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## SHELF-LIFE AND ENZYMATIC OXIDATION IN FRESH CUT ESCAROLE

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### ABSTRACT

Modified atmospheres, saturated in N<sub>2</sub> and Ar, have been studied for their effect on the final quality of six cultivars of fresh-cut escarole. The study has observed that the fresh-like properties and consumer acceptability, relative to browning and, therefore, to shelf-life was good up to 14 days, especially in modified atmospheres, in which PPO reduces the oxidation of polyphenols.

### RESUMEN

Se ha estudiado el efecto de la atmósfera modificada, saturada de N<sub>2</sub> y Ar en la calidad final de seis tipos diferentes de escarola recién cortadas. De dicha investigación, se ha observado que las propiedades de la frescura y la aceptación por parte de los consumidores, relativos al escurecimiento del producto, y, como consecuencia, la conservación en las estanterías frigorífico llegaba a 14 días, y especialmente, las muestras sometidas a la atmósfera modificada, llegando a la conclusión de que, en dichas condiciones, el ppo reduce la acción del oxígeno sobre los polifenoles.

### INTRODUCTION

Fresh-cut vegetable use is rising by 6% annually in Europe and Italy, which is particularly significant since fresh vegetable consumption has seen a downturn in recent years. Escarole (*Cichorium endivia* var. *Latifolium*) is one of the most highly regarded salads because of its colour, slightly bitter flavour and for its toning, purifying and diuretic properties in low-calory diets. Furthermore, fresh-cut escarole has a long shelf-life because it resists browning. This is not entirely understood but may be due to low polyphenoloxidase (PPO) activity, which is responsible for enzymatic browning in many vegetables (Castaner et al., 1999; Matthew et al., 1971; Chazarra et al., 2001; Espin et al., 1996; Dogan et al., 2002) and/or the low level of polyphenol substrate in the vegetable tissue.

Six escarole cultivars (Salanca, Laurv, Davos, Perlita, Lorca, Elsa) grown in Italy were studied, of which only Salanca was studied on its own, the others studied as a mix. Samples were packed in both air atmosphere and nitrogen or argon saturated atmosphere with triple layer film. The phenol content, ppo activity and colour change (l\*, a\* and b\* values) were measured during storage for 14 days at 4°C.

## MATERIALS AND METHODS

### Plant material and preparation

Six escarole cultivars (*Cichorium endivia* var. *Latifolium* cultivar *Salanca*, *Laurv*, *Davos*, *Perlita*, *Lorca*, *Elsa*) were used. The samples were harvest at the commercial maturity stage and they were transported to the laboratory by ventilated car, stored at  $4 \pm 0.5^\circ\text{C}$  and processed the next day.

Having removed the outer leaves, the remainder was washed, cut into 3 x 3 cm pieces, dipped in a 200 ppm chlorine bath for 10 mins and centrifuged. Randomly and in aseptic conditions, bits were subdivided into boxes, *Salanca* on its own (S), the other 5 (*Laurv*, *Davos*, *Perlita*, *Lorca*, *Elsa*) mixed (Mix).

Polyethylene boxes (31x227x35mm.), triple layer film (PET12/EVOH5/PE65, HB 85, oxygen permeability  $<2 \text{ cc/m}^2/24\text{h /atm}$ , carbon dioxide permeability  $12 \text{ cc/m}^2/24\text{h /atm}$ , steam permeability  $7 \text{ g/m}^2/24\text{h}$  at  $38^\circ\text{C}$ ; System Packaging Italia) and air atmosphere (A) and nitrogen (N) or argon (Ar) saturated atmosphere were used. The samples were stored for 14 days at  $4 \pm 0.5^\circ\text{C}$ . Every two-three days, three samples were analyzed for any qualitative variations.

### Extraction PPO

25 g of sample was homogenised with 100 ml 0,05 M sodium-phosphate buffer pH 7.5 and 3.75 g of PVPP for 1 minute with Ultraturrax. Centrifuged at 6000g per 10 minutes at  $4^\circ\text{C}$  (Fukumoto et al., 2002).

### PPO activity

Using 10 ml test tubes, 450  $\mu\text{l}$  of 0.04 M 3,4-Dihydroxyphenylacetic acid (DOPAC) was added to 50 mM phosphoric acid ( $\text{H}_3\text{PO}_4$ ), 50  $\mu\text{l}$  of 2% 3-Methyl-2-benzothiazolinone hydrazone hydrochloride hydrate (MBTH) in methanol, 25  $\mu\text{l}$  of N,N-dimethylformamide, 750  $\mu\text{l}$  of 50 mM sodium-phosphate buffer pH 6,5 and 250  $\mu\text{l}$  of the previously-extracted solution, stirred continuously in a bath at  $20 \pm 0.5^\circ\text{C}$ . The reaction was stopped using 250  $\mu\text{l}$  of 1 N  $\text{H}_2\text{SO}_4$ . The solution was read with a spectrophotometer to 505 nm (Espin et al., 1996).

### Total Phenolics Compounds Extraction

10 g sample was homogenised with 20 ml of 70% ethanol for 1 minute, homogenised for 30 minutes, filtered and centrifuged at 6000g for 10 minutes at  $4^\circ\text{C}$  (Castaner et al., 1999).

### Total Phenolics Compounds Analysis:

200  $\mu\text{l}$  extract were added to 1 ml Folin & Ciocalteu reagent (1:30) and, after 2 minutes, 0,8 ml of sodium carbonate 708 mM. After 1 hours, absorption at 550 and 850 nm range was reported, with a maximum absorption at 760 nm, against at blank (Waterman and Mole, 1994).

### Colour measurements

Colour parameters (cie  $L^*$ ,  $a^*$  and  $b^*$ ) were determined with adobe photoshop in ten replicates (clydesdale, 1969; riva, 2004).

### Statistical analyses

Each analyses were conducts with 3 replicates; the results were subjected to an analysis of variance (ANOVA), and when statistically significant differences were detected, LSD values were calculated at the  $P \leq 0.05$  level.

## RESULTS and DISCUSSION

Figures 1 e 2 show colour variation during storage at 4°C; only L\* e a\* were significant. L\* increase similarly in all samples probably due to the product becoming lighter during storage (Castaner et al., 1999). a\* showed little increase meaning little evident browning. There were no significant differences between samples stored in either atmosphere.

Figures 3 e 4 show polyphenoloxidase (PPO) and polyphenol substrate (TP) variation during storage at 4°C.

In samples S e Mix, PPO initially rises and then falls; the highest rise is after 2 days shelf-life suggesting that tissue wounding causes an increase in PPO activity due to the activation process from latent to fully active (Cantos et al., 2001; Castaner et al., 1999). The subsequent fall may be due to the reduced availability of polyphenol substrate and not to the effective reduction in enzyme, in fact Cantos et al. (2001) report that PPO was only active in the initial phase and not further synthesised.

S e Mix behave similarly and the samples in air atmosphere show a higher curve than those in modified atmosphere, argon revealing higher values than nitrogen.

Figure 3 shows the polyphenol curve peaking at 2 days possibly due to polyphenol biosynthesis as phenylalanine-ammonia-lyase (PAL) (Castaner et al., 1999) increases and then decreases, reducing the polyphenols which are gradually used as an oxidation substrate of PPO.

Figure 3 also shows a higher peak in air than in modified atmosphere, but successively this reduces to zero.

## CONCLUSION

It may be deduced that escarole is well-suited as a fresh-cut salad vegetable with a long shelf-life thanks to its low PPO activity and browning resistance. Nevertheless, atmospheres modified with argon and nitrogen can further improve shelf-life compared with air over 14 days due to reduced PPO biosynthesis and the absence of oxygen which prevents the enzyme oxidizing in turn leading to browning.

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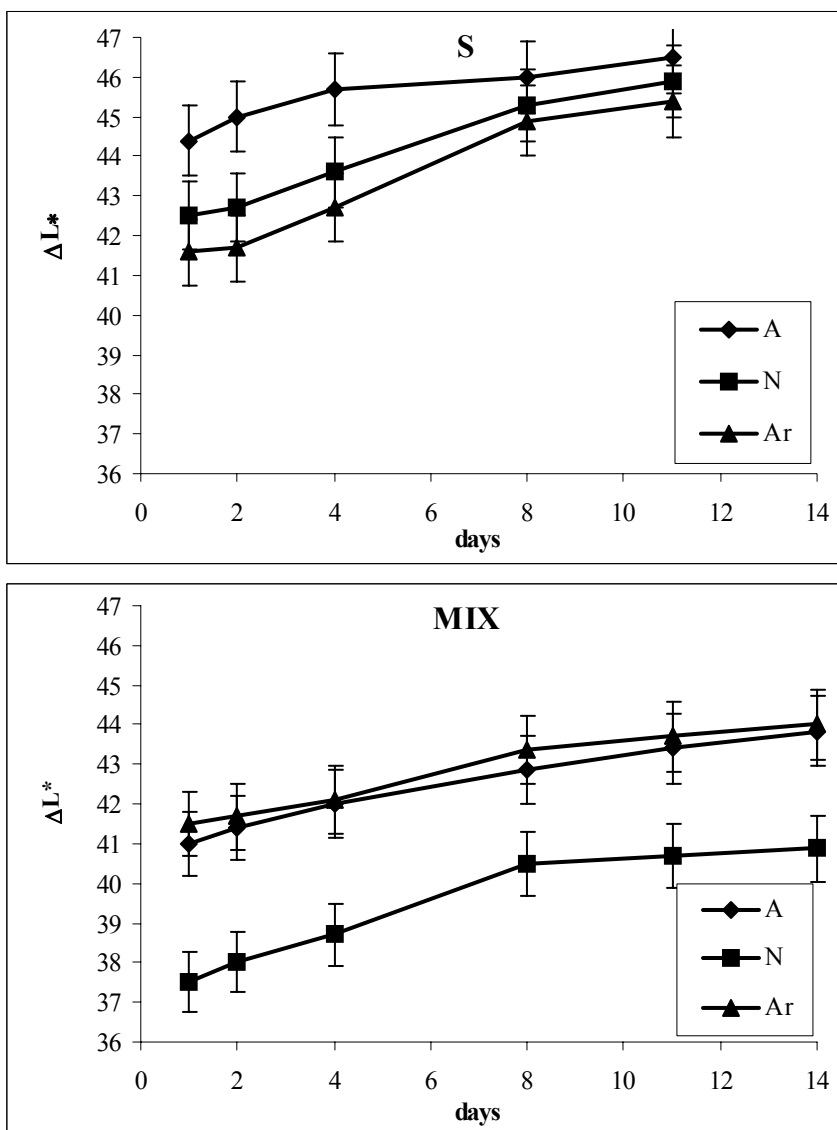


Figure 1 –  $L^*$  values during storage.

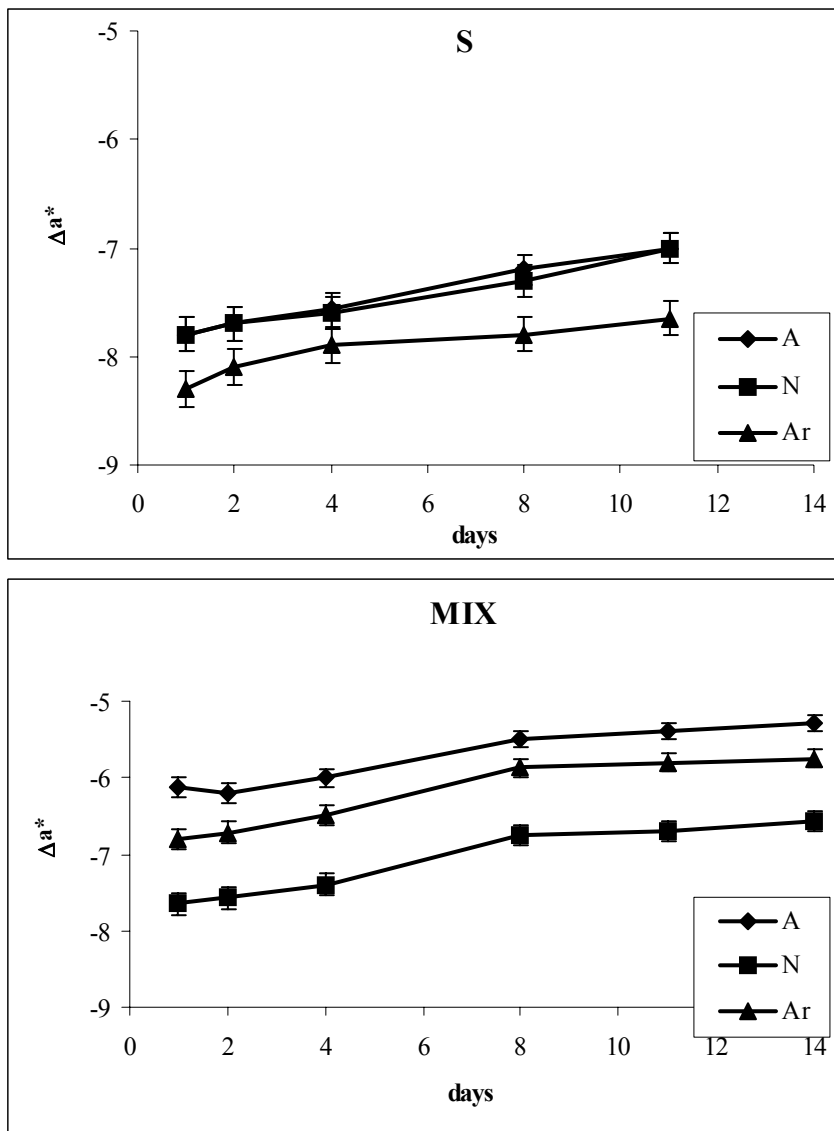


Figure 2 -  $a^*$  values during storage.

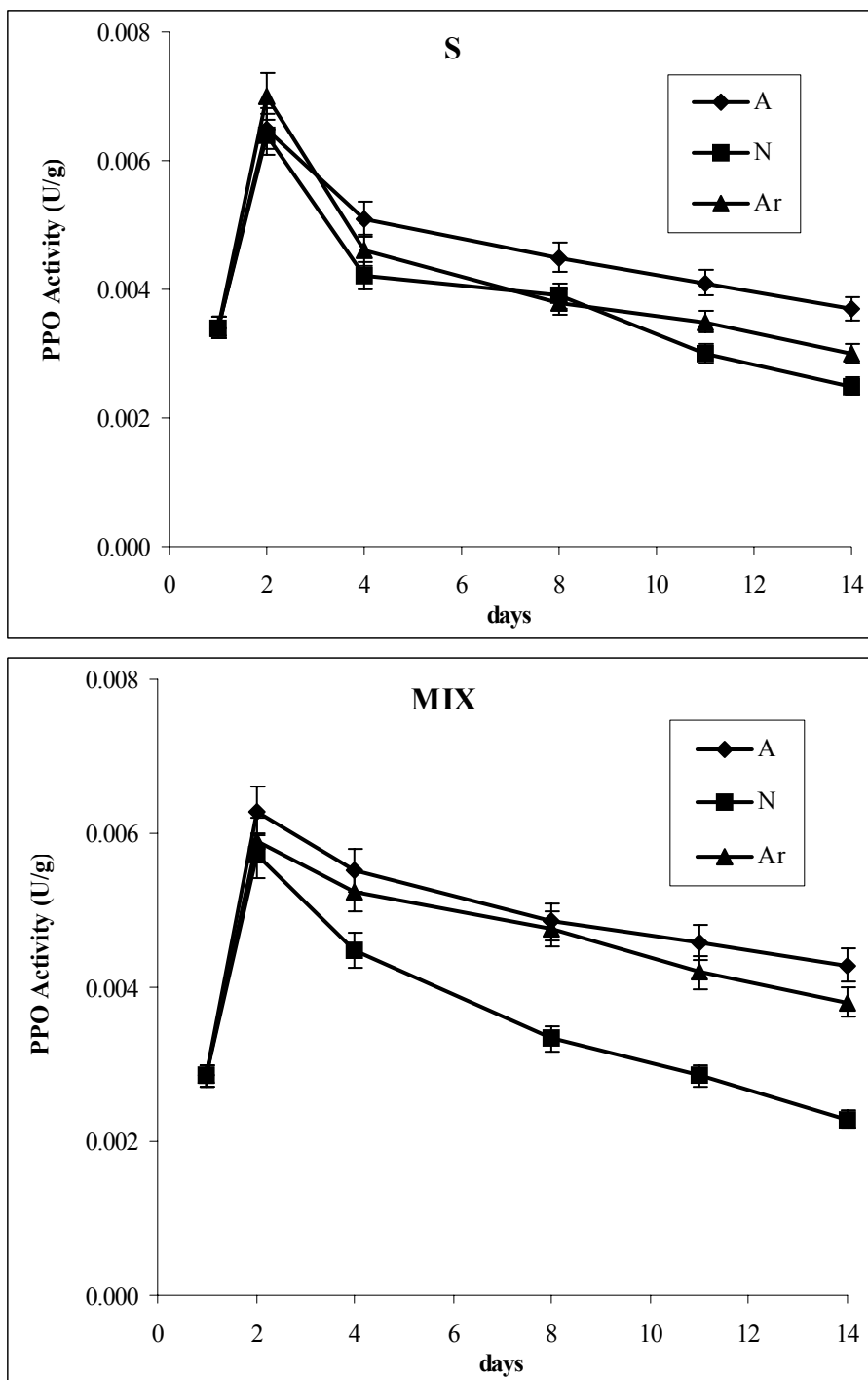
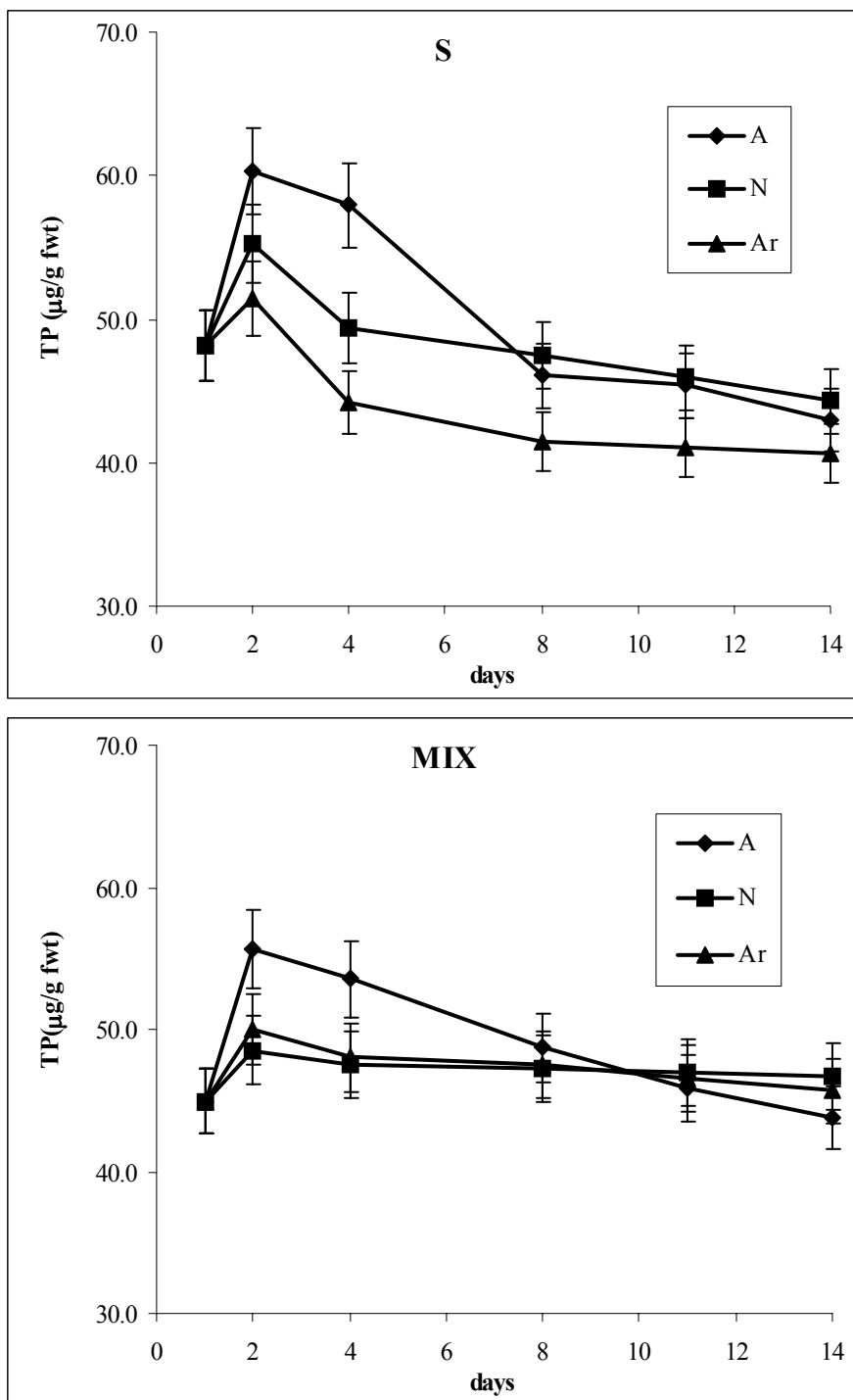


Figure 3 – Polyphenoloxidase (PPO) during storage.



**Figure 4 - Total Phenolics Compounds (TP) during storage.**