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Water Extracts of Cruciferous Vegetable Seeds Inhibit Enzymic Browning of Fresh-Cut Mid Ribs of Romaine Lettuce

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Abstract: Enzymatic browning, occurring on the cut surfaces of many popular fresh-cut fruit and vegetables due to wounding and the activity of endogenous polyphenyloxidase enzymes, is considered as the main reason for their rejection by consumers. In this study, water extracts were obtained from seeds of cabbage, sinapis, and wild rocket at 10 and 20% *w*/*w* seed:water ratios (SWE) and analyzed for total phenolic compounds (TPC) and antioxidant capacity (AC). The extract was then applied on cut surfaces of mid rib segments of lettuce leaves for 1 or 3 min. The segments were stored at 7 °C for 14 days. The SWE's inhibitory capacity on enzymatic browning were measured by CIELAB color coordinates L* a* and b* and expressed as second derivatives, their % inhibition and different indices. An additional visual acceptance measurement and calculation of shelf life was also performed. The seed extracts of cabbage at 10–20% and wild rocket at 20% showed the highest anti-browning efficacy (comparable to 25 mM potassium metabisulfite control) along with TPC and AC. A high % of seed:water extract and increased exposure time led to a considerable increase in shelf life, visual score, % inhibition of browning or whitening index of the extracts of all seed sources. Chromatometric outcome data clearly showed that the visual data were more accurate than the chromatometric procedure (L*, a*, b* values, their derives ΔE, h°, C, Δh° and ΔC or calculated indices), although the latter could detect the differing degrees of browning development or its inhibition in treated and control segments during storage.

Keywords: quality; color; shelf life; cabbage; wild rocket; sinapis

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1. Introduction

Fresh-cut fruit and vegetables suffer from tissue browning as a result of processing (cutting or other mechanical treatment involving breaking of cells) promoted physiological deterioration, biochemical changes and microbial degradation. It is well known that browning and its intensity in the fruit and vegetable tissues is predominately influenced by polyphenoloxidase (PPO) and secondarily by peroxidase (POD) which are wound-response enzymes that oxidize phenolic substrates into brown substances after tissue is damaged [1–3], in addition to phenylalanine ammonia lyase (PAL) which catalyzes phenylalanine into cinnamic acid, generating phenolic substrates [3,4].

The control of browning is one of the most important issues in the fresh-cut fruit and vegetable industry, since color significantly influences consumer decisions and browned foods are perceived as spoiled [5]. Anti-browning agents like sodium bisulfite, L-cysteine, 4-hexylresorcinol, glutathione and ascorbic acid have been extensively studied for the prevention of enzymatic browning [6–8]. Narváez-Cuenca [9] reported that sodium hydrogen sulfite reacts with *o*-quinones generated by PPO, and the sulfo-adducts formed terminate the browning reactions. Sulfites, used as food additives, are industrially employed to

inhibit PPO-induced browning reactions for a wide range of products [10]. However, the use of sulfites is often discouraged due to the associated side effects or toxicity [11].

The dramatic growth of the fresh-cut produce market is a result of consumer demand for fresh, healthy, convenient, and additive-free foods that are safe and nutritious [12]. Currently, other than chemical approaches to prevent the browning of fresh-cut fruit and vegetable tissues, other methods are being implemented to inhibit the activity of PPO and preserve the intrinsic quality of produce, such as physical treatment [12], mild heat treatment [13,14] and different storage temperatures or atmosphere composition [15]. The use of high CO_2 modified atmosphere storage (80% N_2, 20% CO_2) combined with appropriate wrapping films for retail packages has been successfully implemented to fresh-cut lettuce salads [16]; however, recently, a new disorder indicating tissue collapse has been reported in certain lettuce cultivars [17].

Lately, natural anti-browning preserving agents are favored by consumers since they are non-toxic and have no known adverse side effects even though some of these methods are generally constrained by their single-action or organoleptic-impairing properties. All natural anti-browning preserving agents are characterized by increased phenolic compounds content and/or antioxidant activity. Different sources of natural anti-browning agents include pineapple juice [18], green tea extract [19], whey protein concentrate [20], phytoncide essential oil derived from pine leaves [1], onion extract [21], rice bran extracts [22], allium plant [22,23], purslane extract [24], coconut [25], hawthorn leaf extract [26], clove essential oil and eugenol [27] or basil leaves and wheat bran water extracts [28], among others. Lately, diacetyl treatment repressed the browning of fresh-cut stem lettuce by regulating the phenylpropanoid metabolism pathway and antioxidant ability [29]. Decreased PPO activity and therefore extension of the shelf life of fresh-cut produce could be achieved by the integration of mild technologies with synergistic effects [30,31].

Cruciferous vegetable by-products have also been the focus of some research that investigated their potential as natural anti-browning agents in different matrices. Landi [32] reported that the PPO activity of cut rocket was much lower than that in lettuce and that cutting induced an increase in PPO activity only in lettuce. Zocca [33], using extract prepared by cooking young cruciferous leaves with water, found that it has the capacity to inhibit both commercial and grape polyphenol oxidases when applied with ascorbic acid. Broccoli seed supercritical CO_2 extract, when tested with the passive staining of apples, had a better ΔE value compared to that of sodium bisulfite reference solution [34]. Further, cruciferous and allium extracts exerted anti-browning, anti-radical and reducing capacity [35,36], which has been utilized to stabilize refrigerated avocado pulp [37].

Due to PPO-induced browning's impact on the food industry, research into potential inhibitory compounds continues. In this respect, the goal of this study was to investigate the potential of the inhibition of the enzymic browning of wild rocket, cabbage and sinapis seed extracts at two seed:water ratios using a lettuce mid rib segment as model. This approach was expected to increase our understanding on the potential anti-browning properties of the water extracts of these cruciferous seeds, since to the best of our knowledge at this time, no relevant data were reported.

2. Materials and Methods

2.1. Chemicals and Reagents

Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid), DPPH (2,2-diphenyl-picrylhydrazyl) and gallic acid were obtained from Sigma-Aldrich (St. Louis, MO, USA). ABTS (2,2′-azino-bis(3-ethylben-zothiazoline-6-sulfonic acid)) and TPTZ (2,4,6-tripyridyl-s-triazine) were purchased from TCI (Chuo-ku, Tokyo, Japan). Folin–Ciocalteu's phenol reagent, sodium carbonate, sodium acetate, potassium persulfate, ferric chloride hexahydrate, and glacial acetic acid, as well as HPLC grade methanol, were purchased from Chem-Lab (Zedelgen, Belgium).

2.2. Plant Material Handling and Storage

The romaine-type lettuce (*Lactuca sativa* L., var. longifolia Lam.) used in this study was purchased from the local market. The lettuce head outer leaves were removed and discarded, and the remaining leaves were washed with tap water and cut with a sharp stainless-steel knife to obtain the mid rib. Only the white part of the cut ribs was used, and the ribs were further cut into 1 cm segments before being subjected to treatments. To create units for treatments, 12–16 segments were placed in a Petri dish. Four Petri dish replications per each treatment were created. All dishes were stored at 7 °C for 14 days in the dark.

2.3. Seed Water Extracts

Wild sinapis, cabbage and wild rocket seeds were selected to produce water extracts as anti-browning agents of lettuce mid rib segments based on previous pre-experimentation screening. *Sinapis arvensis* seeds were collected from the Aristotle University farm; the local variety of cabbage (*Brassica oleracea*, cv. Kilkis) and wild rocket (*Diplotaxis tenuifolia* (L.) DC. cv. Celebries) were obtained from the local market.

Pre-experimentation also indicated that the maximum and intermediate level of seed:water ratio (w/v) were 20 and 10%, respectively. To obtain water extracts, seeds were weighed and washed in tap water; preparations of 5 or 10 g of seeds were combined with 50 mL deionized water, resulting in proportions of 10% and 20% (w/w), respectively. These mixtures were then homogenized using a T18 Digital Ultra TURRAX (IKA, Staufen, Germany) homogenizer at 7000 rpm for 1.5 min in a water-ice bath (5 °C). The homogenate was then filtered through a 0.5 mm plastic sieve and centrifuged at 6000× g for 20 min (at 25 °C) using a Sigma 3–15 (Sigma Laborzentrifugen GmbH, Osterode, Germany) centrifuge (to result in seed water extracts—SWE) used thereafter. Besides SWEs, two controls were also used; a distilled water control and 25 mM potassium metabisulfite (E224), resulting in 8 final treatment solutions.

2.4. Exposure Time

Lettuce mid rib segments, prepared as previously described, were grouped in random in 15–20 pieces and were submerged in SWE (four pooled batches) or control solutions for 1 or 3 min then rinsed with 100 mL of deionized water, before being placed in Petri dishes. A drop of water was added to each Petri dish to establish a uniform relative humidity environment. The Petri dishes were placed in a refrigerator at 7 °C for 14 days. The chromatometric color as well as the visual estimation of enzymatic browning were recorded at the 0, 3, 7, 10 and 14th day of storage.

2.5. Determination of SWE Phenolics and Antioxidant Capacity

The SWE, comprising four batch-replications per treatment, were produced and utilized as anti-browning treatments on lettuce mid rib segments, and were analyzed for their phenolic compound content as well as antioxidant capacity.

The phenolic compounds (PC) of SWE were determined using Folin–Ciocalteu (FC) reagent according to AOCS [38] and Scalbert [39], with some modifications. A total of 0.6 mL of SWE, or water used as a control, was mixed with Folin–Ciocalteu reagent after 3 min with 1.5 mL of Na_2CO_3 (20% w/v) and kept in a dark environment at room temperature (at 20 °C) for 60 min. The absorbance of the solution was monitored at 760 nm using a Genesys 80 UV-VIS (Thermo Fisher Scientific, Waltham, MA, USA) spectrophotometer. To the control, a 0.6 mL of distilled water was added. The phenolic compounds (PC) of SWE were determined from the linear regression equation of a standard curve ($y = 0.025x - 0.0707$, $R^2 = 0.995$), and the results were expressed as mg of gallic acid equivalent (GAE) per L of SWE.

The non-enzymatic antioxidant activity of SWE was evaluated using ferric-reducing antioxidant power (FRAP) assay by Benzie and Strain [40] with some modifications. SWE and samples, of 120 µL, were mixed with 3 mL of freshly prepared FRAP working solution;

this comprised 0.3 M acetate buffer (pH 3.6) containing 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) and 40 mM $FeCl_3$ $6H_2O$. The mixture was incubated at 37 °C for 4 min and the absorbance was measured at 593 nm using a Genesys 80 UV-VIS (Thermo Fisher Scientific, MA, USA) spectrophotometer. Trolox was used as a standard and results were expressed in mmol L^{-1}.

The antioxidant activity of the SWE samples was also determined using DPPH as a free radical according to Brand-Williams [41] and Nenadis [42]. The 0.6 mL of SWE or methanol used as control were added to 2960 µL of DPPH methanolic solution (100 µM) in a test tube. The tubes were then vortexed and kept in a dark environment at room temperature (at 20 °C) for 30 min. The absorbance of the solution was monitored at 517 nm using a Genesys 80 UV-VIS (Thermo Fisher Scientific, MA, USA) spectrophotometer. The antioxidant activity of SWE (%) values (%RSA) were determined by using the formula %RSA = [Abs515(t = 0) − Abs515(t)] × 100/Abs515(t = 0) after correction with appropriate blanks. Trolox equivalents were obtained using the linear regression equation of a calibration curve (y = 82,239x − 0.3069, R^2 = 0.995) and results were expressed in mmol L^{-1}.

$ABTS^{\bullet+}$ scavenging activity (ABTS) was evaluated according to Re [43] using 10 or 20 µL of diluted SWE (1:10, *v*/*v*, with distilled water). The degree of quenching of the $ABTS^{\bullet+}$ radical, measured as inhibition in percent (% Inh), was calculated using the formula % Inh = [Abs734(t = 0) − Abs734(t)] × 100/Abs734(t = 0) after correction with an appropriate blank [44]. Trolox equivalents were obtained using the linear regression equation of the calibration curve (y = 2.8165x − 0.1907, R^2 = 0.999) and results were expressed in mmol L^{-1}.

2.6. Chromatometric Color Measurement

The surface color of cut mid rib tissue was measured using a chromameter (Minolta CR-400, Minolta, Osaka, Japan), equipped with an 8 mm measuring head and a D65 illuminant. The instrument was calibrated with a white reference tile (L* = 97.52, a* = −5.06, b* = 3.57) prior to measurements.

Four color measurements were taken at four locations of each Petri dish; four Petri dish-replications were employed for each treatment, amounting to 16 readings per treatment at every sampling time.

The L* (0 = black, 100 = white), a* (+red, −green) and b* (+yellow, −blue) color coordinates were determined according to the CIELAB coordinate color space system [45]. For color change during storage, ΔE (total change in color) and the % inhibition of ΔL (percentage change in brightness), Δa (percentage change in green color to red), and Δb (percentage change in blue color to yellow), were calculated to provide normalized indicators for eliminating the heterogeneity among the samples [46]. The method of calculation is shown below:

$$\Delta E = \sqrt{(L*_{end} - L*_{start})^2 + (a*_{end} - a*_{start})^2 + (b*_{end} - b*_{start})^2} \quad (1)$$

$$\Delta L*\% = \frac{(L*_{end} - L*_{start})\ 100}{L*_{start}} \quad (2)$$

$$\Delta a*\% = \frac{(a*_{end} - a*_{start})\ 100}{a*_{start}} \quad (3)$$

$$\Delta b*\% = \frac{(b*_{end} - b*_{start})\ 100}{b*_{start}} \quad (4)$$

where tristimulus color values at day 0 were $L*_{start}$, $a*_{start}$ and $b*_{start}$ and the color values at sampling day were $L*_{end}$, $a*_{end}$, $b*_{end}$.

Positive values of the percentage change (0 and 100) would indicate treatment effectiveness in browning inhibition or even bleaching for values greater than 100%. Negative values would indicate ineffectiveness and even the promotion of browning.

Second derivative parameters of the L*, a*, b* model, hue angle (h°) and color saturation (C) as well as their % inhibition, Δh° (percentage change in hue angle) and ΔC (percentage change in color saturation C) were also calculated, as follows:

$$h^\circ = 180 + tan^{-1}\frac{b*}{a*} \quad (5)$$

When $a^* < 0$ and $b^* \geq 0$: $90^\circ < Hue < 180^\circ$),
When $a^* > 0$ and $b^* \geq 0$: $0^\circ < Hue < 90^\circ$)

$$C = \sqrt{a*^2 + b*^2} \quad (6)$$

$$\Delta h^\circ\% = \frac{\left(h^\circ_{end} - h*^\circ_{start}\right) \times 100}{h^\circ_{start}} \quad (7)$$

$$\Delta C = \sqrt{\left(a*_{end} - a*_{start}\right)^2 + \left(b*_{end} - b*_{start}\right)^2} \quad (8)$$

where h° and C color values at day 0 were h°_{start} and C_{start} and the color values at sampling day were h°_{end} and C_{end}.

Further, were calculated the browning index (BI), whiteness index (WI) saturation index (SI) and color index (CI), as follows [47]:

$$BI = 100\frac{x - 0.31}{0.17} \quad (9)$$

where [48]

$$x = \frac{a* + 1.75L*}{5.64L* + a* - 0.012b*} \quad (10)$$

$$WI = 100 - \sqrt{(100 - L*)^2 + a*^2 + b*^2} \quad (11)$$

$$SI = \sqrt{a*^2 + b*^2} \quad (12)$$

$$CI = 1000\,\frac{a*}{L* * b*} \quad (13)$$

2.7. Visual Evaluation of Browning and Shelf-Life

The degree of browning of the mid rib leaf cut surface was evaluated by three referees using a scale of 1 to 5, where 1 = none, 3 = moderate-limit of marketability, 5 = severe browning. The grading index is shown in Table 1 [17]. A visual score index (VSI) was obtained for each treatment through averaging the three scores (obtained by the three referees) per replication (four replications per treatment). VSI was employed as a reference for the development of browning during storage and was compared to chromatometric raw or processed data.

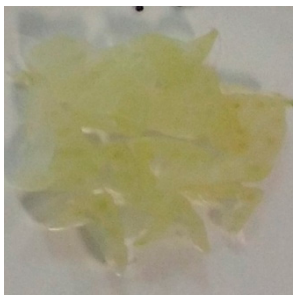
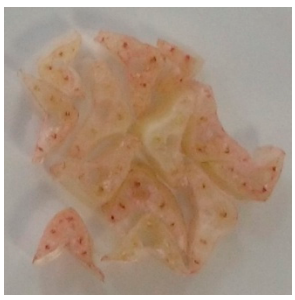
The shelf-life was defined as the total time it took a unit of segments to reach grade 3 for which browning has developed to an unacceptable level. Shelf-life was obtained from four Petri dish/replications, producing the average shelf life for each treatment based on VSI.

2.8. Statistical Analysis

The statistical analysis followed a completely randomized design. An ANOVA with four replications per treatment with 3 independent variables (water seed extract, exposure time and storage time) and 15 dependent variables (L*, a*, b*, ΔL, Δa, Δb, ΔE, h° Δh°, C, ΔC, BI, WI, SI and VSI) was employed. The effect of each factor was evaluated using partial eta squared (η^2) calculated as follows: partial eta^2 = $SS_{effect}/(SS_{effect} + SS_{error})$, where SS = sum of squares. All dependent variables were tested for normality using the Anderson–Darling test; Pearson's correlation coefficient and principal component analysis (PCA) analyses was additionally performed to indicate variable interrelations and the effective expression of

browning during storage. The mean separation of data was based on Duncan's multiple range test or Least Significant Difference (LSD) at $p < 0.05$. All analyses were performed using the SPSS statistic software for Windows (version 29).

Table 1. Visual color evaluation scores of browning and acceptability of cut mid rib lettuce leaves.

Degree of Browning Score		Browning/Acceptability
1		Absolutely acceptable No sign of browning
2		Accepted Evidence of browning beyond doubt
3		Borderline unacceptable Noticeable browning
4		Unacceptable Obvious time wear—inappropriate
5		Unacceptable Decomposition image

3. Results and Discussion

3.1. Phenolics and Antioxidant Capacity of SWE

Total phenolic compounds (TPC) of SWE were different in all samples. SWE obtained from 20% seed to water (*w*/*w*) level were higher in TPC than at the 10% level; highest TPC in all SWE were observed in cabbage (1748 mg GA L^{-1}), followed by wild rocket and sinapis (Table 2). Similar was the pattern in antioxidant capacity evaluation; cab20% showed maximum FRAP antioxidant capacity, followed by cab10% and roc20%, while in DPPH or ABTS, maximum capacity was followed by roc20% and cab10% (Table 2).

Table 2. Total phenolics and antioxidant capacity of seed:water (*w*/*w*) extracts (SWE%) of sinapis (sin10%, sin20%), wild rocket (roc10%, roc20%) or cabbage (cab10%, cab20%).

SWE	% (*w*/*w*)	TPC (mg GA/L)		FRAP (mmol TE/L)		DPPH (mg TE/L)		ABTS (mgTE/L)	
Sinapis	10%	831.3 ± 26.7	F	0.71 ± 0.02F		37.0 ± 0.1	F	639.7 ± 1.5	E
	20%	1448.0 ± 10.0	C	0.89 ± 0.00D		41.2 ± 0.0	D	771.3 ± 5.9	D
Wild rocket	10%	941.3 ± 3.3	E	0.76 ± 0.02E		39.4 ± 0.2	E	682.6 ± 29.6	E
	20%	1498.0 ± 40.0	B	1.24 ± 0.01C		50.1 ± 0.1	B	1191.5 ± 3.0	B
Cabbage	10%	1121.3 ± 16.7	D	1.54 ± 0.01B		47.2 ± 0.4	C	996.2 ± 71.0	C
	20%	1748.0 ± 10.0	A	2.25 ± 0.03A		59.2 ± 0.4	A	1647.1 ± 20.7	A
ANOVA, $p < 0.05$:		<0.0001		<0.0001		<0.0001		<0.0001	
LSD(0.05):		38.43		0.033		0.40		58.06	

Different letters within each factor represent statistically significant difference according to Duncan's multiple range test.

Broccoli seed supercritical CO_2 extract solutions (1%) have been reported to contain 13.25 mg/100 mL TPC (as caffeic acid equivalents) and 15.34 mM Trolox equivalents antioxidant capacity [34]. These values are close to the ones obtained for cabbage or wild rocket seed water extracts. Further, extracts of cruciferous as well as allium vegetables exerted anti-radical and reducing capacity [35,36] and concluded that all natural anti-browning preserving agents are characterized by an increase in phenolic compounds content and antioxidant activity.

3.2. Analysis of Variance

The analysis of variance was performed, using the seed water extract (SWE) including water and sodium metabisulfite (E224) controls, the exposure time of the model plant material (lettuce leaf mid rib segments) to SWE, as well as storage time as the main factors. These main factors are known to affect browning and shelf-life of cut produce, since the development of brown color on cut surfaces during the storage of cut mid rib lettuce segments is mainly limiting self-life [17].

Variables related to color attributes (L*, a*, b*) and their % inhibition, (ΔL, Δa, Δb) or second derivatives (h°, Δh°, C, ΔC) as well as calculated indexes (BI, WI, SI, CI) including visual score index (VSI) were predominantly influenced by the SWE or the storage time factor (Table 3); this was indicated by an increased η^2 accounted for these variables. Color variables L* and a*, ΔE, ΔL, Δa and WI were highly influenced (η^2 = 0.44–0.62) by both SWE and storage time factors, while CI was mainly influenced only by SWE and DE, DC, BI and VSI only by storage time.

SWE exerted a moderate influence (η^2 = 0.19–0.39) on C, Db, Δh°, ΔC and SI and storage time on CI (Table 3). The factor of exposure time influenced most variables very poorly (very small, though statistically significant, η^2 values were accounted for them). A significant interaction of exposure time and storage time was observed; all variables except for DE, h° and VSI were moderately influenced by this interaction (η^2 = 0.32−0.22).

Table 3. Analysis of variance for the variables L*, a*, b* hue angle (h°), saturation (C), total change in color (ΔE), % inhibition (ΔL, Δb, Δa, Δh°, ΔC) as well as indices for browning (BI), whitening (WI), saturation (SI), color (CI) and visual score (VSI) obtained during 14 day/7 °C storage of cut mid rib segments of leaf lettuce previously exposed to seed:water (*w*/*w*) extracts (SWE%) of sinapis (sin10%, sin20%), wild rocket (roc10%, roc20%) and cabbage (cab10%, cab20%), in addition to including water or bisulfite controls for 1 or 3 min.

	DF	L*	a*	b*	h°	C	ΔE	ΔL	Δa	Δb	Δh°	ΔC	BI	WI	SI	CI	VSI
									ETA SQR (η^2)								
SWE (A)	7	0.61 ***	0.53 ***	0.18 ***	0.18 ***	0.19 ***	0.18 ***	0.44 ***	0.51 ***	0.19 ***	0.20 ***	0.20 ***	0.39 ***	0.52 ***	0.19 ***	0.43 ***	0.27 ***
EXPOSURE (B)	1	0.05 ***	0.01 *	0.01 **	0.02 ns	0.01 *	0.01ns	0.01 ***	0.02ns	0.00 ***	0.02 ns	0.00 ns	0.03 ns	0.05 ***	0.01 **	0.00 ns	0.02 ***
STORAGE (C)	2	0.51 ***	0.46 ***	0.14 ***	0.13 ***	0.08 ***	0.47 ***	0.52 ***	0.43 ***	0.11 ***	0.15 ***	0.49 ***	0.51 ***	0.46 ***	0.08 ***	0.31 ***	0.75 ***
A X B	4	0.18 ***	0.01ns	0.08 ***	0.06 *	0.06 ***	0.03 ***	0.11 **	0.02 ns	0.01 ***	0.06 ns	0.03 ns	0.14 ***	0.17 ***	0.06 **	0.00ns	0.04 ***
A X C	7	0.03 ns	0.01 ns	0.01 ns	0.01 ns	0.01 ns	0.28 ns	0.01 ns	0.01 ns	0.00 ns	0.02 ns	0.01 ns	0.01 ***	0.00 ns	0.01 ns	0.00 ns	0.01 ***
B X C	14	0.28 ***	0.31 ***	0.27 ***	0.10 ***	0.29 ***	0.02 ***	0.28 ***	0.29 ***	0.20 ***	0.11 ***	0.22 ***	0.29 ***	0.22 ***	0.29 ***	0.29 ***	0.13 ***
A X B X C	28	0.11 ***	0.02 ns	0.03 **	0.07 ns	0.0 3ns	0.16 ***	0.11 ns	0.01 ns	0.02 ***	0.08ns	0.04 ns	0.09 ***	0.09 **	0.03 ns	0.02 ns	0.03 ***
MAIN EFFECTS									MEANS								
SWE (*w*/*w*)	Water	46.52E	−0.71A	16.55E	92.0C	17.0F	8.08A	−12.44E	−91.4D	1.24B	−20.1C	5.93A	42.4BC	43.8EE	170.0F	−154A	2.73C
	E224	55.36A	−7.27E	17.98CD	112.1A	19.4CD	3.67D	0.02A	28.2A	11.4A	−13.5A	2.76E	27.4E	51.3A	19.4CD	−2381C	2.20D
	Sin10%	51.76B	−0.88A	17.76D	93.6C	18.6E	7.36A	−9.41D	−90.8D	13.9A	−14.8B	5.25AB	40.1C	48.3B	18.5E	−215AB	2.82BC
	Sin20%	47.75D	−1.81B	18.39CD	95.2C	19.0DE	6.83AB	−9.75D	−81.8D	−6.1C	−15.3B	5.23AB	44.7B	44.3CDE	19.0DE	−616B	3.14A
	Roc10%	47.2DE	−1.26AB	19.45A	105.0B	20.1AB	8.15A	−9.28D	−88.5D	4.3B	−6.0A	5.92A	51.0A	43.5E	20.1AB	−382AB	2.93B
	Roc20%	52.21B	−6.11D	18.51BC	108.5AB	19.5BCD	4.46CD	−4.13B	−64.2A	13.4A	−3.0A	3.27DE	33.2D	48.3B	19.5BCD	−2187C	2.15D
	Cab%	48.86C	−5.51CD	19.38A	106.1B	20.3A	5.55BC	−6.91C	−20.7B	14.6A	−5.1A	3.89CD	40.9C	45.0CDE	20.3A	−2177C	2.15
	Cab%	48.89C	−5.11C	19.08AB	105.1B	19.9ABC	8.20A	−6.82C	−34.4C	3.3B	−6.0A	4.33BC	40.5C	45.1C	20.0ABC	−1980C	2.09D
EXPOSURE (min)	1	49.28B	−3.35A	18.64A	99.9B	19.5A	6.23B	−7.24A	−57.3B	6.8A	−11.0B	4.67A	41.6A	45.6B	19.5A	−1218A	2.62A
	3	50.36A	−3.82B	18.14B	104.5A	19.0B	6.85A	−7.44A	−45.5A	7.1A	−6.9A	4.47A	38.5B	46.8A	19.0B	−1305A	2.43B
STORAGE (days)	0	53.78A	−7.17E	17.41C	112.2A	18.8C	5.51B	0.00A	0.0A	0.0D	0.0A	3.75B	27.3E	50.0A	18.8C	−2406D	1.00E
	3	50.88B	−4.95D	17.72C	105.5B	18.5C	4.90CB	−5.34B	−29.3B	3.2CD	−6.0B	3.53BC	33.9D	47.5B	18.5C	−1751C	1.57D
	7	48.91C	−2.73C	18.38B	98.2C	19.0BC	4.29C	−9.02C	−63.9C	6.7BC	−12.5C	3.10C	41.7C	45.4C	19.0CB	−986B	2.65C
	12	48.05D	−1.86B	18.86B	98.3C	19.5B	4.75CB	−10.62D	−76.7D	10.5B	−12.5C	3.59BC	46.3B	44.4D	19.5B	−643A	3.32B
	14	47.47D	−1.19A	19.58A	96.8C	20.2A	1.32A	−11.73E	−87.2E	14.5A	−13.9C	8.89A	50.9A	43.6E	20.2A	−522A	4.09A

*, **, ***: significance levels of 0.05, 0.01 and 0.001, respectively. ns: not statistically significant. Different letters within each factor represent statistically significant difference according to Duncan's multiple range test.

The main effects of SWE treatment including water and E224 controls showed the greatest overall average differences among them in most color variables except for b*. Roc10% and sin20% had increased browning and variable values that were similar or close to water-control while roc20% and cab10% exhibited little browning and variable values that were similar or close to the E224-control (Table 3). Bustos [37] reported that crucifer vegetables, such as cauliflower and Brussels sprout extracts were highly effective as PPO inhibitors, extending the shelf life of refrigerated avocado pulp by up to two weeks. Further, Wessels [34] reported the partial inhibition of the browning of treated fresh-cut apples using 1% broccoli seed extract among many different plant sources. It is well known that cruciferous vegetables contain organosulfur glucosinolates [49,50], to which is attributed the effective inhibition of PPO. However, the precise mechanisms for the enzymatic anti-browning effects have not been yet fully elucidated.

Exposure for 3 min was more effective than 1 min for all treatments as shown by increased L*, a*, Δa, ΔE, ΔL and WI and lower C, BI and VSI. The exposure time of cut lettuce mid ribs with both the extracts and control solutions had a positive effect on browning inhibition due to the increased probability of the interaction of molecules remaining in physical motion in a common space. It thus appears that 1 min was not sufficient time for successful inhibition of browning. Therefore, the longer exposure (of 3 min) of cut segments to SWE resulted in significant inhibition as expressed by most variables (Table 4).

Storage time factor decreased variables L*, h°, ΔE, Δa, or WI and increased a*, C, ΔL, Δa, Δh°, ΔC, BI, SI, CI and VSI. Enzymatic browning is a time-evolving catalytic reaction and studies focusing on its kinetic characteristics are often conducted, e.g., in evaluating eggplant polyphenyloxidase enzymatic activity [51]. The time dependence of the effect is justified and explains why the duration of exposure accumulated the largest percentage of variability in the analysis of variance of the overall experiment (Table 3).

Table 4. Pearson linear correlation coefficients of the variables L*, a*, b* hue angle (h°), saturation (C), total change in color (ΔE), % inhibition (ΔL, Δb, Δa, Δh°, ΔC) as well as indices for browning (BI), whitening (WI), saturation (SI), color (CI) and visual score (VSI).

	L*	a*	b*	h°	C	ΔE	ΔL	Δa	Δb	Δh°	ΔC	BI	WI	SI	CI	VSI
L*	--															
a*	−0.793 **	--														
b*	−0.197	0.024	--													
h°	−0.504 **	0.647 **	−0.244	--												
C	−0.027	−0.186	0.960 **	−0.111	--											
ΔE	−0.392 **	0.478 **	−0.381 **	0.048	−0.480 **	--										
ΔL	0.905 **	−0.924 **	−0.191	−0.589 **	0.033	−0.399 **	--									
Δa	0.795 **	−0.991 **	−0.075	−0.636 **	0.122	−0.482 **	0.916 **	--								
Δb	0.113	0.045	0.708 **	−0.114	0.713 **	0.415 **	−0.087	0.036	--							
Δh°	−0.824 **	0.841 **	−0.050	0.440 **	−0.240	0.473 **	−0.842 **	−0.844 **	−0.124	--						
ΔC	−0.521 **	−0.505 **	−0.260	−0.270 **	0.378 **	0.961 **	0.448 **	−0.514 **	0.458 **	−0.533 *	--					
BI	−0.868 **	0.805 **	0.415 **	0.644 **	0.221	0.176	−0.852 **	−0.819 **	0.153	0.767 **	0.296 *	--				
WI	−0.597 **	0.111	0.273*	0.003	0.177	−0.135	−0.265 *	−0.136	0.347	0.341 *	−0.360 *	0.397 **	--			
SI	−0.021	−0.187	0.752 **	−0.114	0.800 **	0.500 **	0.080	0.127	0.542 **	0.159	0.797 **	0.363 *	−0.183	--		
CI	0.202	0.224	−0.198	−0.290 *	−0.196	−0.121	−0.129	−0.172	0.455 **	−0.083	−0.301 *	−0.006	0.838 **	−0.153		
VSI	−0.678 **	0.811 **	0.318*	0.258 **	0.131	0.154	−0.803 **	−0.804 **	0.242	0.746 **	0.207	0.833 **	0.147 **	0.184	0.191	--

* Correlation is significant at the 0.05 level, ** Correlation is significant at the 0.01 level.

3.3. Pearson Linear Correlation and Principal Component Analysis

The processing of collected data was given the possibility to identify and focus on the chromatometric variable(s) which were meaningful while ignoring others that do not render the enzymic browning as consumers perceive it. For this reason, Pearson's linear correlation coefficient was used; of particular interest was the correlation of the chromatometric variables obtained objectively with the organoleptic variable VSI, which results from a subjective visual evaluation. Table 4 shows the analysis of Pearson coefficients; significant strong to weak correlations have been shown among color variables (L*, a*, b*), their % inhibition (ΔLab) or second derivatives and their % inhibition (h°, Δh°, C, ΔC), as well as calculated indexes (BI) and VSI. VSI, a variable based on visual scoring, strongly correlated with a*, ΔL, Δa, Δh° and BI or a medium with L*. Both correlations were statistically significant ($p < 0.05$).

Variables with high eta^2 were discriminated by principal component analysis (PCA) analysis into two separate groups (Figure 1); group one with VSI, BI and color attribute a* and group two with L*, ΔL and Δa values including a variable Δh° with medium eta^2 values for factors treatment (SWE) and storage time (Table 3). The remaining variables were either grouped differently or remained scattered (Figure 1).

Based on the eta^2 values (Table 3), the Pearson coefficient (Table 4) and PCA (Figure 1), two groups of variables were selected to express browning as an interaction of SWE treatments with storage time: VSI, BI and ΔL, Δa and Δh°.

Further, the PCA of SWEs in day 0 and day 14 of storage revealed two distinct groups within day 14 (Figure 2): the E224-control group which included treatments cab10%, cab20%, roc20% and E224-control, and the water-control group which included sin10%, sin20%, roc10% and water-control). Sin10% was somehow in the border of the two groups, and depending on the variable examined, it occasionally might participate in either the E224-control or the water-control group.

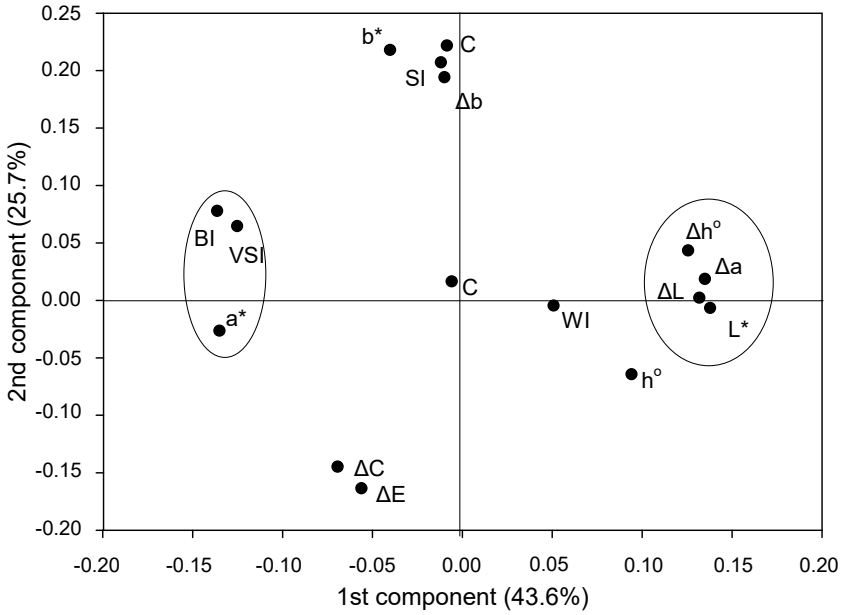


Figure 1. PCA analysis of the variables L*, a*, b* hue angle (h°), total change in color (ΔE), saturation (C), % inhibition (ΔL, Δb, Δa, Δh°, ΔC) as well as indices for browning (BI), whitening (WI), saturation (SI), color (CI) and visual score (VSI).

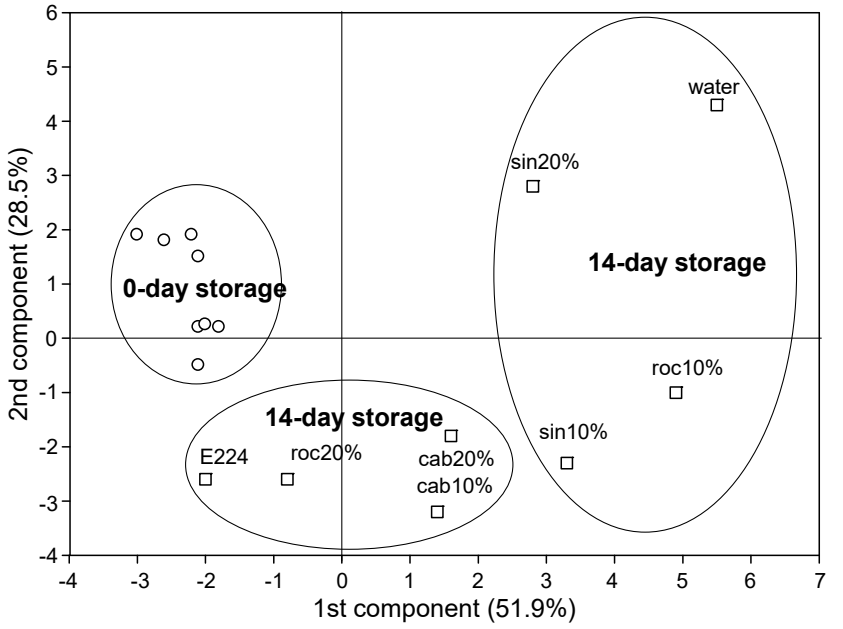


Figure 2. PCA analysis of extracts (SWE) of sinapis (sin10%, sin20%), rocket (roc10%, roc20%) and cabbage (cab10%, cab20%), water or E224 at day 0 and day 14 of storage at 7 °C of cut mid rib segments of leaf lettuce.

3.4. VSI and Browning Index

Figure 3A shows the development of browning, expressed by the VSI, in cut mid rib segments of leaf lettuce for the controls and each SWE during storage for 14 days at 7 °C. The browning of E224 preservative solution-control remained stable until the third day, whereas it increased in water-control or other SWE treatments. This increase continued until the end of the period of storage, but this was lower in E224 compared to the water-control. The differences between E224 and water-controls were found to be statistically significant ($p < 0.05$). The cab10%, cab20% and roc20% group showed a similar pattern

of development during storage to that of the E224-control, while the sin10%, sin20% and roc10% group showed a similar pattern to that of the water-control; the VSI increased above the 3 points of the acceptable sales limit between days 10–12 and 4–6 of storage for the two groups, respectively. The demand for fresh-cut lettuce, among other vegetables, reflects the consumer preferences towards appearance and freshness at the time of purchase as among the main quality criteria [52]. However, the browning of cut edges of romaine lettuce leaves is considered as the most important disorder that limits its shelf life [53]. In this study, the treatment group of cab10%, cab20% and roc20% on fresh-cut lettuce retained the quality for the first 10 days of storage compared to the E224-control.

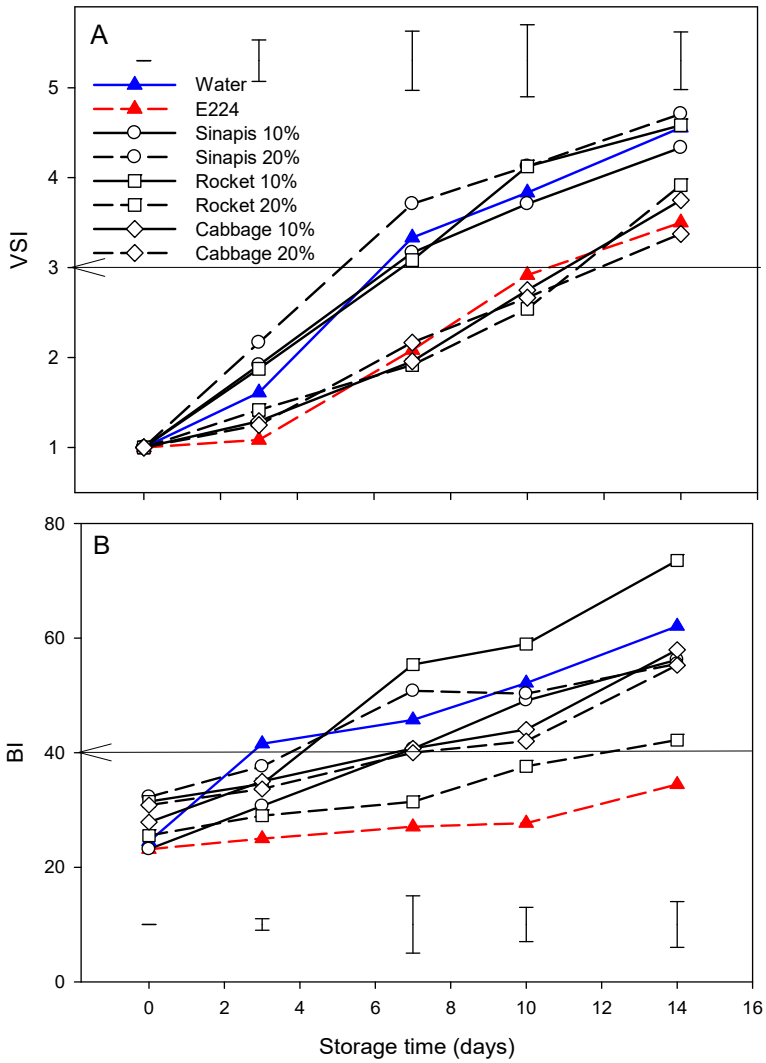


Figure 3. Development of browning based on visual score-VSI (**A**) and browning index-BI (**B**) of cut mid rib segments of leaf lettuce during storage for 14 days at 7 °C, previously exposed to seed:water (*w*/*w*) extracts (SWE) of sinapis (sin10%, sin20%), wild rocket (roc10%, roc20%) and cabbage (cab10%, cab20%), water or bisulfite for 3 min. Vertical bars in each figure represent Least Significant Difference method (LSD) at each sampling date and confidence level of 0.05. Arrows indicate borderline of acceptable/unacceptable color.

A similar pattern of browning development in cut mid rib segments of leaf lettuce for SWE during storage for 14 days at 7 °C was also shown for variables BI (Figure 3B). The correlation of VSI with BI (R^2 = 0.833, $p < 0.05$) indicated that the acceptable sales limit (VSI score point 3) corresponded to a value of 40. The BI was more sensitive than VSI; for BI (Figure 3B), the SWE treatments that formed a group with the water-control,

roc10% and sin 20% increased above the acceptable sales limit as early as day 3, sin10%, cab10% and cab20% at day 14, and roc20% as late as day 14, while the E224 preservative solution-control did not reach a critical level even by day 14th. E224 (sodium hydrogen sulfite) is considered as an anti-browning effective agent since it reacts with o-quinones yielding non-browning sulfo-adducts [54]. Although sulfites have been used in the food industry to inhibit PPO-induced browning of cut produce for a wide range of products [10], its use is associated with side effects or toxicity [11].

The decreased BI value of lettuce cut segments was concomitant with the increments of Δa and ΔL values during storage. BI has been used to describe the browning development of fresh-cut lotus roots treated with ultrasound and/or cysteine following storage at 4 °C for 12 days [55] or fresh-cut carrots with carvacrol-loaded chitosan nanoparticles [56] and cut green beans [57].

3.5. ΔL and Δa and Δh° Values

The lightness % inhibition (ΔL) of fresh-cut lettuce samples in all treatments decreased during storage (Figure 4A). This decrease, however, was lower in the E244-control and roc20% than the other treatments or the water-control and this difference was statistically significant ($p < 0.05$). Both lightness and its % inhibition (ΔL) has been used by researchers as an indicator of vegetable deterioration [58]. In this study, a decrease in the ΔL values of fresh-cut lettuce in all SWE treatments was observed during storage and this was correlated with a decrease in BI or VSI (Table 4).

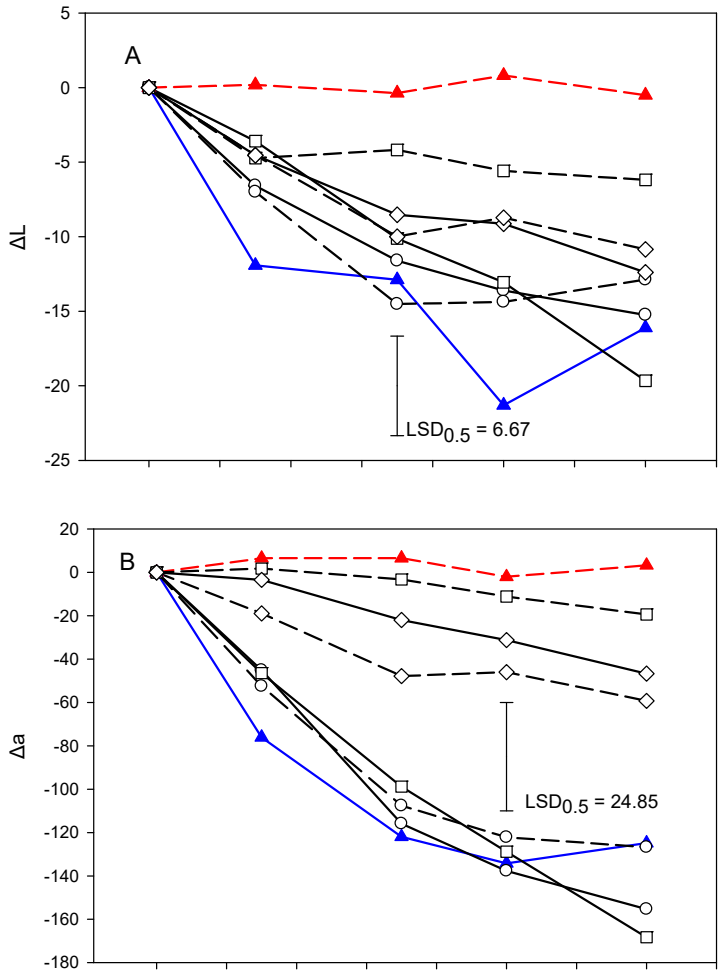


Figure 4. *Cont.*

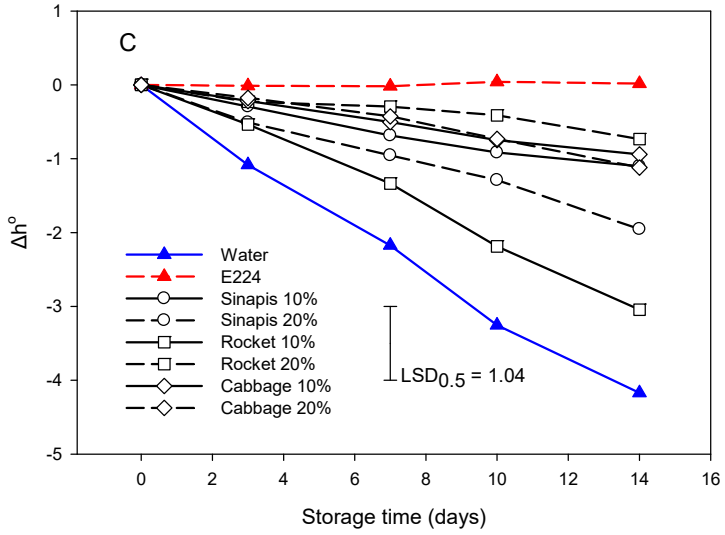


Figure 4. Development of browning based on ΔL (**A**), Δa (**B**) and Δh° (**C**) of cut mid rib segments of leaf lettuce during storage for 14 days at 7 °C, previously exposed to seed:water (*w*/*w*) extracts (SWE) of sinapis (sin10%, sin20%), wild rocket (roc10%, roc20%) and cabbage (cab10%, cab20%), water or bisulfite for 3 min. Mean comparison was performed using the Least Significant Difference method (LSD), at a confidence level of 0.05.

The Δa values (Figure 4B) of fresh-cut lettuce were increased during storage following a pattern similar to VSI in two distinct groups (the E224 group: cab10%, cab20%, roc20% and E224-control, and the water-control group: sin10%, sin20%, roc10% and water-control) to show statistically significant differences at the end of storage ($p < 0.05$). Rocculi [59] used only L* and a to evaluate browning in potato pieces by choosing the parameters they used. Lante [60] used only the total color change, ΔE, of the L*a*b color model to evaluate the effectiveness of UV radiation in the preservation of cut apple and pear varieties.

The pattern for the evolution of SWE treatments was similar in regard to Δh° values during storage (Figure 4C). However, sin10% was grouped with the E224-control group. Hue value reduction indicated that the color change in cut lettuce segments was moved to more red tones.

The SWE treatments and the E224-control group (cab10%, cab20% and roc 20%) could not ameliorate the color attributes of lettuce cut segments during storage. However, it maintained a WI value and delayed the increments of both BI, Δa, and ΔL values compared to the water-control group (sin10%, sin20% and roc10%).

3.6. Shelf-Life

Shelf-life was calculated as the time that VSI or BI (following the correlation of VSI with BI) for browning crossed the threshold of 3 and 40, respectively (Figure 3A,B). Shelf-life based on Δa, Δh° and ΔL were similar to BI. The SWE that showed the highest performance were the E224-control group which included cab10%, cab20% and roc20% with 11.5, 11.3 and 10.4 days of effective acceptable browning levels based on VSI and 9, 8.5 and 11.5 days based on BI, respectively. It should be noted that the shelf-life for the preservative-control was about 10 and 14 days based on VSI and VI, respectively (Figure 5).

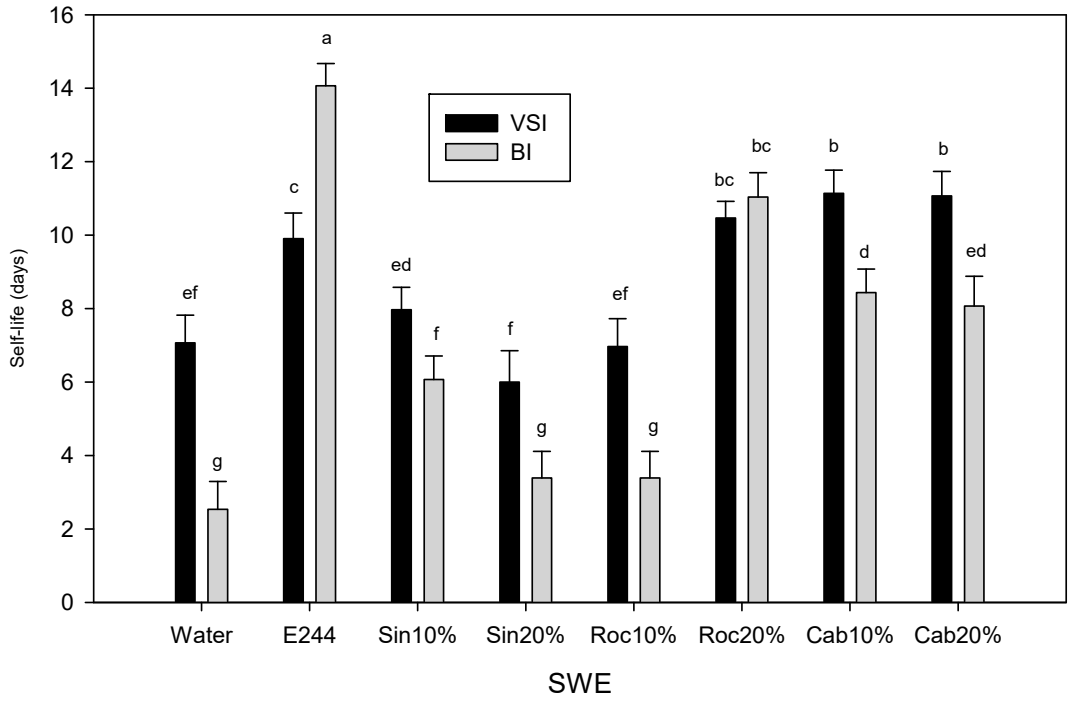


Figure 5. Shelf life (average of 4 replications ± S.D.) of cut mid rib segments of leaf lettuce previously exposed to seed:water (*w*/*w*) extracts (SWE) of sinapis (sin10%, sin20%), rocket (roc10%, roc20%) and cabbage (cab10%, cab20%), water or bisulfite for 3 min during storage for 14 day at 7 °C based on visual score (VSI) and browning index (BI). Different letters on VSI or BI bars represent a statistically significant difference according to Least Significant Difference method at a confidence level of 0.05 ($LSD_{0.05}$ = 1.1546).

All SWE possessing an anti-browning preserving capacity were characterized by increased phenolic compounds content and antioxidant activity (Table 1). The TPC of SWE correlated with the reducing antioxidant capacity content (R^2 = 0.72–0.839, $p < 0.01$) (Table 5). All SWEs had similar polyphenol content, although lettuce mid ribs treated with these extracts showed differences in browning rate delay and therefore in shelf life (Figure 5). Weak and statistically non-significant correlations were observed between the TPC content of SWE and the shelf life based on VSI or BI (Table 5), indicating that phenolic compounds of SWE may contribute only partially to their anti-browning properties. However, strong and statistically significant correlations were observed between the antioxidant activity (DPPH, ABTS and FRAP) of SWE and shelf life based on VSI (R^2 = 0.751–0.766, $p < 0.01$) or BI (R^2 = 0.545–0.638), $p < 0.01$) (Table 5). Rojas-Graü [54] reported that natural sulfur compounds may delay the browning of fresh-cut fruit. Cabbage and wild rocket SWE anti-browning properties might be attributed to glucosinolates, which are natural sulfur- and nitrogen-containing compounds reported to be found in cruciferous vegetables [33,34,61,62]. Further experimentation and analysis of the phenolic and glucosinolate profile of SWE is needed to establish the effects of either or both groups of phytochemicals on the enzymatic browning of fresh cut lettuce or other produce.

Table 5. Pearson linear correlation coefficients of the variables TPC, DPPH, ABTS, FRAP, as well as shelf life based on VSI and BI.

	TPC	DPPH	ABTS	FRAP	SELF $LIFE_{VSI}$	SELF $LIFE_{BI}$
TPC	1					
DPPH	0.839 **	1				
ABTS	0.838 **	0.993 **	1			
FRAP	0.727 **	0.958 **	0.946 **	1		
SELF $LIFE_{VSI}$	0.378	0.760 **	0.751 **	0.766 **	1	
SELF $LIFE_{BI}$	0.386	0.638 **	0.633 **	0.545 *	0.900 **	1

* Correlation is significant at the 0.05 level, ** Correlation is significant at the 0.01 level.

4. Conclusions

This study examined the effect of a water extract of cruciferous seeds to discover whether it possesses a comparable inhibitory activity to a classical sulfate preservatives reference solution when used in a dipping system. With the experimental conditions being as close as possible to an industrial application, the results of the study indicated that the cruciferous seeds under study contain ingredients that inhibit the action of polyphenoloxidase on the cut surfaces of fresh mid rib lettuce segments. Water seed extraction is an efficient method for extracting the above components, and dipping lettuce cut mid rib segments for 3 min in the resulting extract could exert an anti-browning effect.

The independent variables used in our experimental set-up (L*, a*, b*, ΔL, Δa, Δb, ΔE, h° Δh°, C, ΔC, BI, WI, SI, CI and VSI) did not serve well in their role as indicators of developing browning in lettuce mid rib pieces equally. Cabbage or wild rocket seed water extracts had the highest TPC and AC among SWEs and exhibited the highest inhibitory capacity of the browning rate, which was equivalent to 25 mM potassium metabisulfite solution. Sinapis seed extracts had little or no blocking ability.

The research carried out demonstrated the contribution of the constituents contained in the cruciferous seeds to the inhibition of browning reactions in cut mid rib of lettuce leaves. However, the potential anti-browning properties of the water extracts of cabbage, wild rocket or even sinapis seeds among other cruciferous seeds need to be attributed to specific compounds in future studies.

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