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Antioxidant properties of minimally processed endives and escaroles vary as influenced by the cultivation site, cultivar and storage time

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ABSTRACT

The influence of cultivar (CV), growth site (GS) and storage time (ST) on the quality of minimally processed endives was investigated by targeting curly and smooth-leafed cultivars, which were grown in two planting areas (Fiumicino and Fucino) and bagged in modified atmosphere at fixed conditions. The changes of antioxidant properties were examined at one and seven days post-packaging by measuring both contents of total flavonols (Fol), flavonoids (Fid), carotenes (Car) and chlorophylls (Chl) and the antioxidant capacity (AOC) through chemical (ORAC) and erythrocyte-based methods (CAA-RBC and hemolytic assays). Referring to one day of storage, curly types differed from smooth ones due to the total contents of Fid (341.0–891.7 *vs* 312.3–572.3 mg kg^{-1} CE), Fol (312.0–452.7 *vs* 194.3–520.3 mg kg^{-1} QE), Car (72.4–110.5 *vs* 7.3–38.8 mg kg^{-1}) and Chl (342.7–824.6 *vs* 276.5–490.4 mg kg^{-1}). CV and GS majorly affected the content variation, whilst ST did not exert any impact on the amounts of pigments (Chl and Car). As for the AOC at one day post packaging, curly and smooth endives respectively showed ORAC mean values of 5045 ± 2287 and 4822 ± 573 mmol kg^{-1} TE, CAA-RBC units of 27.5 ± 5.4 and 21.1 ± 2.6 µmol kg^{-1} QE, and hemolysis percentage of 62.5 ± 5.9 and 57.9 ± 10.9. The three factors acted on the AOC variation at the single level and CV x GS was the most affecting interaction. The ORAC values showed positive correlations with Fid, Fol and Chl contents as well as those of CAA-RBC *vs* Fid and pigment amounts, while only the Fol raise agreed with increased anti-hemolytic effects. Positive correlations among the AOC assays were significant just for ORAC *vs* CAA-RBC units. Finally, the principal component analysis clearly pointed at the curly types from Fiumicino as bearing the highest antioxidant quality.

1. Introduction

The consumption of minimally processed salads has increased in western societies along with the consumer's enhanced awareness of vegetables as healthy food (Rico et al., 2007). Contextually, the EU market has been relevant in the world charts of fresh-cut sales, and the Italian industry has gained a high rate in the ready-to-eat sector (Pilone et al., 2017). The "bagged salad revolution" has prompted the demand for research-supported information on product quality and effects on health (Rico et al., 2007; Stranieri and Baldi, 2017). Phase-specific processing technologies (Zhang et al., 2015) have been effective to preserve product quality (e.g. inhibition of deleterious microorganisms, control of leaf physiology and chemical degradation); however some procedures (e.g. chopping, shredding, washing, drying) intrinsically affect it.

Endive (*Cichorium endivia*) leaves are used in bagged salads as mono-reference (curly- or smooth-leafed types) or as mixed products (combined with other vegetables). They naturally contain high amounts of polyphenols (Ferioli et al., 2015), which contribute to the anti-oxidant capacity (AOC) and healthy properties (Chen et al., 2011; Papetti et al., 2008). Phenolic content can vary with cultivar and planting site (D'Acunzo et al., 2016; Ferioli et al., 2015), cultivation technique and processing (Degl'Innocenti et al., 2008; Serna et al., 2013). Flavonoids are phenols that include sub-classes such as flavones, flavonols, flavanes, anthocyanins etc. (Kumar and Pandey, 2013). Endive particularly abounds with flavonols mostly represented by kaempferol, which is efficiently absorbed in humans (DuPont et al., 2004; DuPont et al., 2000) and exerts hepatic protection from oxidative

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damage (Chen et al., 2011). Carotenoids are pigments that confer yellow, orange and red colors and have healthy characteristics (Burrows et al., 2017); β-carotene is the major provitamin-A precursor, and zeaxanthin and lutein intake correlate with decreased risks of cataract and macular degeneration (Ma et al., 2012). Endive mainly contains these carotenoids, whose levels vary with leaf maturity, harvesting season, processing and storage (de Azevedo-Meleiro and Rodriguez-Amaya, 2005). Chlorophylls are photosynthetic pigments responsible for the greenness, a crucial organoleptic trait in consumer's acceptance. Chlorophyll content naturally declines with senescence and in response to stress (Thomas and Ougham, 2014); it can be affected by processing (Toivonen and Brummell, 2008) and that of endives is influenced by growing season (Koudela and Petrikova, 2007) and cultivar (Adamczewska-Sowinska and Uklanska, 2010). The AOC measurement is a valid tool to assess food potential beneficial effects and nutritional quality (Carocho and Ferreira, 2013); contextually, several analytical and biological methods have been developed (Alam et al., 2013) and those based on human cells have been successfully used for plant food (Blasa et al., 2011; Wolfe and Liu, 2007).

Generally, stakeholders of the salad processing know that quality of fresh-cut endive changes with several factors (e.g. seasonality, cultivation techniques), however little information has been provided on antioxidant properties of cultivars intended to packaging. In central Italy, Fiumicino and Fucino are two main open-field production areas that are sequentially exploited to span spring-summer cycle; the area change is mandatory because Fucino environment assures summer production that would be of unsuitable quality if held up in Fiumicino. In this context, we addressed, as first aim, whether the endive genotypes grown in the two areas maintained antioxidant quality. Given that antioxidant quality confers important nutritional properties to fresh-cut products, the second objective was to assess the performance of bagged endives during storage by monitoring the variation of major antioxidant compounds (total flavonoids, flavonols, carotenoids and chlorophylls), together with antioxidant capacity by chemical and biological assays. Finally, we intended to select the best performing cultivars.

2. Materials and methods

2.1. Plant growth conditions

'Domari' (D) and 'Cigal' (C) are curly-leafed (*Cichorium endivia* var. *crispum*), 'Kethel' (K) and 'Parmance' (P) are smooth/broad-leafed cultivars (*C. endivia* var. *latifolium*, syn.: escaroles). Enza Zaden (http://www.enzazaden.it) and Rijk Zwaan (http://www.rijkzwaan.it/) companies own D/P and C/K, respectively. These cultivars (syn.: genotypes) were grown in two areas of central Italy; info on environment/cultivation and farming practices are summarised in Tables 1 and 2 (more details are available upon request). Meteorological data are available by web services of Lazio and Abruzzo regions (http://www.arsial.it/portalearsial/agrometeo/E1_2.asp; https://www.wunderground.com; station ILAQUILA4).

2.2. Product processing and sampling criteria and treatment

Sixty plants of each cultivar were delivered to the industry San Lidano (Latina, Italy http://www.sanlidano.it/). After visual selection, 45 plants were processed through standardized mechanical procedures including cut, sanitary treatment (sodium hypochlorite 20–40 mg L^{-1}) and water wash (both at 4–6 °C), the bags were flushed (active modified atmosphere packaging) with a mixture of Argon/CO_2 (ratio 80/20) to reach a final concentration of 7.2% O_2, 8.8% CO_2, 84% N_2 (Portable Gas Analyzer Checkpoint3 Dansensor − Mocon ® Europe). Filling was at calibrated weight of 203–205 g. Automated vertical packaging (Olimpia 4000 Simotion, Miele, Italy) produced bags (size 220 mm x 320 mm) made of anti-fog polypropylene film (thickness 35 μm; density 910 kg m^{-3}; model PP AFM035, Masterpack S.p.A., Milan), which had

Table 1
Cultivation environment and techniques.

	Fiumicino (S1)	Fucino (S2)
Coordinates		
Latitude and longitude	41°49′05.9"N 12°14′36.5"E	41°56′26.8"N 13°35′42.8"E
Altitude (m asl)	3	700
Climate[a]		
Temperature (°C)	14.1 ± 2.7	17.7 ± 2.8
Relative humidity (%)	42.3 ± 27.4	60.1 ± 9.2
Total rain (mm)	249.1	183.8
Soil		
Clay (< 0.002 mm) (%)	3	9.8
Silt (0.05-0.002 mm) (%)	4	68.7
Sand (2-0.05 mm) (%)	93	21.4
Total nitrogen (%)	0.058	0.111
Organic matter (%)	0.85	1.77
P_2O_5 available (mg kg^{-1})	49	23
K_2O exchangeable (mg kg^{-1})	234	277
E.C. (mS cm^{-1})	0.395	0.264
pH	8.00	8.28
Cation Ex. Cap. (meq 100 g^{-1})	11.32	18.19
Cultivation		
Sowing date	27/01/2014	08/05/2014
Transplant date, leaf number	5/3/2014, 3–4	26/05/2014, 3–4
Field density (plants m^{-2})	6.5	6.5
Harvest date	05/05/2014	08/07/2014

[a]Data refer to the period that spans from transplant to harvest.
[b]USDA classification (https://www.nrcs.usda.gov/).

Table 2
Major farming procedures.

Fiumicino			
Operation	Product-type	Dosage	Timing[a]
Basal dressing	Nitrophoska special, EuroChem Agro, IT	500 kg ha^{-1}	7 d bt
Protection	Signum, BASF, UK (a.i. boscalid + pyraclostrobin)	1.00 kg ha^{-1}	2 d pt
	Decisevo Bayer, IT (a.i. delthametrin)	0.50 L ha^{-1}	2 d pt
Fertigation	Calcium nitrate	75.0 kg ha^{-1}	15 d pt
	Hydrofert 14.22.11	100 kg ha^{-1}	25 d pt
	Hydrofert 14.22.11	100 kg ha^{-1}	40 d pt
Fucino			
Operation	Product-type	Dosage	Timing
Basal dressing	Manure [b]	500 kg ha^{-1}	7 d bt
Protection	Signum, BASF, UK (a.i. boscalid + pyraclostrobin)	1.00 kg ha^{-1}	2 d pt
	Decisevo Bayer, IT (a.i. delthametrin)	0.50 L ha^{-1}	2 d pt

[a]bt, before transplant; pt, post-transplant.
[b]Sanitized and stabilized chicken manure, compatible with product specifications.

permeability to: O_2 (method ASTMD3985) of 8.155E–12 mol mm^{-2} s^{-1} Pa^{-1}; CO_2 (ASTM D1434) of 37.69 E–12 mol mm^{-2} s^{-1} Pa^{-1} and H_2O (ASTM F1249) of 22.93 E–12 mol mm^{-2} s^{-1} Pa^{-1}. Mono varietal bags were stored in cold rooms at 7 ± 1 °C in the dark. Bag contents were crunched in liquid nitrogen, stored at −80 °C; an aliquot of 50 g was weighed without thawing, lyophilized at −50 °C for 72 h (laboratory freeze dryer with stoppering tray dryer, FreeZone®, Labconco Corp., Kansas City, MO, USA) and stored at −20 °C. The content of each bag represented a replicate batch; three replicates were used in all assays and all the measurements were in triplicate. The Italian law in force (DM n ° 3746-2014) imposes rules on microbiological parameters, storage (< 8 °C) and the warning on consumption date (2 d after bag opening). Factories conventionally fix the sell-by date at maximum of 7 d pp because shelf-life prolongation appears to negatively affect consumer's acceptance