Relationship between Quality Parameters and the Overall Appearance in Lettuce during Storage

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Abstract: Fresh-cut and whole iceberg lettuce were stored in refrigerated condition and the main sensory (overall appearance: OA, browning index: BI), physical (colour variation: *ΔE**), chemical (respiration activity: RA, ammonia content: A) and biochemical (total chlorophyll: TC, antioxidant activity: AA, total phenols: TP, *o*-quinones*: o*-q, polyphenol oxidase: PPO, phenylalanine ammonia-lyase: PAL and peroxidase: POD) traits were followed during the trial. Significant relationships among these parameters were assessed by principal component analysis (PCA) in order to find the main traits related to OA, in fresh-cut lettuce. Results from PCA showed that OA was inversely correlated with BI (r=-1.00), *ΔE** (r= -0.98), A (r= -0.94), AA (r= -0.86), TP (r= -0.82), *o*-q (r= -0.89), and PAL (r= -0.80). Moreover, OA was positively related with RA (r= 1.00) and PPO activity (r= 0.96). Ammonia resulted significantly correlated with the main sensory, physical, chemical and biochemical parameters. Thus, this parameter, with *ΔE** could be used as indicator of the product quality and acceptability in control quality procedures. In conclusion, rating scale used to estimate OA, resulted a valid method to evaluate the iceberg lettuce quality, since it showed significant relationships with the main analytical parameters.

Keywords: Browning phenomena*, Lactuca sativa* L., Principal component analysis, Rating scale**.**

INTRODUCTION

The assessment of marketability loss in fresh lettuce is generally, carried out by the sensory evaluation of the relative overall appearance (OA), since this aspect has a key role in consumer acceptability [1]. Quality rating methods, have been a popular approach for estimating the OA of fresh fruits and vegetables [1, 2]; five-point rating quality scale (from 5: excellent, to 1: extremely poor) was used by several Authors to estimate the OA of vegetables [3] and fruits [4-6]. Since, this method is dependent on subjective judgments and in the aim to improve the repeatability of a sensory rating scales, the images of each score could be associated with different physical and chemical parameters [7], also by multivariate analysis technique, such as principal component analysis (PCA).

Iceberg (*Lactuca sativa* L.), is one of the most popular lettuce worldwide cultivated and diffused as whole and fresh-cut products. Preparation of fresh-cut lettuce (*i.e*., cutting, shredding) causes disruption of cells, which induces browning, degreening, texture breakdown, and off-flavour formation due to increased respiration activity, water loss, enzymatic activities and/or microbiological contaminations [8, 9].

After cutting, two biochemical processes mainly affected the OA: the increase of phenylalanine ammonialyase (PAL, EC 4.3.1.5) activity with the enhancing of phenylpropanoid metabolism, which results in phenolics accumulation, and in the subsequent exposure of the phenolic substrates to polyphenol oxidase (PPO, EC 1.14.18.1). Polyphenol oxidase catalyzes both hydroxylation of monophenols to *o*-diphenols and oxidation of colourless *o*-diphenols to *o*-quinones (*o*-q) [10] then, the *o*-q condenses spontaneously to give brown, red, or dark pigments in injured vegetable tissues [11, 12]. Even peroxidase (POD, EC 1.11.1.7) was considered to be involved in browning mechanism by oxidation of phenols to quinones in the presence of hydrogen peroxide [13]. Besides, the enzymeetic browning above reported, darkening of vegetable tissues could be also attributable to the interacttions between phenolics and heavy metals especially iron [14]. However, for lettuce and also other kinds of fruits or vegetables, no precise relationship was reported for browning potential, PPO activity, and total or individual phenol accumulation or degradation [15-17].

Starting from these findings, the aim of this work was to investigate significant relationships among sensory, physical, chemical and biochemical parameters followed during lettuce cold storage, in order to assess the traits main related to OA loss and to standardize the sensory evaluation.

MATERIALS AND METHODS

Plant Material and Processing

Iceberg (*Lactuca sativa* L.) was provided by a farm (Ortomad srl) located in Pontecagnano (Salerno, Italy),

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and transported in cold condition to the Postharvest Laboratory of the Institute of Sciences of Food Production. Plants were selected, in order to avoid damaged samples, washed in chlorinate water (100 mg L^{-1}) and drained. Then, fifteen plants were cut (Robot-cup CL 52, Vincennes, France) in pieces (approx. 5 cm); whereas other fifteen were stored as whole items. Whole or fresh-cut lettuce, one head or 300 g for each replicate respectively, were put in polyethylene bags in triplicates, for a total of 15 bags (3 replicates x 5 visual quality score) for each typology (whole or fresh-cut) and stored at 4°C ± 0.5. All items were analysed during storage: after 0, 6, 8, 10 and 16 days for fresh-cut lettuce and after 0, 17, 34, 50 and 71 days for whole heads. During storage, the main sensory (overall appearance: OA and browning index: BI), physical (colour: *ΔE**, respiration activity: RA), chemical (ammonia: A and total chlorophyll content: TC) and biochemical parameters (antioxidant activity: AA, total phenols: TP content and *o*-quinononones: *o*-q, peroxidase: POD, polyphenols oxidase: PPO and phenylalanine ammonia-lyase: PAL assays) were assessed as reported below.

Reagents and Standards

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical and 6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid (Trolox) used for the antioxidant activity, were purchased from Sigma-Aldrich (St. Louis, MO, USA), Folin–Ciocalteu's phenol reagent was purchased from Merck (Germany). Polyvinylpyrrolidone (PVP) and all reagents utilized for enzyme extraction were supplied by Sigma-Aldrich (St. Louis, MO, USA).

Sensory Parameters: Overall Appearance and Browning

A selected group of 10 assessors (made up of 5 females and 5 males, aged between 25 and 55 years old), was trained in three sessions to describe the attributes of lettuce. All evaluation sessions were held in the laboratory of the Institute of Sciences of Food Production. Periodically, samples were evaluated for OA, using subjective rating scales, with 5 = excellent, no defects; 4 = very good, minor defects; 3 = fair, moderate defects (limit of marketability); 2 = poor, major defects (limit of edibility) and 1 = not acceptable for consumption, was used [6]. The evaluation of surface browning index (BI) was based on a subjective 1 to 5 scale, where 1 = none, 2 = slight (up to 5% surface affected), 3 = moderate (5–20% surface affected), 4 = moderately severe (20–50% surface affected) and 5 = extreme (>50% surface affected) [18].

Physical Parameters: Colour and Respiration Activity

Colour parameters *L**, *a** and *b** were measured on 3 random points on external cut surface of whole iceberg and on fresh-cut slices surface (10 for each replicates). A colorimeter (CR-400, Konica Minolta, Osaka, Japan) equipped with a D65 illuminant (6504 K) in the reflectance mode and in the CIE *L* a* b** colour scale was used. The colorimeter was calibrated with a standard reference having values of *L**, *a** and *b** corresponding to 97.55, 1.32 and 1.41 respectively. In order to measure colour variations at each sampling day, *ΔE** was calculated using the following formula:

$$\Delta E^* = [(L_0^* - L^*) + (a_0^* - a^*) + (b_0^* - b^*)]^{1/2}$$

[19], where, subscript 0 represents colour parameters measured on the fresh samples.

The RA of whole and fresh-cut iceberg was measured after 24 h from harvest, when the inside product temperature was 4°C (± 0.5), and during cold storage, using a closed system [1]. Whole or fresh-cut samples were put into 6 L sealed plastic-jars, where carbon dioxide was allowed to accumulate until the value of a standard gas mixture containing carbon dioxide and nitrogen (0.1% - 99.9% Sapio, Milano, Italy). Then, a 1 mL gas sample was taken from the head space through a rubber septum and injected into the gas chromatograph (p200 micro GC Agilent, Santa Clara, CA, USA), equipped with dual columns and thermal conductivity detector.Carbon dioxide was analyzed with a retention time of 16 s and total run time of 120 s on a 10 m PPU column at a constant temperature of 70 °C.

Chemical Parameters: Ammonia and Chlorophyll Content

For A determination, samples were analysed as reported in previous study [20] reading absorbance at 635 nm by using spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan). Concentrations of ammonium sulphate were used as standard.

For the determination of TC, 5 g of lettuce were immersed in 20 mL methanol for 24 h at 20°C in darkness. The quantification of chlorophyll was performed spectrophotometrically at 653, 666 and 470 nm and the content was expressed as milligrams of total chlorophyll per 100 grams of fresh weight (fw) using the equation reported by Cefola *et al*. [20].

Biochemical Parameters: Antioxidant Activity, Total Phenols Content, *O*-Quinones, POD, PPO and PAL Assays

Antioxidant Activity and Total Phenols Content

For AA and TP determinations 5 grams of whole or fresh-cut iceberg for each replication were homogenized (Ultraturrax T-25, IKA Staufen Germany) in a methanol:water solution (80:20) for 1 min, and then centrifuged at 5°C at 6,440 *xg* for 5 min.

Antioxidant assay was performed following the procedure described by Brand-Williams *et al*. 1995 [21] with minor modifications. Extract obtained as previously reported, diluted in water was pipetted into 0.95 mL of DPPH solution to initiate the reaction. After about 30 min the absorbance was read at 515 nm. Trolox was used as a standard and the antioxidant activity was reported in milligrams of Trolox equivalents per 100 grams of fw.

TP were determined according to the method of Singleton & Rossi 1965 [22] by mixing 100 µL of the extract with 1.58 mL of water, 100 µL of Folin–Ciocalteu reagent and 300 µL of sodium carbonate solution (200 g L^{-1}). Samples were kept in dark for 2 h, then the absorbance was read at 765 nm (UV-1800, Shimadzu, Kyoto, Japan). The content of TP was calculated on the basis of the calibration curve of gallic acid and was expressed as milligrams of gallic acid per 100 grams of fw.

O-Quinones Determination

Soluble *o*-q of vegetable tissues, were extracted as described by Ke & Saltveit, 1986 [23] with low modifications by homogenized 5 g of tissues with 20 mL of methanol (80%). The homogenate was filtered through four layers of cheesecloth and centrifuged at 6,440 *xg* for 5 min. The supernatant was used directly to measure the soluble *o*-q at a wavelength of 437 nm.

POD, PPO and PAL Assays

For POD and PPO extraction, iceberg lettuce (whole or fresh cut) were chopped into small pieces and about 10 g were homogenized for 3 min with 20 mL of 0.05 M sodium phosphate buffer, pH 6.2 and 2 % PVP. The homogenate was centrifuged at 15,000 *xg* at 4°C for 10 min.

For PPO assay, the enzyme activity was determined by using 10 mM chlorogenic acid in 0.05 M sodium phosphate buffer, pH 6.2, following the oxidation of 10 mM chlorogenic acid at 410 nm at 25°C. One enzyme unit is defined as the absorbance change per minute per gram of fw under the above assay conditions.

POD activity in the supernatant fractions was determined with 20 mM guaiacol as reducing substrate in a reaction mixture containing 0.05 M sodium phosphate buffer, pH 6.2 and 30 mM H_2O_2. The oxidation of guaiacol was followed by observing the increase in absorbance at 470 nm at 25°C. One enzyme unit is defined as the absorbance change per minute per gram of fw under the above assay conditions.

For the PAL extraction, 5 g of sample were homogenized for 1 min with 15 mL of 0.2 M boric acid-NaOH buffer (pH 8.8) containing 5 mM 2-mercaptoethanol and 30 g L^{-1} of PVP. The homogenate was filtered through four layers of cotton gauze, and the resulting filtrate was centrifuged at 15,000 *xg* at 4°C for 10 min [24]. The enzyme activity was measured using 10 mM phenylalanine in 0.05 M borate buffer (pH 8.8) and following the increase in cinnamic acid by a spectrophotometric method at 290 nm according to the procedure of Ke & Saltveit, 1989 [25]. One unit of PAL activity was defined as the formation of 1 µmol of cinnamic acid per hour.

Statistical Analysis

The effect of the storage of whole and fresh-cut samples on qualitative parameters were tested by performing a one-way ANOVA, with data means arranged in a completely randomized design. The mean values were separated using the Student–Newman–Keuls (SNK) test. Principal component analysis and linear regressions were run using the software Statistica 6.0. Standardization of data was necessary for the different magnitudes of the parameters used in the analysis; after this operation, each parameter contributes equally to data set variance and carries equal weight in principal component calculation.

RESULTS

Changes in Sensory and Physical Parameters Occurring during Storage

Fresh-cut lettuce showed a decrease in OA during storage (Figure **1A**), reaching the marketable limit (OA=3) after 8 days, resulting not acceptable for consumption at sixteenth day (OA=1) whereas whole lettuce showed a slighter decrease in OA from the first sample day to the end of the storage period (Figure **1B**). At the marketability limit (after 8 days), fresh-cut samples were scored moderate browned (5-20%

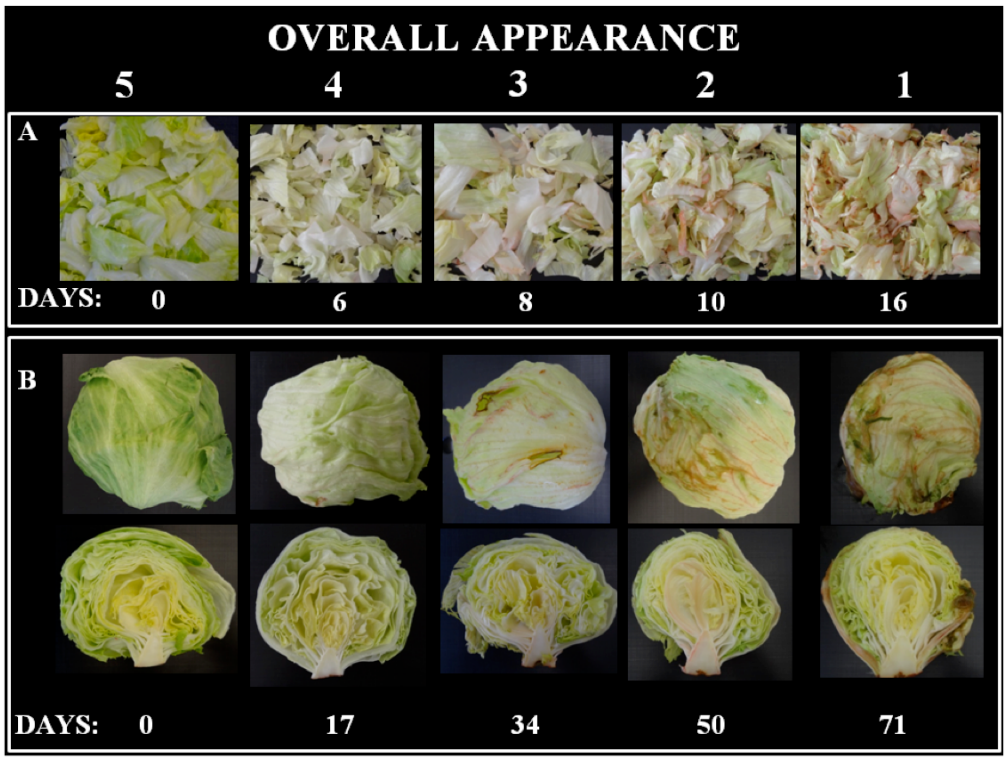

Figure 1: Overall Appearance of fresh-cut **(A)** and whole **(B)** iceberg lettuce during storage.

Table 1: Quality Parameters for Fresh-Cut and Whole Iceberg Lettuce during Storage at 4°C

Quality Parameters	Fresh-Cut Storage Days						Whole Storage Days					
	0	6	8	10	16	P	0	17	34	50	71	P
Overall appearance (5-1)	5.00a	400b	3.00c	2.00d	1.00e	****	5.00a	4.00b	3.00c	2.00d	1.00e	****
Browning index(1-5)	1.00d	2.20d	3.07c	3.93b	5.00a	****	1.00d	1.83c	3.50b	4.57a	5.00a	****
Colour (ΔE^*)	0.00d	6.50c	7.78c	11.82b	15.99a	****	0.00c	4.62b	4.89b	7.53b	11.87a	****
Total Chlorophyll (mg $100g^{-1}$)	0.38	0.41	0.34	0.39	0.39	ns	0.38a	0.27b	0.27b	0.21b	0.20b	***
Ammonia content (µmole $NH_4^+g^{-1}$)	0.05c	0.07c	0.10c	0.25b	0.37a	****	0.04c	0.04c	0.06c	0.11b	0.25a	****
Antioxidant activity (mg Trolox $100g^{-1}$ fw)	11.57b	12.85b	12.21b	14.63b	20.24a	****	14.80b	14.35b	19.63a	14.32b	15.09b	****
Total phenols (mg gallic acid $100g^{-1}$ fw)	30.04b	30.29b	30.35b	30.88b	33.81a	***	31.21	30.08	32.53	29.90	29.95	ns
O – quinones (OD_{437} $5g^{-1}$ fw)	0.04c	0.09bc	0.09bc	0.12b	0.24a	****	0.04B	0.03B	0.05B	0.05B	0.08A	**
PPO (Ug^{-1} fw)	21.14a	19.72a	18.92a	14.75b	10.66b	****	19.83a	13.15b	19.35a	20.94ab	23.68a	***
PAL (Ug^{-1} fw)	0.04b	0.04b	0.05b	0.05b	0.08a	****	0.00b	0.05a	0.01b	0.01b	0.01b	****
POD (Ug^{-1} fw)	1.94b	2.65b	2.90a	2.18c	2.66b	****	1.92c	2.05bc	2.48ab	2.54ab	2.69a	**

Significance: ns, **, *** and **** = not significant, significant at $P \leq 0.01$, 0.001 and 0.0001, respectively. Mean values of 3 samples. For each parameter, means followed by a different letter are significantly different ($P < 0.05$) according to the SNK test.

surface affected) by panellists (Table **1**). In whole lettuce, an increase of brown area on the external surface, scored by panellists from slight (up to 5% surface affected) after about 2 weeks, to extreme (>50% surface affected) at the end of the storage, was observed (Table **1**).

In fresh-cut samples, data from colour analysis, showed an increase in ΔE^* from 0.0 to about 8.0, after 8 days (OA = 3) and to 16.0 (± 3.3) at the end of the storage (OA = 1) (Table **1**). A slighter increase in whole heads was registered in external section, where ΔE^* was 4.9 (± 0.6) after 34 days of storage, reaching 11.9

(± 2.6) at the end of the storage (Table **1**). In addition, significant linear regressions were found between OA loss or BI development and Δ*E** as in fresh-cut (R^2=0.97, P<0.01) as in whole (R^2=0.98, P<0.001) lettuce. A transitory increase in respiration activity (RA) was observed in fresh-cut samples; in fact the respiretion decreased sharply after 1 day of storage, reaching at the end of the storage a value of about 50 mL CO_2 Kg^{-1} h^{-1}. On the other hand, whole lettuce showed a low respiretion activity (on average 19.27 mL CO_2 Kg^{-1} h^{-1}), which remain roughly constant during storage (data not shown).

Changes in Chemical and Biochemical Parameters Occurring during Storage

In fresh-cut samples no significant chlorophyll changes were observed. Furthermore, in whole samples, the incoming senescence bring about a chlorophyll degradation of about 30% after 17 days respect to the fresh ones (Table **1**); moreover, the chlorophyll content remained constant until the end of the storage. For fresh-cut and whole lettuce, ammonia content significantly increased of about 4-fold from the marketability limits (after 8 and 34 days, respectively), at the end of the storage (Table **1**).

In addition, a significant increase in TP was achieved in fresh-cut samples at the end of the storage (33.8 ± 0.9 mg gallic acid 100 g^{-1} fw),, while the AA doubled after 16 days, reaching the final value of 20.2 ± 2.8 mg Trolox 100 g^{-1} fw (Table **1**). Conversely, in whole samples TP content and AA remained almost unchanged during all the storage (Table **1**). Regarding the *o*-q, the content was low and relatively constant in whole sample, whereas, at the end of the storage, a six-fold increase was measured in fresh-cut item, respect to the fresh samples (Table **1**).

Fresh-cut lettuce showed an induction of PAL activity during storage. In particular, a slow increment, until the tenth day, was observed, followed by a sudden increase until the end of storage (Table **1**). In the whole product, instead, the enzyme activity increased within the first 17 days, then, there was a decrease until a level similar to the beginning of the experiment. Afterwards, the enzyme maintained a constant activity throughout the remaining period (Table **1**). Regarding PPO, in fresh-cut products, the enzyme showed insignificant reduction for the first 8 days, followed by a significant decrease until the end of the storage (Table **1**). In the whole, instead, after a very slow decrease, PPO activity raised with different rates, during the entire storage period (71 days). Finally, POD activity in fresh-cut underwent to several changes showing a slight tendency to augment during all the storage (Table **1**). In whole lettuce, instead, for the first 17 days, the POD activity remained almost constant with a very slow trend of raise until to the end of the storage (Table **1**).

Principal Component Analysis (PCA) on Fresh-Cut Lettuce

PCA analysis was performed with the aim to study the relationships among the quality parameters affectting the loss in OA of fresh-cut lettuce. (Table **1**). The whole lettuce were not considered in this analysis, since, in this sample, the loss of OA was mainly attributable to the senescence processes and to the long storage time, needed to have significant change in quality parameters. The first principal component (PC1) explained 78.4 % of the total variance in the data set, while the second principal component (PC2) explained 12.5 %. The loading plot of PC1 versus PC2 from selected data, is presented in Figure **2A** and the score Plot of PC1 versus PC2, in Figure **2B**. The two PC1 and PC2 factors in the resulting model described 90.6 % of total variance in the data. RA, PPO activity, A, AA, TP, *o*-q, PAL activity, Δ*E*,* and BI, were manly representted with PC1, whereas TC and POD activity mainly accounted with PC2, as was shown in Figure **2A.**

PCA allowed to discriminate samples in three groups: acceptable, not acceptable and at marketable limit. Acceptable samples at high OA are located in the right of the score plot (Figure **2B**), whereas not acceptable samples were clustered in opposite positions respect to the fresh samples (left upper quadrant). Whereas samples at marketability limit (OA = 3) are situated in the lower part of the graph close to PC2 (Figure **2B**).

The different position of the samples belonging to each group is due to the different effect of quality parameters on the OA. The parameters that mainly contributed to the loss in OA are located in the left part of the graph. The main correlations among the considered parameters were showed in Table **2**. In particular, OA was significantly and inversely correlated with BI (r=-1.00), *ΔE** (r= -0.98), A (r= -0.94), AA (r= -0.86), TP (r=-0.82), *o*-q (r= -0.89), and PAL (r= -0.80). Moreover, OA was positively correlated with RA (r= 1.00) and PPO activity (r= 0.96).

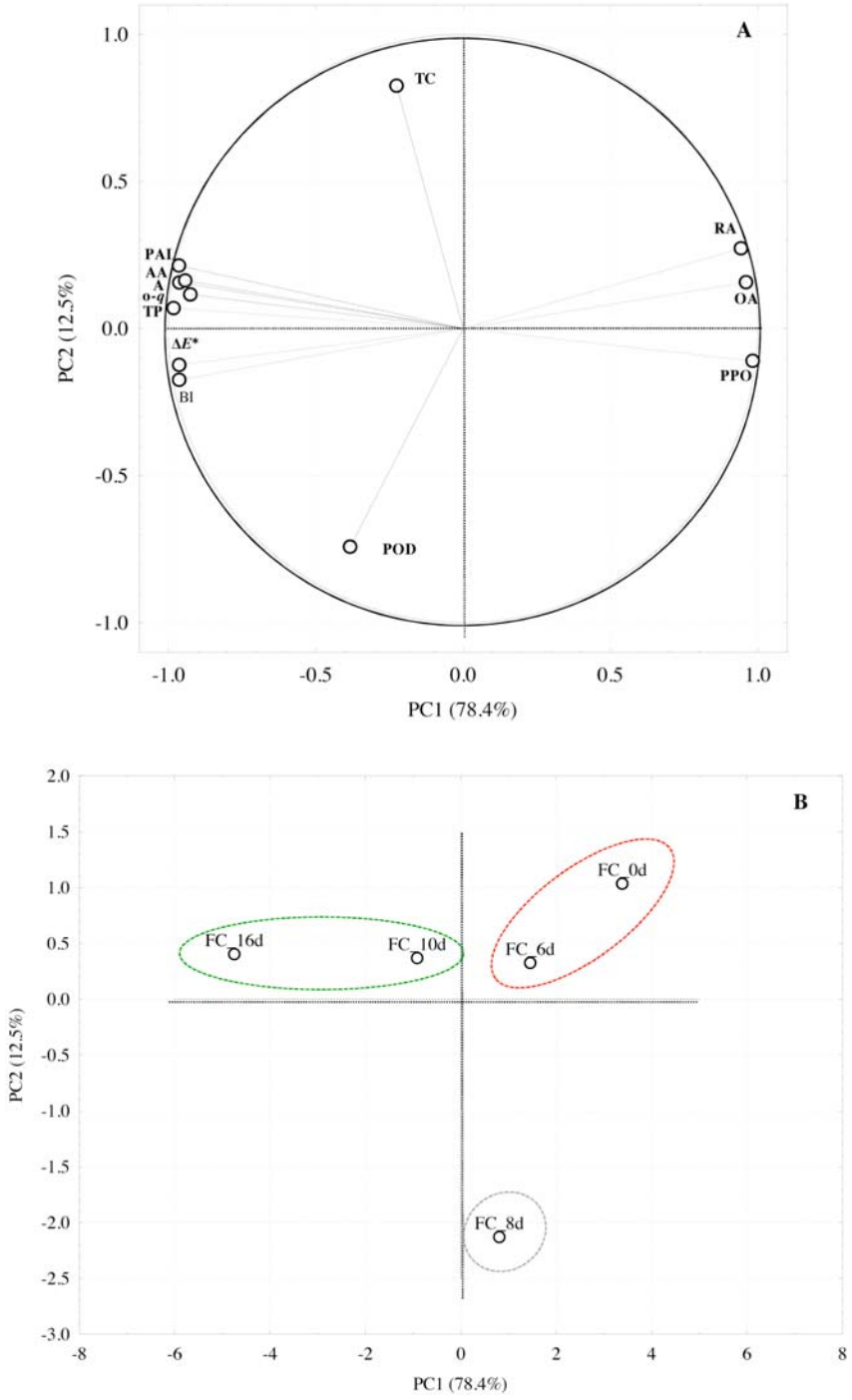

Figure 2: Principal component analysis loading **(A)** and score plot **(B)** for fresh-cut iceberg lettuce at different storage days. Fc_0d, fc_6d, fc_8d, fc_10, fc_16d (fresh-cut lettuce after 0, 6, 8, 10 and 16 days of cold storage); the parameters reported in loading plot (a) are coded in Table **2.**

DISCUSSION AND CONCLUSION

The evaluation of the OA in fresh-cut was mainly influenced by leaf browning; whereas in whole lettuce ruptures and dark necrotic stains on leaf tissues, indicating an increase in mechanical fragility of leaves and a severe pathological process of rots, affected panellist scores. Similarly symptoms were observed by others authors [26, 27] on butter head and iceberg lettuce, during storage. These sensory changes occurring during fresh-cut and whole lettuce storage resulted well described by colour analysis, as already suggested by Pace *et al*., 2011 [6]. In addition, the loss of sensory quality in fresh-cut and whole lettuce, during storage, could be explainable by changes in the main chemical and biochemical parameters followed during

Table 2: Pearson Correlation Matrix between the Analysed Parameters, Obtained by Principal Component Analysis

	OA	BI	ΔE^*	RA	TC	A	AA	TP	*o*-q	PPO	PAL	POD
Overall appearance (OA)	-											
Browning index (BI)	-1.00 ***	-										
Colour (ΔE^*)	-0.98 **	0.99 ***	-									
Respiration rate (RA)	1.00 **	-0.99 ***	-0.99 **	-								
Total Chlorophyll (TC)	-0.07 ns	0.08 ns	-0.18 ns	-0.02ns	-							
Ammonia (A)	-0.94 *	0.93 *	0.91 *	-0.88 *	0.26 ns	-						
Antioxidant activity (AA)	-0.86 ns	0.86 ns	0.87 ns	-0.82 ns	0.38 ns	0.95 *	-					
Total phenols (TP)	-0.82 ns	0.83 ns	0.82 ns	-0.79 ns	0.29 ns	0.91 *	0.99 ***	-				
***O* –quinones** (*o*-q)	-0.89 *	0.90 *	0.91 *	-0.88 *	0.29 ns	0.93 ***	0.98 **	0.98 **	-			
PPO	0.96 *	-0.95 *	-0.94 *	0.91	-0.27 ns	-1.00 ***	-0.10 *	-0.92 *	-0.95 *	-		
PAL	-0.80 ns	0.80 ns	0.80 ns	-0.77 ns	0.29 ns	0.87 ns	0.97 **	1.00 ***	0.98 **	-0.89 *	-	
POD	-0.39 ns	0.44 ns	0.46 ns	-0.53 ns	-0.29 ns	0.13 ns	0.24 ns	0.28 ns	0.39 ns	-0.22 ns	0.34 ns	-

ns: not significant; ** Significant at $p< 0.01$; *** Significant at $p<0.001$.

the trial. Just below the marketability limit (OA= 2), in both fresh-cut and whole samples, the incoming senescence phenomena (mainly characterized by protein catabolism) caused an increase in A. A reduction in TC only in whole heads was also measured, with no significant changes in fresh-cut items [28]. The reported pathway for the chlorophyll degradation could be related to chemical, enzymatic and possible gene expression changes, which induce protein synthesis, degradation, and a variety of other modifications, as reported by Heaton & Marangoni 1996 [29]. The increase in A is related to senescence and protein catabolism, as already detected by Chandra *et al*. 2006 [30] on lettuce; therefore it was found that ammonia production is also a consequence of stressful condition as reported by Cantwell *et al*. 2010 [31] on romaine lettuce and Cefola *et al*. 2010 [20] on broccoli raab. Wounding, which represents a stress, removes the natural protection of vegetables; hence, tissues of fresh-cut products, are more perishable and senescence-prone, than the whole part from which they are obtained [32]. Thus, this shows how the measure of ammonia could be a real indicator of product quality as for the whole as the for fresh-cut lettuce, useful in quality control procedures.

During storage the increment of the TP in fresh-cut lettuce, with a corresponding increase in the AA was probably related to the increased phenolic metabolism [33, 34]. Moreover, the enzymes (PAL, PPO, and POD) involved in browning phenomena in lettuce, were investigated. In particular, the results on PAL activity, in fresh-cut lettuce, indicated that the phenol metabolism was enhanced during storage, inducing the conversion of the amino acid L-phenylalanine to trans-cinnamic acid. Moreover, phenols resulted oxidised by PPO, in good agreement with the data reported by Tavarini *et al*. 2007 [17]. In whole lettuce changes in enzymatic activities were also measured during storage. In particular, the decrease in PAL activity could be due to a *de novo* synthesis of a PAL-inactivating factor [35]. Whereas, the PPO activation might be an event that is associated with senescence, storage and pathogenic attack; in addition, the activation of a latent PPO form could be supposed [36]. This latent form of PPO could catalyze the oxidation of the phenolic compounds to give rise to the reactive *o*-q, that non enzymatically polymerize to melanins [37].

Finally, POD activity seems did not be influenced by the wounding. This last result is in agreement with data reported by Degl'Innocenti *et al*. 2007 [38] on rocket salad, and with the hypothesis of a *de novo* POD isoenzymes synthesis during storage. This induction of new POD isoenzymes could be related with lignifications processes to repair cell walls after tissue wounding [39]. In addition, regarding browning reactions, that generally have been assumed to be a direct consequence of PPO action on polyphenols [40], the possible involvement of POD, cannot be excluded [41,42]. In fact, the synergistic PPO-POD effect through the generation of H_2O_2 in the oxidation of some phenolics catalyzed by PPO, can suggest the involvement of POD in browning processes [39].

The relationships highlighted by PCA allowed to indicate the quality parameters main related with the OA loss and to consumer acceptability of fresh-cut lettuce iceberg. The negative correlations between OA with *ΔE** confirmed the above reported results regarding the use of colour analysis as an objective method for fresh-cut lettuce marketability assessment. Moreover, the correlations between OA and PAL-activity, TP, and AA, could be explained as consequence of the stress conditions occurred after wounding. The latter changes could be trigged by ROS productions that hence, might stimulate the scavenger activity of polyphenols [43]. In addition, the positive correlation between A and the main sensory (OA, BI), physical (*ΔE**), chemical (RA) and biochemical parameters (AA, TP, *o*-q, PPO), suggested A as indicator of the overall product quality, as already above reported. In fact, during storage, physiological changes, occurring in the samples, lead to ammonia accumulation with consequent decay, which could stimulate the following phenomena: development of rots, H_2O_2 generation, activation of a latent form of POD, production of *o*-q that polymerize to brown pigments [23, 25].

In conclusion, the relationships highlighted by principal component analysis allowed to indicate colour change and ammonia as quality parameters highly correlated with the fresh-cut lettuce overall appearance assessment. Moreover, ammonia and colour analysis can be used as objective indicators of the overall appearance that might be implemented in control quality procedures, to standardize the sensory rating scales. In addition, the trust of the overall appearance assessment through sensory rating scales was assessed. Finally, rating scales resulted a valid method to evaluate the marketability loss of fresh-cut and whole lettuce iceberg.

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