

Nutritive Parameters and Antioxidant Quality of Minimally Processed “Cime di Rapa” (*Brassica rapa* subsp. *sylvestris*) Vary as Influenced by Genotype and Storage Time

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In order to assess the quality and performance of bagged broccoli-raab, a recently marketed product, several nutritive parameters were determined in novel hybrid and conventional cultivars at pre- and post-packaging stages in the industrial environment. The characterization of shoots and composing organs at post-cut stage included contents of dietary fibre (DF), glycaemic carbohydrates (GC), antioxidant compounds (ACC) and capacity (AOC), which were determined by chromatographic methods and spectrophotometric assays. ACC and AOC were analysed during shelf life of bagged products. Genotype and storage effects were addressed as variability factors at fixed packaging conditions. Contents of DF and GC (39.64–34.57; 7.56–2.21 g/100 g), glucosinolates (37.47–24.63 mg/g SIN), and ACC (total phenolics: 18.64–14.92 mg GAE/g; flavonoids: 34.74–30.96 mg/g CE; flavonols: 14.62–14.08 mg QE/g), and AOC (Oxygen Radical Absorbance Capacity: 354.62–293.25 $\mu\text{mol/g TE}$; DPPH[•] scavenging activity: 59.35–46.14) were lower in shoots of the hybrid than marketed cultivar. In both genotypes, AOC was maximal in leaves, followed by florets and stems. The integrated analyses suggested that the hybrid genotype was better suited for fresh consumption and that increased ratio of florets/leaves vs. stem is expected to raise product antioxidant properties. The comparison of unprocessed and bagged products pointed at a value decay of most parameters except for glucosinolates and correlation analyses supported the necessity of performing multiple antioxidant assays to enhance product quality evaluation. As for shelf life, storage time was the major factor affecting antioxidant properties, while genotype and interaction effects were minimal.

ABBREVIATIONS

TPC – total polyphenol content; FID – flavonoids; FOL – flavonols; GSL – glucosinolates; AOC – antioxidant capacity; ACC – antioxidant compound content; DW – dry weight; FW – fresh weight; hpp, hours post packaging.

INTRODUCTION

Cruciferous vegetables encompass nutritionally and economically important crops worldwide and the “broccoletto” group (*Brassica rapa* L.) was proposed based on morphologi-

cal traits [Bonnema *et al.*, 2011] and molecular phylogeny. It includes “cime di rapa” and “friariello” vegetables from south Italy [Elia & Santamaria, 2013], also known as broccoli-raab or rapini [PlantUse, 2020] and consumed as cooked or raw (salads or sauces).

Botanically, broccoli-raab falls into the subspecies *sylvestris* Janch var. *esculenta* Hort. [Romani *et al.*, 2006], and the edible products are shoots bearing leaves, stems, and corymb-inflorescences (florets). Local farmers usually select parental lines phenotypically, aiming at progenies with superior traits and minimal inbreeding depression. Seed companies exploit these local selections to develop synthetic varieties or to create inbred lines for F₁ hybrids with new traits such as regrowth attitude (for multiple cutting), product tenderness and antioxidant content. Landraces cultivation spans fall-winter cycles of 50–90 days in open field [Elia & Santa-

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maria, 2013] with minimal agronomic practices and pesticide treatments; greenhouse and hydroponic systems are also feasible [De Pascale *et al.*, 2007].

Nutritional quality of broccoli-raab relies on dietary fibre [U.S.D.A., 2019] and on bioactive phytochemicals, such as tocopherols, ascorbate (vitamin C) [Annunziata *et al.*, 2012; Cefola *et al.*, 2010; Conversa *et al.*, 2016], carotenoids [https://fdc.nal.usda.gov], phenolics [De Pascale *et al.*, 2007; Romani *et al.*, 2006], and glucosinolates (GSL) [Annunziata *et al.*, 2012; Cefola *et al.*, 2010; Conversa *et al.*, 2016]. These latter are typical of *Brassicaceae* and some specific classes could fingerprint “cime” ecotypes. The GSL breakdown products exert effects against different cancer types and their bioavailability is affected by product processing [Barbieri *et al.*, 2008]. As for phenolics (PP), broccoli-raab abound in phenolic acids (chlorogenic and ferulic) and flavonols (a flavonoid subclass) that are mainly quercetin, kaempferol, isorhamnetin, and respective conjugates [De Pascale *et al.*, 2007; Romani *et al.*, 2006]. Phenolics health-promoting action is mediated by interactions with gut microbiota and potential direct effects of *Brassica* phenolics are reported [Cartea *et al.*, 2010]. Chemical antioxidant capacity (AOC) of foods can be assessed by *in vitro* assays that detect various scavenging mechanisms. The AOC measurement is often unlinked to *in vivo* outcomes and feeble to provide health claims, though valid to assess potential effects in *B. rapa* vegetables [Soengas *et al.*, 2011].

Found that consumers associate local products to quality, tradition and health, industries were prompted to release bagged broccoli-raab into the ready-to-use market, favouring the *ex situ* consumption; with nutritional value preservation, undisclosed florets, stay-green leaves, typical taste without off-odours being the major quality parameters. Some factors (*e.g.*, seasonality, cultivation techniques) affect these characteristics during conservation [Barbieri *et al.*, 2009] and airflow modifications were effective to prolong floret storage [Cefola *et al.*, 2010]. Moreover, increased sulphur fertilization during cultivation and light exposure during storage enhanced the antioxidant quality of film-and-tray packaged product [Barbieri *et al.*, 2009]. Lab-scale results identified suitable gas equilibria and wrapping material for modified atmosphere packaging [Conte *et al.*, 2011; Torrieri *et al.*, 2010].

This work intended to gain novel information on dietary fibre and glycaemic carbohydrate content of two broccoli-raab genotypes, a novel F₁ hybrid and a marketed cultivar, and to provide information on the antioxidant quality of bagged products derived from an industrial process by monitoring ACC and AOC. Genotype and storage effects were the studied variability factors at fixed packaging conditions.

MATERIALS AND METHODS

Plant materials and growth conditions

Plants of ‘Broccoletto di rapa novantino riccio San Marzano’ (BSM90) were marketed by “Domenico Pagano e Figli” (Scafati, SA, IT); plants of the unpatented ‘F₁ Hybrid 39’ (H39) were provided by a seed company. They were grown under a 90-day cycle at the “Di Pastina and Meletti” farm (LT, Italy); Table 1 reports information on environment, cultivation, and farming practices. The parental lines

TABLE 1. Environment and cultivation parameters of two genotypes of *Brassica rapa* subsp. *Sylvestris*.

Murillo Sezze, Latina	
Coordinates	
Latitude	N 41° 27' 7.382"
Longitude	E 13° 3' 45.666"
Altitude (m asl)	5.0
Climate ¹	
Temp. min (°C)	8.7±3.0
Temp. max (°C)	17.2±3.0
Temp. mean (°C)	12.2±2.6
Relative humidity (%)	77.7±9.7
Total rain (mm)	127.0
Soil ²	
Clay (<0.002 mm) (%)	440
Silt (0.05–0.002 mm) (%)	220
Sand (2–0.05 mm) (%)	340
Total nitrogen (%)	1.40
Organic matter (%)	24.9
P ₂ O ₅ available (mg/kg)	462.6
K ₂ O exchangeable (mg/kg)	450.6
E.C. (mS/cm)	0.480
pH	7.84
Cation Ex. Cap. (meq/100 g)	30.3
Cultivation	dates
Di-ammonium phosphate (100 kg/ha) Panfertil, IT, 18 % P ₂ O ₅ , 46 % NH ₄ ⁺	05/11/2015
Sowing – density 70 plants/m ²	07/11/2015
Harvest	12/02/2016
Farm delivery	15/02/2016
Processing	16/02/2016

¹sowing-harvest; www.arsial.it/portalearsial/agrometeo; ²USDA classification [U.S.D.A., 2006]

of BSM90 and H39 derive from landraces of south Italy (Apulia and Campania regions) and the breeding schemes are undisclosed. Phenotypically, H39 showed less fringed margin leaves (Figure 1f), synchronous flower disclosure, and erect habitus with a primary axis prevailing over secondary branching (not shown) as compared to BSM90 (Figure 1e).

Product processing, sampling criteria, and sample treatment

Seventy plants were processed by “San Lidano” company (http://www.sanlidano.it/) according to quality management standard of standard for quality [ISO 9001, 2008] and traceability [ISO 22005, 2007]. Personnel performed manual selection and cutting to produce 10–20 cm long shoots; the av-

erage yield was *ca.* 45 g out of 100 g of starting material. Sanitary treatment (sodium hypochlorite 30 mg/L), water wash (both at 4–6°C), mechanized drying (automatic electric spin dryer extra compact – Turatti Group, Venice, Italy) at 4400/5000 round per minutes using a load of 5–8 kg. The bags were flushed (active modified atmosphere packaging) to reach a final concentration of 7.2% O₂, 8.8% CO₂, and 84% N₂, measured by Portable Gas Analyzer Checkpoint3 Dansensor (Mocon® Europe, Milan, Italy). Filling was at calibrated weight of 500 g±2%. Weight parameters (g) were measured using ten bags (gros: 514.51±9.33, net: 503.8±10.33, **bag + humidity**: 10.83±1.27, **inflorescences**: 28.67±4.49, **stems**: 101.83±7.43, **leaves**: 351.56±10.09; **i+s+l**: 482.06±11.85, **b+h+i+s+l**: 492.19±10.24). Automated vertical packaging (Olimpia 4000 Simotion, Miele, Italy) produced bags made of anti-fog polypropylene film (size 645 mm x 380 mm, thickness 35 mm; density 910 kg/m³; model PP AFM035, Masterpack S.p.A., Milan, Italy). Film permeability parameters were 8.155 (standard test method ASTM D3985), 37.69 (ASTM D1434) and 22.93 (ASTM F1249) 10⁻¹² mol/mm² s Pa respectively for O₂, CO₂, and H₂O. Filled-in bags were stored in cold rooms at 7±1°C in the dark.

As for sampling of unprocessed material (Figure 1 a–f), three bulks (each of 1 kg, biological replicate) of delivered shoots were used to generate four batches, each consisting of entire shoots (*ca.* 15 cm), flowers, stems, and leaves. Dietary fibre and glycaemic carbohydrates were determined in entire shoots, while antioxidant parameters were assessed in entire shoots and composing organs. As for the packaged material, the content of each bag represented a replicate batch (Figure 1 g–h). All samples were immediately frozen and crunched in liquid nitrogen, stored at -80°C. An aliquot of 100 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. Three replicates were used in all assays and all the measurements were in triplicate. The Italian law imposes consumption within 2 days after bag opening (DM n° 3746–2014); factories have conventionally fixed the sell-by dates at 7 days post packaging (pp) because consumer's choice is affected by storage prolongation [Stranieri & Baldi, 2017]. In this work, the storage time analyses of antioxidant parameters included plant material before processing

and bagged shoots at 1, 48, and 96 h pp (hpp), considering this lapse as the one during which the consumer most likely eats the product.

Contents of extractive, dietary fibre, glycaemic carbohydrate, and ashes

Entire shoots were sampled at the post-cut stage; quantification of cellulose, hemicellulose, and lignin was performed on ground lyophilized tissue (0.3 g) after separation of extractive; it was based on acid treatment to depolymerize polysaccharide and release Klason and soluble lignin fractions [Sluiter *et al.*, 2008b]. Monomeric sugars were analysed by HPAE chromatography, using pulsed amperometric detection (Thermo Scientific™ Dionex™ ICS-5000, Sunnyvale, CA U.S.A.) and CarboPac PA20 (4 mm x 250 mm)/dedicated guard columns [Dionex Technical note 40, 2016].

Runs were carried out at 30 °C, flow rate of 0.4 mL/min, and a sodium hydroxide/sodium acetate-stepped gradient that consisted of: 2.4/0 mM, 0–18 min; 100/0 mM, 18–20 min; 100/0 mM, 20–30 min; 100/100 mM, 30–30.1 min; 100/100 mM, 30.1–46 min; 200/0 mM, 46–46.1 min; 200/0 mM, 46.1–50 min; 2.4/0 mM, 50–50.1 min. The eluents and the standard solutions were prepared using HPLC grade reagents (Sigma, Steinheim, Germany). Data were processed by Chromeleon software versions 6.8. Dietary fibre was calculated as the sum of total lignin, hemicellulose, pectin, and cellulose. Extractive separation and ashes determination were carried out exactly as described [Sluiter *et al.*, 2008a,c]. Starch and soluble sugars (glucose, fructose and sucrose) were determined as previously reported [Moscatello *et al.*, 2017] with minor modifications. Briefly, 10 mg of lyophilized tissue powder was added to 1.5 mL of an extraction ethanol-buffer mixture – 80% ethanol and 20% buffer (Hepes-KOH 100 mM, 10 mM MgCl₂, pH 7.1), gently shaken (80°C, 45 min) and centrifuged (14,000×g, 5 min). The supernatant was collected for soluble sugars analysis (5 min centrifugation at 16,000×g). The pellet containing starch was washed four times with 1 mL of acetate buffer (40 mM, pH 4.5), autoclaved (1 mL of washing buffer, 45 min, 120°C) to solubilize starch, and finally incubated with α-amylase and amyloglucosidase (4 and 40 U, respectively) for 1 h at 50°C up to complete hydrolysis into glucose. The quantification of glucose, fructose, and sucrose from the extraction supernatant



FIGURE 1. Industrial process chain: “cime di rapa” at San Lidano industry (a). Batches of flowers (b), leaves (c), and stems (d) that derived from shoots of BSM90 before processing (e). Shoots of H39 (f), arrows indicate leaf margin that is less fringed than BSM90. Packaged product (g) and inner content (h). Dietary fibre and glycaemic carbohydrates were determined in shoots (e and f), while antioxidant parameters were assessed in all samples.

and of glucose from starch hydrolysis (after centrifugation, $16,000\times g$, 5 min) was achieved by spectrophotometric coupled enzymes assays, using a Plate reader (Spectrotar Nano BMG, Labatech GmbH, Ortenberg, Germany).

Extraction and determination of total contents of phenolics, flavonoids, flavonols, and glucosinolates

Extraction was carried out based on previous protocols [Li *et al.*, 2011]. Briefly, lyophilized material (0.2 g) was added to (final concentration of 10 mg/mL) a water-methanol mixture (20–80%, v/v). The suspension was shaken for 3 h in the dark at 4°C, and then centrifuged at $3,000\times g$ for 10 min at 4°C. The supernatant was recovered, aliquoted at 4°C for the content determinations, and stored at –80°C. Phenolic compounds were measured spectrophotometrically at 760 nm according to the Folin–Ciocalteu method [Li *et al.*, 2011] and the total phenolic content was expressed as milligrams of gallic acid equivalents per gram (mg GAE/g) of dry weight (DW). Total flavonoid amount determination followed a method used for fruit [Kim *et al.*, 2003] using absorbance at 430 nm, and values were converted into milligrams of catechin equivalents per gram of DW (mg/g CE). Total flavonol quantification was at 360 nm [Castillo-Muñoz *et al.*, 2009] using quercetin as standard and expressed in milligram of quercetin equivalents per gram of DW (mg QE/g). Total glucosinolates were determined at 365 nm [Giorgetti *et al.*, 2018]. Briefly, aliquots (0.5 mL) from extracts were vacuum-dried, the pellet was re-dissolved in 0.2 mL distilled sterile water and treated with myrosinase (28 U/mL) for 1 h at 37°C to catalyse the glucosinolates conversion into isothiocyanates. Cyclo-condensation of these was achieved by adding a pre-dissolved mixture of 80 mM 1,2-benzenedithiol (0.1 mL), methanol (0.9 mL), and 0.1 M potassium phosphate buffer (0.78 mL) in a final volume of 2 mL and incubated at 64°C for 1 h. The reaction generated 1,3-benzenedithiol-2-thione from 1,2-benzenedithiol ($\epsilon=23,000$ /M cm at 365 nm) and glucosinolates were quantified referring to the calibration curve of sinigrin and expressed as milligram of sinigrin equivalents per gram of DW (mg/g SIN).

Antioxidant activity by chemical assays

The oxygen radical absorbance capacity (ORAC) assay was performed by minor modifications [Ninfali *et al.*, 2005]. Briefly, 100 μ L of the extract was diluted (1:10, 1:100: 1:1000, v/v) and added to a mixture of 1 mL final volume. The mixture contained 800 μ L sodium phosphate buffer (75 mM, pH 7.0) with fluorescein sodium salt (0.05 μ M) plus a 100 μ L solution of 2,2'-azobis(2-amidinopropane) dihydrochloride (400 mM). The standard mixture consisted of 100 μ L of 50 μ M 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox); the control consisted of sodium phosphate buffer (75 mM, pH 7.0). The Perkin-Elmer Victor™ X3 apparatus (Waltham, MA) measured fluorescence every 5 min at 37°C at 485 nm excitation, 520 nm emission for 60 cycles. The ORAC values were calculated by the formula $(As-Ab/At-Ab)*KA$, where: As, area subtended by the curve (AUC) of fluorescein in the sample (Perkin Elmer 2030 Work Station); At and Ab, Trolox and control AUCs, respectively. K, dilution factor; and A, Trolox concen-

tration (μ M). The ORAC unit was expressed in micromoles of Trolox equivalents per g of DW (μ mol/g TE). The radical scavenging activity was also determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH•) assay [Boudjou *et al.*, 2013]. The extract (50 μ L) was added to a solution of 60 μ M methanol dissolved DPPH• (final volume 2 mL), vortexed and incubated at 25°C for 60 m in the dark. Blank consisting of pure methanol and control was ethanol (50 μ L) instead of extract; absorbance was measured at 517 nm. The anti-radical activity (ARA) was expressed as percentage of DPPH inhibition relative to the control by the equation: $ARA = [1-(As/Ac)] \times 100$, with As and Ac as absorbance values of sample and control, respectively.

Statistical analysis

All parameters were analysed according to a completely randomised design with three replications. ANOVA was carried out by a General Linear Model (GLM, SAS Software, Cary, NC, USA). Fibre and sugar content data were analysed in relation to genotypes by a one-way ANOVA, while two-way ANOVA was applied to the antioxidant data deriving from post-cut fresh product (genotype/organs) and from packaged product (genotype/storage). The Least Significant Difference test ($\alpha=0.05$) was used to separate means. PROC CORR in SAS performed variable correlation analyses on samples of entire shoots and composing parts at the time 0 plus bagged products sampled at 1, 48, and 96 h of storage for both genotypes analysed.

RESULTS AND DISCUSSION

Dietary fibre and glycaemic carbohydrates of pre-packaged shoots

The H39 and BSM90 entire shoots were sampled after cutting during the industrial process (Figure 1) and assayed for the contents of sugars, dietary fibre, extractives, and ashes (Table 2). Overall, the extractives (polar and non-polar soluble fractions included sugars, organic acids, lipids, phenolics, soluble proteins, and pigments) were the most abundant category and the genotypes showed no significant difference. Sugars (Table 2) were grouped into dietary fibre (cell wall components and lignin, DF) and glycaemic carbohydrates (GC) following their nutritional function [EFSA Panel on Dietetic Products Nutrition and Allergies, 2010]. DF was over 5 times higher than GC and higher in BSM90 than H39 (39.64 vs. 34.57 gram per 100 grams of DW). As for total lignin, the genotypes shared similar contents (9.21–9.56 g/100 g DW), though the soluble component was higher in BSM90 (4.11 vs. 3.07 g/100 g DW), while the Klason fraction was higher in H39 (6.13 vs. 5.45 g/100 g DW) leading to a Klason/soluble ratio of 2.0 and 1.3 in H39 and BSM90, respectively. Galactose, arabinose, xylose, and mannose participated to the hemicellulose polymer, which had contents of 5.67 and 7.61 g/100 g DW in H39 and BMS90, respectively. The most abundant monomer was galactose followed by arabinose, xylose, and mannose, respectively representing the 35%, 29–34%, 19–24%, and 12–13% fractions of the total content. The BSM90 genotype was ca. 1.3-fold richer in total hemicellulose than H39 due to

TABLE 2. Fibre and sugar contents of entire shoots of “cime di rapa” after cutting.

Compounds ¹	BSM90 (g/100 g)	H39 (g/100 g)	Signif.
Lignin (Klason)	5.45±0.03 ^b	6.13±0.32 ^a	*
Soluble lignin	4.11±0.22 ^a	3.07±0.2 ^b	**
<i>Total lignin</i>	9.56±0.19	9.21±0.13	<i>n.s.</i>
Arabinose	2.58±0.01 ^a	1.63±0.11 ^b	***
Galactose	2.67±0.03 ^a	1.97±0.18 ^b	**
Xylose	1.48±0.04 ^a	1.34±0.07 ^b	*
Mannose	0.89±0.06 ^a	0.73±0.05 ^b	*
<i>Total hemicellulose</i>	7.61±0.15 ^a	5.67±0.4 ^b	**
Ramnose	0.79±0.05 ^a	0.70±0.04 ^b	*
Galacturonic acid	11.20±0.40 ^a	9.82±0.49 ^b	*
<i>Total pectin</i>	11.99±0.45 ^a	10.52±0.53 ^b	*
Cellulose	10.48±0.35 ^a	9.18±0.70 ^b	*
Dietary fibre	39.64±1.14 ^a	34.57±1.69 ^b	**
Glucose	3.48±0.16 ^a	0.80±0.08 ^b	***
Fructose	2.80±0.15 ^a	0.99±0.15 ^b	***
Sucrose	0.70±0.01 ^a	0.27±0.03 ^b	***
<i>Soluble sugars</i>	6.97±0.32 ^a	2.06±0.26 ^b	***
Starch	0.59±0.04 ^a	0.15±0.05 ^b	***
Glycaemic carbohydrates	7.56±0.28 ^a	2.21±0.30 ^b	***
Other parameters			
Residual humidity	3.58±0.05	3.27±0.07	<i>n.s.</i>
Ashes	10.84±0.02 ^b	13.15±0.17 ^a	**
Extractives	47.41±5.07	46.75±1.36	<i>n.s.</i>

¹ mean ± standard deviation; g, grams of dry weight. Significance letters refer to the genotype incidence; *n.s.*, non-significant; *, **, *** = significant at $P \leq 0.05$, 0.01, and 0.001, respectively. Mean ratios (percentage) of dry (lyophilized) vs. fresh weight tissues were 11.75±1.03 and 10.58±0.43 with no significant difference for BSM90 and H39, respectively. Division factors of 8.5 and 9.5 were used to convert data into g/100 g fresh weight for BSM90 and H39, respectively.

the higher level of each monosaccharide, and particularly of arabinose and galactose. The pectin group consisted of galacturonic acid and rhamnose (93 vs. 7% of the total pectin, respectively) and total contents were higher in BSM90 (11.99 vs. 10.52 g/100 g DW). BSM90 also had a higher amount of cellulose (10.48 vs. 9.18 g/100 g DW) and, overall, the great DF content was due to the major contribution of hemicellulose. GC included glucose fructose, sucrose, and starch and showed abundance differences between the two genotypes. Looking at mean values of soluble sugars in H39 and BSM 90, glucose (0.80 vs. 3.48 g/100 g DW) and fructose (0.99 vs. 2.80 g/100 g DW) prevailed on sucrose contents (0.27 vs. 0.70 g/100 g DW). The amounts of soluble sugars and starch (0.15 vs. 0.59 g/100 g DW) were

at least 2.5-fold higher in BSM90 than H39 shoots, accounting for the higher total carbohydrate content in the former (7.56 vs. 2.21 g/100 g DW). Finally, ashes were more abundant in H39 than BSM90 (13.15 vs. 10.84 g/100 g DW, respectively).

Rapini nutrient contents are expressed as g/100 g fresh weight (FW) in the USDA database [https://fdc.nal.usda.gov] and those of BSM90 and H39 were converted accordingly (see notes of Tables 2 and 3 for DW/FW ratios and conversion factors). The comparisons of BSM90-H39 vs. USDA showed that values of the former were higher as for ashes (1.3–1.4 vs 0.9) and fibre (4.7–3.6 vs. 2.7), lower as for total carbohydrates (0.9–0.2 vs. 2.9), and in comparable ranges as for soluble sub-fraction (0.8–0.2 vs. 0.4). Quantification of DF components was previously undescribed in broccoli-*raab* (*B. rapa* subsp. *sylvestris*) that showed higher levels than turnip greens of *B. rapa* subsp. *rapa* [Francisco *et al.*, 2011] and comparable to that of broccoli (*B. oleracea*), which have positive impact on human microbiota with a 200 g daily consumption [Kaczmarek *et al.*, 2019]. Given that a 25 g daily consumption of DF is optimal [EFSA Panel on Dietetic Products Nutrition and Allergies, 2010], 200 g serve of BSM90 and H39 would respectively provide 37% and 22% of the requirement. Content variations of lignin, pectin, and hemicellulose monomers were under genotype effects as also reported in turnip greens [Francisco *et al.*, 2011]. Galactose, arabinose, xylose, and mannose were here included as hemicellulose components, however the abundance of arabinose and galactose may also derive from arabinogalactans. The total content of pectin was underestimated because only galacturonic acid and rhamnose were quantified; however they were the most abundant fraction of DF. The GC higher content in BSM90 vs. H39 may confer a sweeter flavour (appreciated by consumers) that counterbalances the bitterness associated to glucosinolates [Schonhof *et al.*, 2004]. Finally, starch quantification enriched information on “cime” nutrient compared to the USDA database.

Antioxidant compounds and capacity of shoots and composing organs before packaging

Comparing BSM90 with H39 (Table 3), mean values from entire shoots were 18.64–14.92 mg GAE/g of total phenolic compounds (TPC) content, 34.74–30.96 mg/g CE of flavonoids (FID) content, 14.62–14.08 mg QE/g of flavonols (FOL) content, 37.47–24.63 mg/g SIN of glucosinolates (GSL) content, 354.62–293.25 $\mu\text{mol/g}$ TE of ORAC, and 59.35–46.14 % ARA against DPPH[•]. The sum of values measured in each shoot component was not always consistent with the values of entire shoots (*e.g.*, +48–56% for FOL content, +12–20% for ORAC, +10–14% for ARA against DPPH[•]), likely because of the variability of organ frequency in the shoots. The leaves of both genotypes showed the highest values of all parameters, followed by those of florets and stems. Specifically, leaves had on average double ORAC and contents of FID, FOL, and GSL than florets, and at least five-fold higher levels of FID and GSL than stems. The ANOVA pointed at the genotype (G) effects on TPC, GSL, ARA against DPPH[•] and ORAC levels ($P \leq 0.01$ and 0.001) that were recurrently higher in BSM90 than H39. The organ type (O) significantly

TABLE 3. Antioxidant compound and glucosinolate contents and antioxidant capacity in shoots and organs of H39 and BMS90 “cime di rapa” at post-cut phase¹.

Genotype	Organ	TPC (mg/g GAE)	FID (mg/g CE)	FOL (mg/g QE)	GSL (mg/g SIN)	ORAC (μ mol/g TE)	ARA (%)
BMS90	Shoot	18.64 \pm 1.29	34.74 \pm 8.51	14.62 \pm 2.76	37.47 \pm 6.39 ^a	354.62 \pm 39.21	59.35 \pm 5.51 ^a
	Leaves	9.85 \pm 1.82	33.07 \pm 12.42	19.51 \pm 5.42	28.60 \pm 7.37 ^b	242.97 \pm 21.19	29.57 \pm 3.44 ^c
	Flowers	7.47 \pm 2.10	17.03 \pm 2.82	10.21 \pm 1.46	8.70 \pm 4.57 ^{cd}	125.22 \pm 4.26	26.17 \pm 0.11 ^c
	Stems	3.15 \pm 0.88	5.69 \pm 1.54	3.27 \pm 0.44	3.21 \pm 1.14 ^d	73.17 \pm 5.07	10.43 \pm 0.39 ^c
H39	Shoot	14.92 \pm 0.98	30.96 \pm 9.07	14.08 \pm 2.54	24.63 \pm 2.04 ^b	293.25 \pm 18.14	46.14 \pm 0.56 ^b
	Leaves	7.66 \pm 1.13	36.03 \pm 23.36	16.21 \pm 4.82	14.69 \pm 3.12 ^c	190.48 \pm 5.13	25.36 \pm 6.61 ^c
	Flowers	5.89 \pm 2.24	12.94 \pm 2.91	7.73 \pm 2.20	6.3 \pm 4.89 ^d	82.66 \pm 1.53	18.54 \pm 1.46 ^d
	Stems	2.35 \pm 0.40	4.30 \pm 0.61	2.93 \pm 1.15	3.18 \pm 1.16 ^d	61.25 \pm 2.80	9.86 \pm 0.89 ^c
Signif.	G	**	n.s.	n.s.	***	***	***
	O	***	***	***	***	***	***
	G x O	n.s.	n.s.	n.s.	*	n.s.	*

¹mean \pm standard deviation; g, grams of dry weight. G, genotype; O, organ. Significance letters refer to G \times O interactions; n.s., non-significant; * ** *** = significant at P \leq 0.05, 0.01 and 0.001, respectively. TPC, total phenolic compounds; FID, flavonoids; FOL, flavonols; GSL, glucosinolates; ORAC, oxygen radical absorbance capacity; ARA, DPPH^{*}-based antiradical activity. Other abbreviations are in Materials and methods. See Table 2 for division factors to convert into mg/g fresh weight.

influenced all parameters (P \leq 0.001), whereas G \times O interactions affected only GSL and ARA against DPPH^{*} variations (P \leq 0.05). The higher contents of TPC were likely to explain the 1.2-fold higher antioxidant activity (ARA against DPPH^{*}, ORAC) of BMS90 vs. H39 shoots.

Hereafter we refer to literature results based on methods and units comparable to this work. As for the entire shoot, TPC ranges of “friariello” were 89–118 and 220–250 mg GAE/100 g FW [De Pascale *et al.*, 2009; Romani *et al.*, 2006] and are consistent with those of Table 3, converted into 157–219 mg GAE/100 g FW. FID determination included values of 39–104 mg CE/g FW from *B. oleracea* vegetables [Reilly *et al.*, 2014], namely over four times lower than broccoli-raab shoots (325–408 mg CE/g FW). As for the shoot components, FOL content ranges of *B. oleracea* florets were 4.5–5.4 mg QE/g DW [Naguib *et al.*, 2012] and lower than those of “cime di rapa” (Table 3). These FOL content differences (higher contents in *B. rapa* “friariello” than *B. oleracea*) were also observed by chromatographic quantifications of quercetin, kaempferol, and isorhamnetin forms [Romani *et al.*, 2006]. As for GSL, total sums of different classes revealed similar contents between leaves (10.6–39.8 μ mol/g DW) and entire shoots (20.9–31.4 μ mol/g DW) in “friariello” [Barbieri *et al.*, 2008; De Pascale *et al.*, 2007], while in this study, leaf GSL content was 60–76% of the entire shoot. The discrepancies may be due to different type of sampling or genotypes. Moreover, GSL amounts of rapini florets (66.32–102.35 mg SIN/100 g FW) were comparable with those of broccoli [Fernández-León *et al.*, 2013]. Regarding ORAC values, those previously reported [Wu *et al.*, 2004] for broccoli-raab fell in the ranges of this work (30.8 vs. 30.8–41.7 μ mol/g TE FW); ARA percentages of *B. rapa* var. *rapa* leaves [Iqbal *et al.*, 2013] were higher than stems, 25–35% vs. 20–25% similarly to “cime di rapa”. The G, E, and G \times E effects were reported for phenolic

compound contents and antioxidant properties of “friariello” [Barbieri *et al.*, 2009; De Pascale *et al.*, 2007], while GSL contents were under control of these factors in several *Brassica* vegetables [Reilly *et al.*, 2014], consistently with results of this work. “Cime di rapa” contained higher TPC and AOC values in leaves than flowers similarly to other *Brassica* vegetables [Bhandari & Kwak, 2015]. Finally, leaves, stems, and florets represented the 72.95 \pm 2.08, 21.12 \pm 1.28 and 5.94 \pm 0.83% of the packaged product. Considering that florets have higher TPC/AOC values than stems and are much enjoyed by consumers, increasing the flower/stem ratio (by selective cutting or genetic improvement) can improve the final product antioxidant quality.

Antioxidant quality profile of bagged shoots during shelf life

The product at 48 hpp was assumed as the most probably consumed; the mean values for BMS90 and H39 were respectively: 16.07–14.54 mg GAE/g of TPC content, 12.17–9.39 mg/g CE of FID content, 2.96–4.29 mg QE/g of FOL content, 34.55–24.71 mg/g SIN of GSL content, 185.49–178.36 μ mol/g TE of ORAC, and 86.80–90.44% of ARA against DPPH^{*} (Table 4). During the 1 to 96 hpp lapse, the storage time (ST) effects were strongly significant on FID, FOL, and ORAC variables (P \leq 0.001), less on TPC and ARA ones (P \leq 0.05), and unrevealed on GSL (Table 4). More precisely, ST effects were linear in ORAC (-25%) along the whole time lapse, and oscillatory for FID and FOL contents (*e.g.*, for FOL -50 % at 48 hpp and -10% at 96 vs. 1 hpp). The genotype had a modest influence on the variation of TPC and ORAC values and the G \times ST interaction mildly affected FID variations, essentially due to an increase in FID content in H39 at 96 hpp, opposite to the general decreasing trend. Finally, looking at the compound con-

TABLE 4. Variation of compound contents and antioxidant capacity during storage of “cime di rapa” bagged products¹.

Genotype	Storage (hour pp)	TPC (mg/g GAE)	FID (mg/g CE)	FOL (mg/g QE)	GSL (mg/g SIN)	ORAC ($\mu\text{mol/g TE}$)	ARA (%)
BSM90	1	15.21 \pm 1.67	21.85 \pm 5.81 ^a	8.51 \pm 2.11	31.00 \pm 8.43	248.13 \pm 35.77	87.13 \pm 1.29
	48	16.07 \pm 1.00	12.17 \pm 2.12 ^b	2.96 \pm 0.31	34.55 \pm 6.02	185.49 \pm 16.17	86.80 \pm 2.57
	96	12.43 \pm 1.16	14.34 \pm 2.73 ^b	5.38 \pm 0.76	26.86 \pm 11.32	195.26 \pm 13.46	90.26 \pm 1.07
H39	1	12.71 \pm 1.27	20.52 \pm 4.91 ^{ab}	8.44 \pm 1.51	22.81 \pm 1.19	222.37 \pm 28.94	87.07 \pm 1.06
	48	14.54 \pm 0.13	9.39 \pm 1.52 ^b	4.29 \pm 1.01	24.71 \pm 1.19	178.36 \pm 3.56	90.44 \pm 0.99
	96	11.79 \pm 2.63	23.36 \pm 2.16 ^a	5.97 \pm 0.63	26.50 \pm 6.68	156.46 \pm 3.42	89.34 \pm 1.48
Signif.	G	*	n.s.	n.s.	n.s.	*	n.s.
	ST	*	***	***	n.s.	***	*
	G x ST	n.s.	*	n.s.	n.s.	n.s.	n.s.

¹mean \pm standard deviation; g, grams of dry weight. Mean ratios of dry vs. fresh weight (percentage) calculated for the 1–48–96 hpp timing were 9.15 \pm 0.39 and 7.86 \pm 0.21, with no significant difference for BSM90 and H39, respectively. Division factors of 10.9 and 12.7 were used to convert data into mg/g fresh weight for BSM90 and H39, respectively. Conversion factors specifically refer to packaged material. TPC, total phenolic compounds; FID, flavonoids; FOL, flavonols; GSL, glucosinolates; ORAC, oxygen radical absorbance capacity; ARA, DPPH[•]-based antiradical activity. G, genotype; ST, storage time. Significance letters refer to G \times ST interactions; n.s., non-significant; *, **, *** = significant at P \leq 0.05, 0.01 and 0.001, respectively.

tents and chemical antioxidant capacity, the ORAC positive correlation with TPC was very strong ($r \geq 0.80$) and strong ($0.60 \leq r < 0.80$) with GSL and FID. The ARA against DPPH[•] antiradical activity showed strong positive correlation with TPC and GSL amounts, however a significant weak positive correlation ($r = 0.45$) occurred between the two antioxidant assays (Table 5).

Processing steps are known to alter nutritive contents of packaged vegetables and, consistently, the comparison of shoots at the post-cut phase vs. bagged products at 48 hpp (Tabs 3 and 4) pointed at level drops of most variables (3–14% for TPC, 65–70% for FID, 70–80% for FOL, 0–8% for GSL, and 39–48% for ORAC). Storage time behaves as an additive factor affecting ACC and AOC of *B. rapa* [Pouria & Seid Mahdi, 2019] and its effects on FID, FOL, and ORAC values were significant in this work. The FID fall-and-raise trend of packaged broccoli-raab recalled that of kale at similar modified atmosphere packaging (MAP) and storage conditions [Kobori et al., 2011]; this behaviour may reflect time-regulated interactions between stress response and senescence pathways as described in lettuce leaves [Ripoll et al., 2019]. Moreover, specific FID classes occur in distinct *Brassica* spp. tissues [Fernandes et al., 2007], and fluctuations may also derive from organ-specific stress responses. Regarding GSL, the O₂/CO₂ equilibria (3–10% vs. 5–10%) in MAP are crucial for brassica product quality and specific conditions for broccoli-raab (8% O₂ and 2% CO₂) increased shelf-life, while higher CO₂ was envisaged to improve it [Conte et al., 2011]. Here, the 7.2% O₂ and 8.8% CO₂ contributed to stabilize the GSL content until 96 hpp and, concurrently, off-odours (not quantified in this work) were unperceived by subjective sensorial analyses. The genotype effects on TPC and ORAC were non- or lowly-significant in pre-processed shoots vs. bagged products; the mitigation of differences between H39 and BSM90 might have been due to diversified responses to work steps before packaging. TPC levels showed a positive

TABLE 5. Correlations between antioxidant activities and compound contents¹ in *B. rapa* subsp. *sylvestris*.

	TPC	FID	FOL	GSL	ARA	ORAC
TPC	1.00	0.37*	0.21n.s.	0.83***	0.77***	0.82***
FID		1.00	0.82***	0.34*	0.05n.s.	0.64***
FOL			1.00	0.25n.s.	-0.22n.s.	0.58***
GSL				1.00	0.71***	0.79***
DPPH					1.00	0.45**
ORAC						1.00

¹Pearson's correlation coefficients (r) and related significance (asterisks). The correlation was established using the whole data set of unprocessed and bagged materials (n=42). TPC, total phenolic compounds; FID, flavonoids; FOL, flavonols; GSL, glucosinolates; ORAC, oxygen radical absorbance capacity; ARA, DPPH[•]-based antiradical activity. n.s., not significant; * P \leq 0.05; ** P \leq 0.01; *** P \leq 0.001.

correlation with those from both AOC assays, in agreement with several studies on *Brassica* vegetables [Li et al., 2018]. Focusing on GSL, their radical scavenging activity depends on the chemical assay and accounts for a small percentage of the total antiradical activity [Cabello-Hurtado et al., 2012]; the GSL positive correlations vs. both AOC assays confirm their (minimal) contribution. In addition, the modest positive correlation between ORAC/ARA against DPPH[•] assays has been previously observed and rely in different sensitivity to diverse antioxidant compounds and/or their mixtures [Floegel et al., 2011], some of which were untested in this work (e.g., vitamin C, carotenoids etc.). Finally, the ORAC value drop and the moderate ARA against DPPH[•] value raise during storage may reflect this differential sensitivity and confirm the necessity of multiple and combined assays to assess antioxidant quality criteria.

CONCLUSIONS

Genotype-dependent differences were observed in fresh unprocessed “cime di rapa” as for the contents of dietary fibre, glycaemic sugars, phenolics, flavonoids, flavonols, glucosinolates, and antioxidant capacity; most values were lower in the hybrid genotype, which may specifically target fresh consumption considering that low fibre levels enhance tenderness. Reducing the stem component in favour of leaves and florets will enhance the antioxidant properties of the product. Level decay of most parameters (except for glucosinolates) occurred in the minimally processed products compared to the pre-packaged ones. The antioxidant quality of packaged product was strongly affected by storage time while feebly by genotype (and factor interactions). Proper gas composition in MAP may have compensated genotype differences and stabilized glucosinolates content variation. Correlation analyses between antioxidant contents and antioxidant capacity support the necessity of performing multiple antioxidant assays to evaluate the product quality.

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CONFLICT OF INTERESTS

The authors report no conflicts of interest and are responsible for the content of this article.

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REFERENCES

- Annunziata, M.G., Attico, A., Woodrow, P., Oliva, M.A., Fuggi, A., Carillo, P. (2012). An improved fluorimetric HPLC method for quantifying tocopherols in *Brassica rapa* L. subsp *sylvestris*

- after harvest. *Journal of Food Composition and Analysis*, 27(2), 145–150.
<https://doi.org/10.1016/j.jfca.2012.05.006>
- Barbieri, G., Bottino, A., Orsini, F., De Pascale, S. (2009). Sulfur fertilization and light exposure during storage are critical determinants of the nutritional value of ready-to-eat friariello campano (*Brassica rapa* L. subsp *sylvestris*). *Journal of the Science of Food and Agriculture*, 89(13), 2261–2266.
<https://doi.org/10.1002/jsfa.3718>
- Barbieri, G., Pernice, R., Maggio, A., De Pascale, S., Fogliano, V. (2008). Glucosinolates profile of *Brassica rapa* L. subsp *sylvestris* L. Janch. var. *esculenta* Hort. *Food Chemistry*, 107(4), 1687–1691.
<https://doi.org/10.1016/j.foodchem.2007.09.054>
- Bhandari, S.R., Kwak, J.-H. (2015). Chemical composition and antioxidant activity in different tissues of brassica vegetables. *Molecules*, 20(1), 1228–1243.
<https://doi.org/10.3390/molecules20011228>
- Bonnema, G., Del Carpio, D.P., Zhao, J. (2011). Diversity analysis and molecular taxonomy of brassica vegetable crops. In Sadowski J. and Kole C. (Eds.), *Genetics, Genomics and Breeding of Vegetable Brassicas*, 1 ed, CRC Press, Boca Raton, Florida, USA, pp. 81–124.
- Boudjou, S., Oomah, B.D., Zaidi, F., Hosseinian, F. (2013). Phenolics content and antioxidant and anti-inflammatory activities of legume fractions. *Food Chemistry*, 138(2–3), 1543–1550.
<https://doi.org/10.1016/j.foodchem.2012.11.108>
- Cabello-Hurtado, F., Gicquel, M., Esnault, M.-A. (2012). Evaluation of the antioxidant potential of cauliflower (*Brassica oleracea*) from a glucosinolate content perspective. *Food Chemistry*, 132(2), 1003–1009.
<https://doi.org/10.1016/j.foodchem.2011.11.086>
- Cartea, M.E., Francisco, M., Soengas, P., Velasco, P. (2010). Phenolic compounds in Brassica vegetables. *Molecules*, 16(1), 251–280.
<https://doi.org/10.3390/molecules16010251>
- Castillo-Muñoz, N., Gómez-Alonso, S., García-Romero, E., Gómez, M.V., Velders, A.H., Hermosín-Gutiérrez, I. (2009). Flavonol 3-O-glycosides series of *Vitis vinifera* Cv. Petit Verdot red wine grapes. *Journal of Agricultural and Food Chemistry*, 57(1), 209–219.
<https://doi.org/10.1021/jf802863g>
- Cefola, M., Amodio, M.L., Cornacchia, R., Rinaldi, R., Vanadia, S., Colelli, G. (2010). Effect of atmosphere composition on the quality of ready-to-use broccoli raab (*Brassica rapa* L.). *Journal of the Science of Food and Agriculture*, 90(5), 789–797.
<https://doi.org/10.1002/jsfa.3885>
- Conte, A., Scrocco, C., Brescia, I., Mastromatteo, M., Del Nobile, M.A. (2011). Shelf life of fresh-cut Cime di rapa (*Brassica rapa* L.) as affected by packaging. *LWT – Food Science and Technology*, 44(4), 1218–1225.
<https://doi.org/10.1016/j.lwt.2010.11.006>
- Conversa, G., Bonasia, A., Lazzizzera, C., Elia, A. (2016). Biophysical, physiological, and nutritional aspects of ready-to-use cima di rapa (*Brassica rapa* L. subsp. *sylvestris* L. Janch. var. *esculenta* Hort.) as affected by conventional and organic growing systems and storage time. *Scientia Horticulturae*, 213, 76–86.
<https://doi.org/10.1016/j.scienta.2016.10.021>
- De Pascale, S., Maggio, A., Pernice, R., Fogliano, V., Barbieri, G. (2007). Sulphur fertilization may improve the nutritional value

- of *Brassica rapa* L. subsp. *sylvestris*. *European Journal of Agronomy*, 26(4), 418–424.
<https://doi.org/10.1016/j.eja.2006.12.009>
14. De Pascale, S., Orsini, F., Vallone, S., Barbieri, G. (2009). Crop season effects on yield and quality of hydroponically grown *Brassica rapa* var. *sylvestris*. *Acta Horticulturae*, 87, 427–432.
<https://doi.org/10.17660/ActaHortic.2009.807.60>
 15. Dionex Technical note 40 (2016). Glycoprotein Monosaccharide Analysis Using HPAE-PAD with Eluent Generation. in Inc., T.F.S. Ed., Sunnyvale, CA, USA, p. 10.
 16. EFSA Panel on Dietetic Products Nutrition and Allergies (2010). Scientific Opinion on Dietary Reference Values for carbohydrates and dietary fibre. *EFSA Journal*, 8(3), 1462–1539.
<https://doi.org/10.2903/j.efsa.2010.1462>
 17. Elia, A., Santamaria, P. (2013). Biodiversity in vegetable crops, a heritage to save: the case of Puglia region. *Italian Journal of Agronomy*, 8(1), 21–34.
<https://doi.org/10.4081/ija.2013.e4>
 18. Fernandes, F., Valentão, P., Sousa, C., Pereira, J.A., Seabra, R.M., Andrade, P.B. (2007). Chemical and antioxidative assessment of dietary turnip (*Brassica rapa* var. *rapa* L.). *Food Chemistry*, 105(3), 1003–1010.
<https://doi.org/10.1016/j.foodchem.2007.04.063>
 19. Fernández-León, M.F., Fernández-León, A.M., Lozano, M., Ayuso, M.C., Amodio, M.L., Colelli, G., González-Gómez, D. (2013). Retention of quality and functional values of broccoli ‘Parthenon’ stored in modified atmosphere packaging. *Food Control*, 31(2), 302–313.
<https://doi.org/10.1016/j.foodcont.2012.10.012>
 20. Floegel, A., Kim, D.-O., Chung, S.-J., Koo, S.I., Chun, O.K. (2011). Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. *Journal of Food Composition and Analysis*, 24(7), 1043–1048.
<https://doi.org/10.1016/j.jfca.2011.01.008>
 21. Francisco, M., Velasco, P., Lema, M., Cartea, M.E. (2011). Genotypic and environmental effects on agronomic and nutritional value of *Brassica rapa*. *Agronomy Journal*, 103(3), 735–742.
<https://doi.org/10.2134/agronj2010.0439>
 22. Giorgetti, L., Giorgi, G., Cherubini, E., Gervasi, P.G., Della Croce, C.M., Longo, V., Bellani, L. (2018). Screening and identification of major phytochemical compounds in seeds, sprouts and leaves of Tuscan black kale *Brassica oleracea* (L.) ssp. *acephala* (DC) var. *sabellica* L. *Natural Product Research*, 32(14), 1617–1626.
<https://doi.org/10.1080/14786419.2017.1392953>
 23. Iqbal, S., Younas, U., Chan, K.W., Saeed, Z., Shaheen, M.A., Akhtar, N., Majeed, A. (2013). Growth and antioxidant response of *Brassica rapa* var. *rapa* L. (turnip) irrigated with different compositions of paper and board mill (PBM) effluent. *Chemosphere*, 91(8), 1196–1202.
<https://doi.org/10.1016/j.chemosphere.2013.01.035>
 24. ISO 9001 (2008). Quality management systems – Requirements. [<https://www.iso.org/standard/46486.html>], Last accessed 26/08/2020.
 25. ISO 22005 (2007). Traceability in the feed and food chain – General principles and basic requirements for system design and implementation. [<https://www.iso.org/standard/36297.html>], Last accessed 26/08/2020.
 26. Kaczmarek, J.L., Liu, X., Charron, C.S., Novotny, J.A., Jeffery, E.H., Seifried, H.E., Ross, S.A., Miller, M.J., Swanson, K.S., Holscher, H.D. (2019). Broccoli consumption affects the human gastrointestinal microbiota. *The Journal of Nutritional Biochemistry*, 63, 27–34.
<https://doi.org/10.1016/j.jnutbio.2018.09.015>
 27. Kim, D.-O., Chun, O.K., Kim, Y.J., Moon, H.-Y., Lee, C.Y. (2003). Quantification of polyphenolics and their antioxidant capacity in fresh plums. *Journal of Agricultural and Food Chemistry*, 51(22), 6509–6515.
<https://doi.org/10.1021/jf0343074>
 28. Kobori, C.N., Huber, L.S., Sarantópoulos, C.I.G.L., Rodriguez-Amaya, D.B. (2011). Behavior of flavonols and carotenoids of minimally processed kale leaves during storage in passive modified atmosphere packaging. *Journal of Food Science*, 76(2), H31–H37.
<https://doi.org/10.1111/j.1750-3841.2010.01988.x>
 29. Li, H., Deng, Z., Liu, R., Young, J.C., Zhu, H., Loewen, S., Tsao, R. (2011). Characterization of phytochemicals and antioxidant activities of a purple tomato (*Solanum lycopersicum* L.). *Journal of Agricultural and Food Chemistry*, 59(21), 11803–11811.
<https://doi.org/10.1021/jf202364v>
 30. Li, Z., Lee, W.H., Liang, X., Liang, D., Wang, Q., Huang, D., Ong, N.C. (2018). Profiling of phenolic compounds and antioxidant activity of 12 Cruciferous vegetables. *Molecules*, 23(5), art. no. 1139.
<https://doi.org/10.3390/molecules23051139>
 31. Moscatello, S., Proietti, S., Buonauro, R., Famiani, F., Raggi, V., Walker, R.P., Battistelli, A. (2017). Peach leaf curl disease shifts sugar metabolism in severely infected leaves from source to sink. *Plant Physiology and Biochemistry*, 112, 9–18.
<https://doi.org/10.1016/j.plaphy.2016.12.001>
 32. Naguib, A.E.-M.M., El-Baz, F.K., Salama, Z.A., Abd El Baky Hanaa, H., Ali, H.F., Gaafar, A.A. (2012). Enhancement of phenolics, flavonoids and glucosinolates of Broccoli (*Brassica oleracea*, var. *italica*) as antioxidants in response to organic and bio-organic fertilizers. *Journal of the Saudi Society of Agricultural Sciences*, 11(2), 135–142.
<https://doi.org/10.1016/j.jssas.2012.03.001>
 33. Ninfali, P., Mea, G., Giorgini, S., Rocchi, M., Bacchiocca, M. (2005). Antioxidant capacity of vegetables, spices and dressings relevant to nutrition. *British Journal of Nutrition*, 93(2), 257–266.
<https://doi.org/10.1079/BJN20041327>
 34. Pouria, G., Seid Mahdi, J. (2019). Antioxidant components of brassica vegetables including turnip and the influence of processing and storage on their anti-oxidative properties. *Current Medicinal Chemistry*, 26(24), 4559–4572.
<http://doi.org/10.2174/0929867325666181115111040>
 35. Reilly, K., Valverde, J., Finn, L., Rai, D.K., Brunton, N., Sorensen, J.C., Sorensen, H., Gaffney, M. (2014). Potential of cultivar and crop management to affect phytochemical content in winter-grown sprouting broccoli (*Brassica oleracea* L. var. *italica*). *Journal of the Science of Food and Agriculture*, 94(2), 322–330.
<https://doi.org/10.1002/jsfa.6263>
 36. Ripoll, J., Charles, F., Vidal, V., Laurent, S., Klopp, C., Lauri, F., Sallanon, H., Roux, D. (2019). Transcriptomic view of detached lettuce leaves during storage: a crosstalk between wounding, dehydration and senescence. *Postharvest Biology and Technology*, 152, 73–88.
<https://doi.org/10.1016/j.postharvbio.2019.02.004>

37. Romani, A., Vignolini, P., Isolani, L., Ieri, F., Heimler, D. (2006). HPLC-DAD/MS characterization of flavonoids and hydroxycinnamic derivatives in turnip tops (*Brassica rapa* L. subsp. *sylvestris* L.). *Journal of Agricultural and Food Chemistry*, 54(4), 1342–1346.
<https://doi.org/10.1021/jf052629x>
38. Schonhof, I., Krumbein, A., Brückner, B. (2004). Genotypic effects on glucosinolates and sensory properties of broccoli and cauliflower. *Nahrung/Food*, 48(1), 25–33.
<https://doi.org/10.1002/food.200300329>
39. Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D. (2008a). Determination of ash in biomass. Technical Report NREL/TP-510-42622, in *Laboratory*, N.R.E. (Ed. *Laboratory Analytical Procedure (LAP)*), Cole Boulevard, Golden, Colorado.
40. Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., Crocker, D. (2008b). Determination of structural carbohydrates and lignin in biomass. NREL Laboratory Analytical Procedure NREL/TP-510-42618, in *Laboratory*, N.R.E. (Ed. *Laboratory Analytical Procedure (LAP)*), Cole Boulevard, Golden, Colorado.
41. Sluiter, A., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D. (2008c). Determination of extractives in biomass. Technical Report NREL/TP-510-42619, in *Laboratory*, N.R.E. (Ed. *Laboratory Analytical Procedure (LAP)*), Cole Boulevard, Golden, Colorado, p. 12.
42. Soengas, P., Sotelo, T., Velasco, P., Cartea, M.E. (2011). Antioxidant properties of Brassica vegetables. In Books, G.S. (Ed.) *Functional Plant Science and Biotechnology*, 1 ed, Isleworth, pp 43–55.
43. Stranieri, S., Baldi, L. (2017). Shelf life date extension of fresh-cut salad: a consumer perspective. *Journal of Food Products Marketing*, 23(8), 939–954.
<https://doi.org/10.1080/10454446.2017.1266545>
44. Torrieri, E., Perone, N., Cavella, S., Masi, P. (2010). Modelling the respiration rate of minimally processed broccoli (*Brassica rapa* var. *sylvestris*) for modified atmosphere package design. *International Journal of Food Science and Technology*, 45(10), 2186–2193.
<https://doi.org/10.1111/j.1365-2621.2010.02387.x>
45. U.S.D.A. (2006). Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. Official Soil Series Descriptions. [<https://www.nrcs.usda.gov>], Last accessed 26/8/2020.
46. U.S.D.A. (2019). FoodData Central, 2019. United States Department of Agriculture, Agricultural Research Service. [<https://fdc.nal.usda.gov/>], Last accessed 26/8/2020.
47. Wu, X., Beecher, G.R., Holden, J.M., Haytowitz, D.B., Gebhardt, S.E., Prior, R.L. (2004). Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. *Journal of Agricultural and Food Chemistry*, 52(12), 4026–4037.
<https://doi.org/10.1021/jf049696w>

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