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Nutritional and sensory evaluation of ready-to-eat salads during shelf life

KEYWORDS: Ready-to-eat salad, total phenolics, antioxidant capacity, ascorbic acid, sensory quality, principal component analysis.

Abstract The evolution of the antioxidant and sensory properties of six commercially available ready-to-eat salads, rocket, iceberg lettuce, baby lettuce, lamb's lettuce, curly endive and radicchio, was studied throughout the shelf life. Both the storage under modified atmosphere and in unsealed pouch in a domestic refrigerator were considered and compared. Ascorbic acid, total phenolics content and antioxidant capacity were chosen as the most representative nutritional parameters for this purpose. The data obtained were analyzed by Principal Component Analysis (PCA). Radicchio, lamb's lettuce and rocket showed an initial higher content of the quality parameters studied and demonstrated a better resistance to air exposure than the other salads, being ascorbic acid the most affected parameter. Modified atmosphere packaging demonstrated to be a very useful and reliable technology to extend nutritional and sensory properties during the shelf life period for all the vegetables studied.

INTRODUCTION

Fruits and vegetables are rich sources of different various bioactive compounds with antioxidant activities, such as vitamins A, C and E and phenolic compounds, which recent studies have shown to be good contributors to the total antioxidant capacity of the foods that contain them (1). Antioxidant components provide protection against harmful free radicals, and have been strongly associated with reduced risk of chronic diseases (2). These facts have led the World Health Organization (WHO) and many health authorities in various countries, to recommend the intake of 400 g of vegetables and fruits, equivalent to 5 portions per day (3). In particular, recent studies have shown the health effects of lettuce and rocket in preventing cardiovascular diseases and cancer in rats and humans, the healthy properties are attributed to a large supply of antioxidant compounds mainly vitamin C and total phenolics as well as the fibre content (4). Ready-to-eat vegetables typically involve peeling, slicing, dicing or shredding prior to packaging and storage. Wounding and other minimal processing procedures can cause physiological changes, including ethylene production, increase in respiration rate, membrane deterioration, water loss, increased rate of vitamin loss, rapid softening, shrinkage, loss of chlorophyll, loss of acidity, increase in sweetness, formation of flavour volatiles, enzymatic browning, lipolysis and lipid oxidation and a shorter storage life (5). Most such changes may impact adversely on the antioxidant status of these products. Decrease in the antioxidant activity after processing was reported for fresh-cut spinach (6) and fresh-cut mandarin (7). Otherwise, wounding caused an increase in antioxidant activity of Iceberg and Romaine lettuce (8) and in total

phenols in peppers (9). Levels of ascorbic acid, carotenoids or phenolics can reflect the variations in antioxidant capacity of fruit and vegetables (10, 11). The processing procedures also affect the sensory quality of ready-to-eat salads that is the most important factor for consumer purchasing choice and therefore its changes over shelf life must be taken into account for a complete evaluation (12).

Modified atmosphere packaging (MAP) is a preservation technique already in use by the fresh-cut industry. Low levels of O₂ and high levels of CO₂ reduce the produce respiration rate, with the benefit of delaying senescence, thus extending the storage life of the fresh produce (12).

The aim of this work was to evaluate the quality changes of popularly consumed ready-to-eat salads during domestic storage and use, by the examination of three of the most interesting functional parameters for this kind of fresh-cut vegetables: antioxidant activity, phenolics and ascorbic acid content. Six vegetable types were considered, in order to assess also different storage behaviours related to the species of origin. The results of the chemical analysis were compared to those from the sensory evaluation.

MATERIALS AND METHODS

Samples

Six fresh vegetables ready-to-eat salads were examined: rocket (*Eruca sativa* Miller.), iceberg lettuce (*Lactuca sativa* L. var. capitata), lamb's lettuce (*Valerianella olerifolia* L.), curly endive (*Cichorium endivia* var. crispata), radicchio (*Cichorium intybus* L. var. "chioggia tondo"), baby lettuce (*Lactuca sativa* L. var. saladbowl). The salads were purchased in

local supermarkets in Rome, Italy, at the first day of their commercial life (T0), 24 h after packaging. For each vegetable 12 bags were analyzed. To mimic the common domestic use, six bags were opened at T0, immediately analyzed and then closed manually, kept in a domestic refrigerator at 4°C, and then reopened and analyzed after 2 days (T1), 4 days (T2), and at expiration date (TF), seven days after the date of packaging. The remaining six bags were kept at 4°C sealed, opened and analyzed only at expiration date (TFC).

Commercial modified atmosphere pre-packed ready-to-eat salads came all from the same vegetable processing company and were produced using industrial standard packaging conditions (5% O₂, 5% CO₂, 90% N₂). They were packed in 125 g bags, with three of them (rocket, lamb's lettuce and baby lettuce) uncut, the other three included undergone cutting process.

Analytical methods

Sample extraction. Sample extractions for the antioxidant capacity and the phenols content were prepared from 3g of salad, randomly sampled for each bag, in 25ml of methanol (Labscan, Ireland). Samples were homogenized in a Ultra-Turrax for 3 min, then ultrasonicated for 3 min and then centrifuged at 3000 rpm for 5 min.

Determination of Antioxidant Capacity. The DPPH free radical scavenging activity of the sample extract was evaluated by measuring the decrease in absorbance at 515 nm as previously reported (13). The absorbance was measured against methanol using a Perkin Elmer 554 UV-Vis spectrophotometer with 1-cm path length cuvettes. Antioxidant capacity was determined also by the ABTS assay conducted by the method by Thaipong et al. (14). The disappearance of ABTS^{•+} was determined by measuring the decrease in absorbance at 734 nm. Results were calculated using the inhibition rate (I) of the radical cation for both the assays, according to the following equation:

$$I\% = \frac{A_0 - A_f}{A_0} \cdot 100$$

A₀: radical cation initial absorbance

A_f: absorbance after sample extract adding

Determination of total phenolics content. Total phenolics content was determined using the Folin-Ciocalteu method according to Singleton et al. (1999) (15). Total phenolics content was expressed as milligrams GA equivalent (GAE) per 100 g fresh weight.

Ascorbic acid determination. The vegetable samples were extracted by the procedure described for phenols with the exception of 3% phosphoric acid as extraction solution instead of methanol. The final extracts were analyzed by high performance-liquid chromatography (HPLC) with UV-Vis detector (Shimadzu, Kyoto, Japan) equipped with a Supelcosil LC-18 column (4.6mm x 250mm x 5µm particle size) (Supelco Inc., Bellefont, PA, USA), using a 0.1% phosphoric acid as the mobile phase at a flow rate of 1.0 ml/min with UV detection of 254 nm.

All the determinations described were carried on three samples of each bag and all samples were run in triplicate on freshly made up solutions.

Sensory evaluation. For a simple sensory evaluation of vegetables, a panel of ten expert judges assessed the Total Visual Quality (TVQ) of minimally processed salads in

modified atmosphere packaging, evaluating color, texture and overall acceptance (including absence of off-flavours) as quality attributes, by comparing results at the beginning and at the end of storage period. The scale is a numerical representation of a subjective evaluation that considers the severity of the symptom (16): rating from 1 (excellent fresh), 2 (very fresh), 3 (fresh), 4 (acceptable), 5 (just acceptable), 6 (just unacceptable), 7 (unacceptable), 8 (deteriorated), 9 (completely deteriorated) and 10 (extremely deteriorated). The cut-off score was fixed at 5. The sample was considered as unacceptable when a mean score above five was reached.

Statistical analysis

One-way analysis of variance (ANOVA) was performed for each ready-to-eat salad to obtain a statistical assessment of the evolution of the physicochemical parameters during storage. Means were separated by Tuckey's test at $p \leq 0.05$ and at $p \leq 0.01$ significance, using the Statistical Analysis System (SAS Ver. 9.0). Correlation analysis was performed and the Pearson correlation coefficient was calculated. Principal component analysis (PCA), was applied in order to highlight a natural grouping of samples depending on their different antioxidant capacity and antioxidants content in relation to the different salad types, and to visualize the high dimensional data with respect to the influence of storage on the parameters studied. All the computations were performed using V-PARVUS (17).

RESULTS AND DISCUSSION

Nutritional properties at the beginning of ready-to-eat salads shelf life

Changes of antioxidant capacity for the six ready-to-eat salads studied, rocket, iceberg lettuce, baby lettuce, radicchio, lamb's lettuce and curly endive are reported in

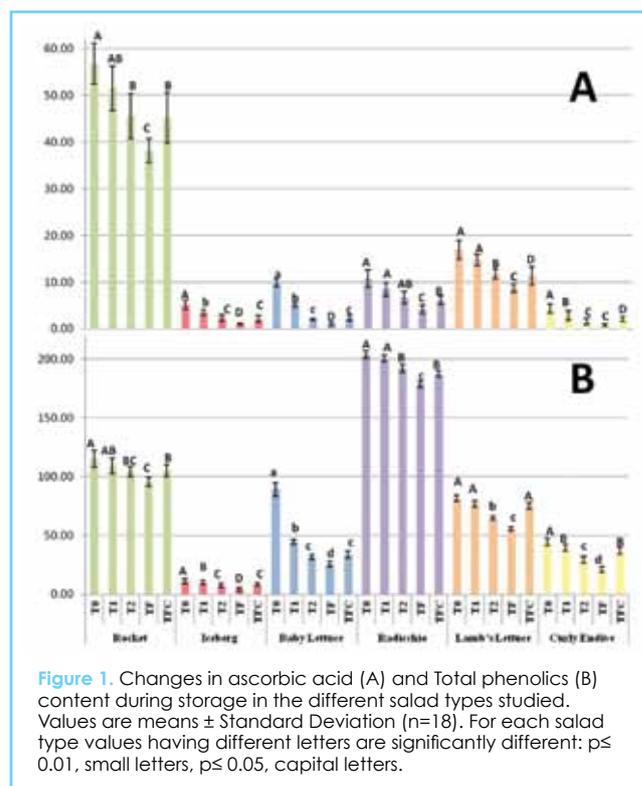


Table 1, while Figure 1 shows the evolution of ascorbic acid (Figure 1A) and total phenolics (Figure 1B)

The antioxidant activity of the six ready-to-eat salads during their shelf life has been assessed by two different assays, measuring the scavenging activity of methanol extracts with different free radicals, DPPH and ABTS.

The ranking of antioxidant capacity of individual vegetables samples was comparable for the two methods. At T0 the DPPH assay antioxidant activity values of radicchio and lamb's lettuce and of rocket and curly endive were similar ($p>0.05$) (Table 1). Radicchio showed a much higher antioxidant capacity in accordance to literature data for red varieties of salads ($p<0.01$), due to the high presence of anthocyanins (18).

Sample	T	DPPH (% I)	ABTS (% I)
Rocket	T0	72.10 ± 3.12 A	36.33 ± 3.53 A
	T1	66.67 ± 3.48 B	35.61 ± 3.31 A
	T2	62.49 ± 4.09 B	33.47 ± 3.57 A
	TF	57.73 ± 2.80 c	28.22 ± 2.65 B
	TFC	64.24 ± 3.03 b	33.55 ± 5.29 A
Iceberg	T0	11.25 ± 1.69 a	10.25 ± 2.14 A
	T1	10.19 ± 1.81 a	9.24 ± 1.14 b
	T2	7.26 ± 1.48 b	6.43 ± 0.94 c
	TF	4.60 ± 1.74 c	4.91 ± 0.56 d
	TFC	7.38 ± 0.99 b	5.89 ± 0.83 c
Baby Lettuce	T0	52.89 ± 3.95 a	31.86 ± 2.14 A
	T1	54.41 ± 5.46 a	26.95 ± 2.11 B
	T2	52.00 ± 3.23 a	21.91 ± 1.99 C
	TF	25.73 ± 3.46 b	17.37 ± 2.86 D
	TFC	36.40 ± 3.54 c	22.83 ± 2.49 B
Radicchio	T0	90.10 ± 3.61 A	95.13 ± 3.23 A
	T1	85.63 ± 3.22 B	93.45 ± 3.12 A
	T2	84.06 ± 2.82 B	89.85 ± 2.85 A
	TF	78.08 ± 3.88 C	84.06 ± 3.56 B
	TFC	83.89 ± 3.31 B	91.68 ± 3.84 A
Lamb's Lettuce	T0	89.38 ± 2.79 A	88.28 ± 2.79 A
	T1	85.71 ± 2.98 A	83.09 ± 2.72 A
	T2	80.20 ± 2.46 B	81.41 ± 2.16 B
	TF	71.00 ± 1.80 C	71.40 ± 1.68 C
	TFC	81.1 ± 2.03 B	83.11 ± 2.31 A
Curly Endive	T0	72.94 ± 2.55 A	73.94 ± 2.55 A
	T1	67.46 ± 2.11 B	67.84 ± 2.45 B
	T2	60.47 ± 1.98 c	61.06 ± 1.66 C
	TF	40.23 ± 2.69 d	39.21 ± 3.24 D
	TFC	69.19 ± 2.82 B	69.02 ± 2.08 B

Table 1. Change of the antioxidant capacity of different salad types during storage. Values are means ± Standard Deviation (n=18).

Values in the same column for each salad type having different letters are significantly different: $p \leq 0.01$, small letters, $p \leq 0.05$, capital letters.

Inhibition percentages of the two free radicals are quite similar for all the species except for rocket and baby lettuce, whose values for the ABTS assay are almost the 50% those

from DPPH assay. These results suggest the presence of different kind of antioxidants, more active in DPPH free radical scavenging, that depends mainly on the number of hydroxyl groups present in molecules and on their electro donating ability, or the presence of interfering substances (19).

The phenols content of plants depends both qualitatively and quantitatively on variety, with red leafed-vegetables showing a higher content than green leafed varieties (20). These literature data are hereby confirmed (Figure 1 B), radicchio is the richest in phenols with double than rocket, baby lettuce and lamb's lettuce content, and twenty-fold in comparison with iceberg lettuce. Baby lettuce and lamb's lettuce show a similar content in phenols ($p>0.05$).

Results for ascorbic acid content in the samples analyzed are described in Figure 1A. At T0 ascorbic acid levels ranged between 56.7 (rocket) to 4.3 (curly endive) mg/100 g fresh weight. Remarkable also lamb's lettuce with 17.00 mg/100 g fresh weight. After ANOVA test baby lettuce and radicchio, curly endive and iceberg lettuce showed a similar ascorbic acid content ($p>0.05$). These results highlight rocket as a good source of vitamin C, useful to reach by one only portion the recommended daily intake suggested by FAO (between 25 and 70 mg per day). (21). The ascorbic acid contents are in the range of previously reported for minimally processed vegetables (18, 22).

Evolution during storage

The evolution of the antioxidant capacity during storage was quite similar between the two assays (ABTS and DPPH) for all the salads ($p>0.05$), indicating that all the antioxidant compounds were equally affected both in air and in modified atmosphere packaging. Looking at DPPH variation from T0 to TF, the variety most affected by storage was iceberg (59%, $p < 0.05$) followed by baby lettuce (51%, $p < 0.01$) curly endive (45%, $p < 0.01$), lamb's lettuce (21%, $p < 0.01$), rocket (20%, $p < 0.05$) and radicchio (13%, $p < 0.01$). This ranking reflects quite well the content at T0, demonstrating that poorer initial quality will result in a shorter preservation.

The decrease of phenolics during shelf life in unsealed pouch in domestic conservation was evident in all the vegetables considered. The decline was more rapid in baby lettuce, where the phenolics content at T1 is 50% T0 ($p < 0.01$), and at T2 is 30% T0 ($p < 0.01$). Also curly endive and iceberg lettuce had a loss of 10% from T0 to T1 ($p < 0.05$), while radicchio, rocket and lamb's lettuce succeeded in maintaining the phenolics content at T0 till T2 ($p < 0.05$), probably due to a synergistic and protective effect of the higher content in antioxidants and ascorbic acid. At the end of the shelf life period (TF), all the vegetables showed a significant decay that was near 70% for baby lettuce and iceberg, 50% for curly endive, 30% for lamb's lettuce and 15% for radicchio and rocket.

On the contrary, the vegetables maintained under modified atmosphere packaging showed a phenolics content at the end of the shelf life period (TFC) not significantly different of that at T1 for rocket, lamb's lettuce and curly endive ($p < 0.05$) and that at T2 for the remaining samples.

The results for ascorbic acid are in accordance with previous findings in fresh-cut spinach under modified atmosphere packaging (6). The ascorbic acid content was the parameter most affected by storage, showing a decrease from T0 to TF of 90% in iceberg and baby lettuce, 80% for curly endive, 62% for radicchio, 49% for lamb's lettuce and 31% for rocket. Therefore ascorbic acid loss during storage followed the same trend of the other parameters, with the exception of

Ready-to-eat salad	Colour					Texture					General appearance				
	T0	T1	T2	TF	TFC	T0	T1	T2	TF	TFC	T0	T1	T2	TF	TFC
						1 ±									
Rocket	1.0 ± 0.00	1.0 ± 0.0	1.75 ± 1.0	3.0 ± 0.25	2.0 ± 0.25	0.0	1.0 ± 0.0	1.5 ± 0.5	3.0 ± 0.75	2.5 ± 0.5	1 ± 0.00	1.0 ± 0.0	2.0 ± 1.0	4.0 ± 0.5	2.5 ± 0.25
Iceberg lettuce	1.0 ± 0.00	2.0 ± 0.5	4.0 ± 0.5	5.5 ± 0.5	4.25 ± 0.5	0	2.0 ± 0.75	3.75 ± 1.0	6.0 ± 1.25	4.5 ± 1.0	1 ± 0.5	2.0 ± 0.75	3.5 ± 0.5	6.75 ± 0.5	3.75 ± 0.75
Baby lettuce	1.0 ± 0.25	1.5 ± 0.25	4.5 ± 0.75	3.0 ± 0.75	3.0 ± 0.00	0.2	1.5 ± 0.5	3.25 ± 0.5	5.0 ± 0.5	4.0 ± 0.25	1 ± 0.5	1.5 ± 0.5	3.0 ± 0.75	5.5 ± 0.00	3.5 ± 0.5
Radiccchio	1.0 ± 0.00	1.0 ± 0.25	2.5 ± 0.00	4.0 ± 1.0	3.0 ± 0.5	0.0	1.0 ± 0.00	2.5 ± 0.25	3.5 ± 0.25	2.5 ± 0.5	1 ± 0.00	1.0 ± 0.00	2.5 ± 0.5	4.0 ± 0.5	2.75 ± 0.25
Lamb's lettuce	1.0 ± 0.00	1.0 ± 0.00	2.0 ± 0.5	3.5 ± 0.5	2.5 ± 0.25	0.0	1.0 ± 0.25	3.0 ± 1.0	3.5 ± 0.5	3.0 ± 0.25	1.0 ± 0.00	1.0 ± 0.25	3.0 ± 0.25	5.0 ± 0.00	3.0 ± 0.5
Curly endive	1.0 ± 0.5	1.5 ± 0.25	5.0 ± 0.75	3.5 ± 1.0	3.5 ± 1.0	0.0	1.5 ± 0.25	3.75 ± 0.5	4.5 ± 0.75	4.0 ± 1.0	1.0 ± 0.25	1.5 ± 0.25	3.5 ± 0.00	5.5 ± 0.5	3.25 ± 0.25

Table 2. Total Visual Quality (TVQ) of ready-to-eat salads during shelf life assessed by ten judges. Scores ranges on a scale from 1 to 10, where 1= excellent, fresh appearance, 5= just acceptable (cut off shore), 10= unusable.

respectively. Figure 2 shows the loading (in blue) and score plot and well substantiates that the first component is mainly related to the antioxidant capacity assays and to phenolics results whereas the second component is mainly related to ascorbic acid.

The score plot of the first two components, allows for the characterization of the salad variety according to the first and the second component. The samples were well separated according to their variety with no overlapping, and the vegetable groups are spread along the first component, except the group of rocket samples that is characterized by ascorbic acid. Therefore, PCA results confirm the preliminary evidences of the previous section, rocket has the highest content in ascorbic acid and this parameter can differentiate very well this vegetable variety from the others, radicchio had the highest content in phenolics and antioxidant capacity followed by rocket, baby and

radicchio whose ascorbic acid decay was more dramatic than the other parameters. The fast loss of ascorbic acid in fruit and vegetables during postharvest handling procedures and its relation to plant genotype has been reported in other papers, with temperature, mechanical injuries and long storage duration the most affecting factors (23)

Evaluation of sensory quality

The sensory quality was assessed to compare the main visual quality attributes for the consumer purchasing choice with the functional parameters studied and their respective evolution during the shelf life of the vegetables (Table 2). The judges evaluations during storage showed good correlations with changes of tested chemical parameters ($r > 0.77$, $p < 0.05$). In particular, salads with a bigger decay in functional parameters, iceberg, baby lettuce and curly endive were judged unacceptable at the end of the under air storage period for all the visual properties tested. The most critical property was the texture and subsequent the general appearance, especially when stored unsealed. Another problem occurring during storage was the leaves browning that was more evident in shredded salads, such as iceberg, curly endive and radicchio, and led to high scores at T2. The salads in sealed bags had scores at the shelf life end period (TFC) comparable to those of T2, so modified atmosphere packaging preserves the vegetables slowing the natural visual quality decay until four days. The agreement between the sensory-based spoilage and the decay in functional properties in all the salads studied might be interesting in promoting the idea of fruit and vegetables freshness associated to healthiness in consumer perception of ready-to-eat salads.

Chemometric evaluation

Principal component analysis (PCA) was used as unsupervised pattern recognition technique to visualize the data set. After autoscaling, two significant components were identified giving account for 64.6% and 26.5% of the total variance,

lamb's lettuce, curly endive and iceberg lettuce. The interesting result coming from this PCA is that the samples are spread along the principal components in order of storage time. In particular, the sample at T0 of each salad has always the highest score on the component that differentiates the respective salad type, followed by the T1, T2 and TF samples, in decreasing order. TFC and T2 scores are quite close for all the salad types except for curly endive and baby lettuce whose TFC score are nearer to T1 and in the middle between T2 and TF, respectively.

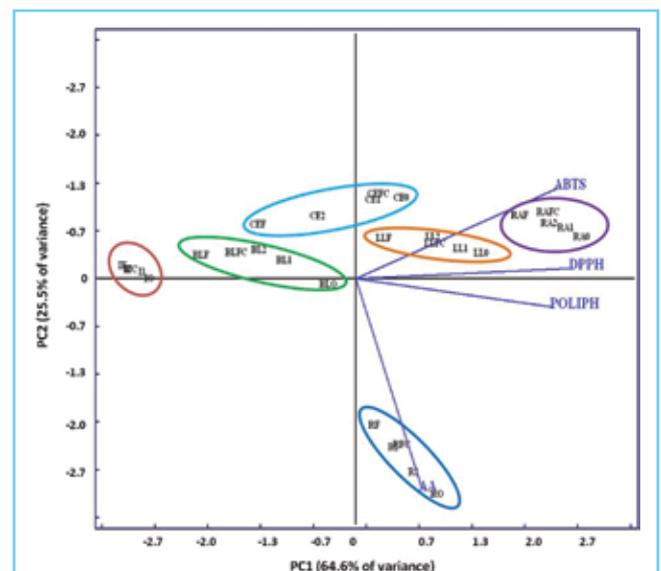


Figure 2. Principal component analysis (PCA) of studied parameters in ready-to-eat salads. Sample code: LL Lamb's lettuce, R: rocket, RA: radicchio, CE: curly endive, I: Iceberg lettuce, BL: baby lettuce. Scores and loadings on data set in Table 1. The number or letter after the sample code refers to the storage time 0= T0, 1= T1, 2= T2, F= TF, FC= TFC.

parameters studied and their behaviour during shelf life. Among the vegetables studied, radicchio, lamb's lettuce and rocket, showed high content in functional quality parameters that was maintained throughout the shelf life period even in domestic storage, such as to be considered "naturally functional foods" for high content in ascorbic acid in rocket and polyphenols in radicchio. Iceberg and curly endive showed a faster decay, probably due to the cutting operations they undergo during processing. Modified atmosphere packaging demonstrated to be a very useful and reliable technology to extend functional and sensory quality during the shelf life period for all ready-to-eat salads.

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