresinol	11.51a	12.39 a	16.88b	18.08 b	1.637	0.983	0.410
Cinnamic acid	28.77 a	29.86 a	35.20 a	34.86 a	3.920	0.168	0.920
1-Acetoxypinoresinol	0.36 a	0.20 a	0.85 a	1.96 b	0.341	1.482	0.240
Luteonin	6.96 a	7.64 a	8.32 a	6.89 a	0.710	0.208	0.890
AOA	11.46 b	11.54 b	4.98 a	3.74 a	0.877	9.883	0.000
Apigenin	4.71 a	4.97 a	6.07 b	6.56 b	0.465	0.884	0.460
ALA	5.12 a	5.55 a	7.16 b	4.92 a	0.532	0.904	0.450

Mean sample size = 36. Means followed by the same roman letter within each row do not present significant differences (Duncan's multiple range test $\alpha = 0.05$)

HtyAc Hydroxytyrosol Acetate, *DDOA* dialdehydic form of decarboxymethyl oleuropein aglycone, *DOA* dialdehydic form of oleuropein aglycone, *DDLA* dialdehydic form of decarboxymethyl ligstroside aglycone, *AOA* aldehydic form of oleuropein aglycone, *ALA* aldehydic form of ligstroside aglycone

[a] *F* tests the effect of time between frost damage and processing

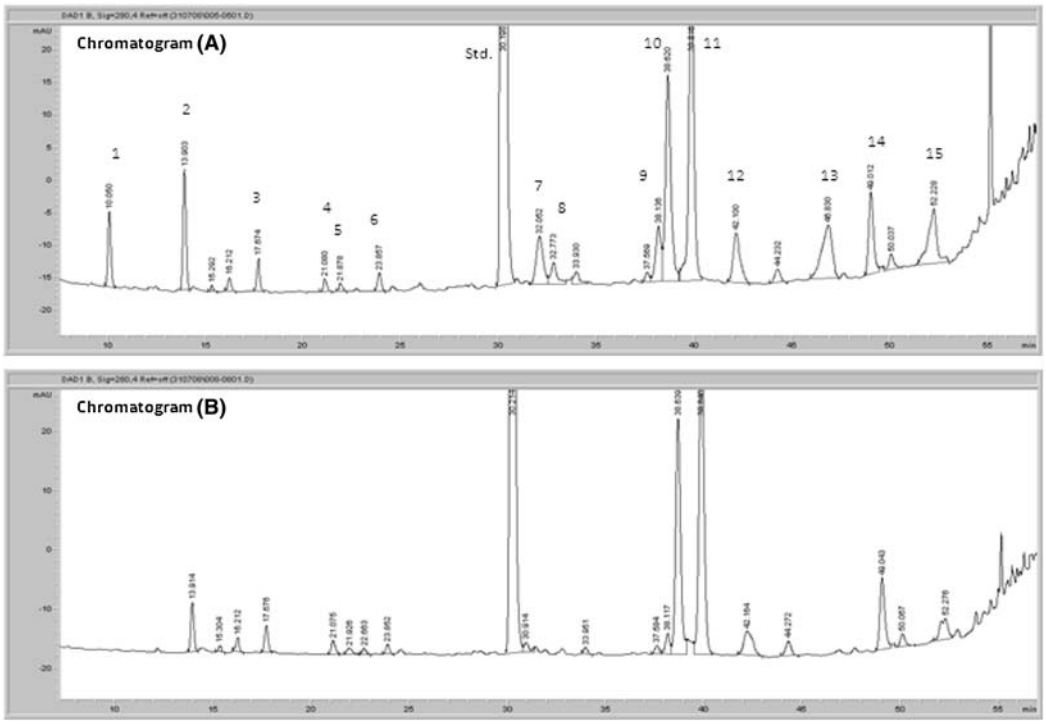

Fig. 1 HPLC chromatograms at 280 nm of phenolic extracts from before frost oil (**a**) and 4 weeks after frost (**b**). *1* Hydroxytyrosol, *2* Tyrosol, *3* Vanillic acid, *4* Vanillin, *5* *p*-coumaric acid, *6* Hydroxytyrosol acetate, *7* DDOA, *8* DAO, *9* DLA, *10* Pinoresinol, *11* Cinnamic acid, *12* Luteolin, *13* AOA, *14* Apigenin, *15* ALA

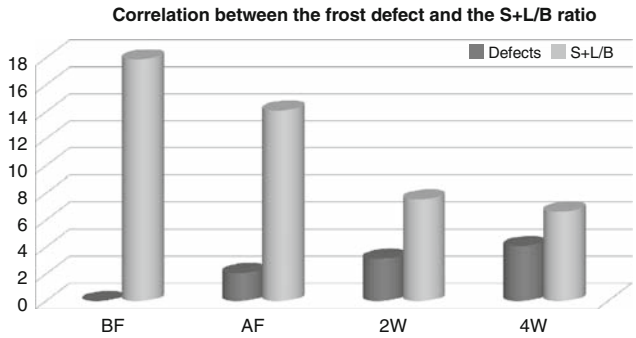

Fig. 2 Correlation between the frost defect and the S + L/B ratio

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