

# Variation for Health-Enhancing Compounds and Traits in Onion (*Allium cepa* L.) Germplasm

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## Abstract

Consumption of onion has been associated with reduced incidence of chronic diseases. Phenolic, organosulfur and carbohydrate compounds present are largely responsible for these effects. This study examined compositional variation for health-enhancing compounds in a genetically diverse collection of onion cultivars. Total antioxidant activity and aroma profiles were characterized. Significant variation in bulb concentration for total and individual phenolic compounds, thiosulfinates, carbohydrates, and total and soluble solids was found. The range of variation was particularly large (>50-fold difference between the cultivars with the highest and lowest content) for fructo-oligosaccharides (FOS) and the polyphenols quercetin, epigallocatechin gallate and epicatechin gallate. Amino acid profiles varied significantly as well with substantial variation (~10 fold) observed in both total and essential amino acids. Total antioxidant activity was positively correlated with polyphenols content, and quercetin in particular ( $r = 0.83$ ), suggesting a major contribution from phenolic compounds to onion antioxidant properties. Significant positive correlation was also found between solids and thiosulfinates content ( $r = 0.74$ ) and between solids and FOS ( $r = 0.81$ ), suggesting a dilution/concentration effect for organosulfur compounds and FOS in onion bulbs. The present study revealed broad variation for health-enhancing compounds content in

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**onion germplasm, which can be exploited in breeding programs aiming at increasing onion nutraceutical value.**

## Keywords

**Onion, Polyphenols, Carbohydrates, Amino Acids, Aroma Profiles**

## 1. Introduction

Consumption of *Allium* species, such as onion (*A. cepa* L.), has been associated with reduced incidence of cardiovascular disease [1] and some types of cancer [2]. These general health-protective effects may be attributed to a few well-demonstrated biological properties of alliums, such as antiplatelet, antioxidant, anti-inflammatory, anti-tumor and hypolipidemic activities [3]. Several *Allium* compounds, or groups of related compounds, have been recognized as major contributors in exerting these effects.

One source of such phytochemicals is polyphenols. Polyphenols contribute to controlling oxidative stress [4]. Their consumption has been associated with reduced risk of developing some chronic diseases of high prevalence [5]. Immuno-regulatory effects have also been reported [6]. Quercetin is the predominant polyphenol in onion bulbs [7].

Organosulfur compounds (OSC) found in *Allium* plants have protective effects against cardiovascular disease. *Allium* OSC have been reported to relieve chronic ailments, such as cancer, diabetes, and cardiovascular disease [8]. Thiosulfinates (TSs) are the major *Allium* OSC, and they are formed when fresh tissues are crushed allowing the vacuolar enzyme alliinase to interact with cytoplasmic precursors collectively known as ACSOs [for S-alk(en)yl-L-cysteine sulfoxides] giving rise to TSs, pyruvate and ammonia (for a comprehensive review on *Allium* biochemistry see Block [9]). Due to stoichiometric amounts of TSs and pyruvate are produced in the ACSOs-alliinase reaction, pyruvate and TSs contents are positively correlated [10], pyruvate analyses, which are relatively easy to perform, are often used to estimate total TSs or total OSC content [11] [12].

The main carbohydrates in onion bulbs are fructose, glucose, sucrose and low molecular weight fructans [13]. Fructans are polymers of fructose molecules derived from sucrose. Low molecular weight fructans, also known as fructo-oligosaccharides (FOS), are the primary storage carbohydrates in onion bulbs [14]. Onions FOS are an important source of soluble dietary fiber. FOS, along with trans-galactooligosaccharides (TOS), are the only dietary compounds that fulfill the classification criteria for prebiotics [15]. Consumption of prebiotics has been associated with reduced incidence of colon cancer, type-2 diabetes and obesity, modulation of the immune system, amelioration of the clinical manifestations of inflammatory bowel disease and irritable bowel syndrome, and increased calcium absorption and bone mineral density [16].

Free Amino Acids (AAs) play a crucial role in biosynthesis of both structural and functional proteins (*i.e.*, enzymes). In addition, complex roles in various metabolic processes including satiety, gastrointestinal function, glucose homeostasis and cell signaling have been described for free AAs [17]. Thus, AAs profile in foods is a relevant feature with impact on health.

In the plant consumed organs, the type and concentration of different bioactive compounds within each major chemical group will determine the type and intensity of biological activities and the overall value as a functional food.

The present study characterized bulb compositional variation, including many health-enhancing compounds, for polyphenols, OSC, carbohydrates and AAs in a genetically diverse onion collection evaluated during two growing seasons. In addition, Total Antioxidant Activity (TAA) and Aroma Profiles (AP) were determined and associated with the bulb chemical composition. To our knowledge, this study represents the most complete compositional characterization of an onion germplasm collection published to date, in terms of number of bioactive compounds evaluated. Furthermore, relationships among bioactive compounds content and TAA and aroma profiles were investigated and quantified, suggesting an important role for some of these compounds in onion flavor and antioxidant activity. Characterization of these germplasm on the basis of their health-enhancing compounds content will benefit both consumers and onion breeding programs aiming at increasing onion nutraceutical value.

## 2. Materials and Methods

### 2.1. Plant Materials

Eight onion cultivars developed by the onion breeding program at INTA-Argentina were used (Table 1). Onions were field-grown at the experimental station of INTA La Consulta in two growing seasons referred as “year 1” and “year 2”, using a completely randomized design with four replicates. The onion bulbs were obtained from seeds and the crop was established by transplant. Onion bulbs were harvested at vegetative maturity and stored in a cool storage room. The germplasms are named as X-INTA, but hereafter they are mentioned without the word INTA for a more fluent reading.

### 2.2. Sample Preparation

Aqueous extracts from ten unsprouted bulbs per replicate were obtained as follows. The dry outer scales were removed and each bulb was cut in half along the longitudinal axis to obtain a representative sample of outer and inner scales. One half from each of the ten bulbs was combined, weighed, juiced, centrifuged and the clear supernatant was aliquoted and stored at  $-80^{\circ}\text{C}$ . The other halves were combined and used for determinations of Dry Matter (DM) and Soluble Solids (SS) content. For DM determinations, bulb tissues were crushed, weighted and dried at  $50^{\circ}\text{C}$  until constant weight was reached. SS content was measured on an aliquot of fresh onion juice using a hand refractometer.

### 2.3. Analysis of Total and Individual Polyphenols

Total polyphenols (TPs) content was quantified by the Folin-Ciocalteu method of Singleton *et al.* [18]. Absorbance was measured at 760 nm in a UV-Vis spectrophotometer (Lambda Bio 20, Perkin Elmer). Results were expressed as mg-gallic Acid Equivalents—GAE·g<sup>-1</sup> dw and mg·gAE·g<sup>-1</sup>·fw.

HPLC analysis of eight individual polyphenols was carried out in an HPLC system (Thermo Separation Products Inc., USA) with an ODS-Hypersil C18 column (250 × 4.6 mm, particle size 5 μm). Compound detection was performed with a fluorescence detector (FL 3000) at 280 (excitation)-310 nm (emission) and a UV-Vis Diode Array Detector (UV 6000 LP) at 280, 306 and 369 nm. The mobile phase used for separation was a linear gradient of 0.1% trifluoroacetic acid and acetonitrile: 0.1% trifluoroacetic acid 80:20.

Calibration curves with pure compounds for catechin, epigallocatechin gallate (EGCg), epicatechin gallate (ECg), kaempferol aglicon, quercetin aglicon and myricetin were used as standards (Sigma-Aldrich, St. Louis, USA). Results were expressed as μg·g<sup>-1</sup> fw. Quercetin and kaempferol glycosides were evaluated by chemical hydrolysis. Briefly, 0.1 ml of HCl 0.25 M was added to 0.9 ml of onion juice in sealed vials and incubated for 2 h at  $100^{\circ}\text{C}$ . When samples reached ambient temperature they were filtered through polytetrafluoroethylene (PTFE) membranes (pore diameter 0.45 μm) and analysed by HPLC using the same chromatographic conditions as described above.

**Table 1.** Morphological and quantitative agronomic characteristics of eight onion cultivars.

Cultivar	Bulb color	Cultivar type <sup>a</sup>	Market end-purpose	Dry matter (g·kg <sup>-1</sup> )		Soluble solids (g·kg <sup>-1</sup> )	
				Year 1	Year 2	Year 1	Year 2
Valcatorce	yellow	long day	fresh	104	104	84	87
Cobriza	yellow	long day	fresh	110	115	92	90
Valuno	yellow	long day	fresh	96	92	77	80
Angaco	yellow	short day	fresh	90	80	81	70
Navideña	yellow	intermediate	fresh	89	89	81	75
Antártica	white	long day	fresh	164	161	150	145
Ancasti	white	short day	dehydration industry	174	215	158	201
Refinta20	white	long day	dehydration industry	221	242	216	223

<sup>a</sup>Classification based on the photoperiod requirements for bulbification (long-, intermediate-and short-day onions require a minimum of 14, 13 and 12 hours of day light, respectively).

## 2.4. Total Antioxidant Activity

Total antioxidant activity (TAA) of bulb samples from year 2 was determined using an ABTS [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation decolorization assay as described previously [19]. All samples were run twice. Results were expressed as mmoles Trolox equivalents·kg<sup>-1</sup> fw.

## 2.5. Total Thiosulfinates Analysis

Determinations of pyruvate content, an estimator of *Allium*'s total thiosulfinates (TSS) content were performed according to the method of Schwimmer and Weston [20]. The pyruvate concentration in the juice was calculated based on calibration curves obtained with pyruvate standards. Values were expressed as μmoles pyruvate g<sup>-1</sup> fw.

## 2.6. Carbohydrates

Carbohydrate analyses by HPLC used the same apparatus as described above for polyphenols, with a Prevail™ Carbohydrate ES Column (5 μm, 250 × 4.6 mm) and an Evaporative Light Scattering Detector (ELSD) (Alltech Associates, Inc., Deerfield, USA). For the ELSD, a drift tube temperature of 85°C, a gas flow rate of 2.2 liters min<sup>-1</sup> and gas pressure of 0.11 MPa were used. High-purity N<sub>2</sub> was used as nebulizer gas. Under these conditions, no vaporization of the solute was observed and a stable baseline with attenuation factor of 0.5 was achieved. For compound separation, a linear gradient with a mobile phase of acetonitrile and water was used.

Calibration curves with commercially available standards [Sigma (St. Louis, MO) and Wako Pure Chemical Industries Ltd. (Japan)] for glucose, fructose, sucrose, 1-kestose, nystose and fructo-furanosyl-nystose were used for quantification. In addition, the indirect method of Hoebregs [21] was used for estimation of total oligosaccharides content (for an in-depth analysis of the method used see Prosky and hoebregs [22]) in onion bulbs from year 2.

## 2.7. Amino Acid Determinations

Onion amino acids (AAs) profiles were investigated by ion exchange chromatography (IEC) analysis in bulb samples from year 2. For this, bulb aqueous extracts were acid hydrolyzed in 6 M HCl for 16 h at 110°C under constant vacuum [23]. After hydrolysis, the pH of the extracts was adjusted to 2.2. IEC was performed in a Biochrom 30 apparatus (Biochrom Ltd.) equipped with a cationic exchange column. L-Norleucin was used as an internal standard and a solution of eighteen pure amino acid standards was used for identification and quantification.

## 2.8. Aroma Profiles

Onion bulb samples from the year 2 harvest were analyzed using an 'electronic nose' (EN) Alpha Fox 4000 (Alpha MOS®, France) with 18 metal oxide sensors (MOS) coupled to an autosampler (HS 100, Alpha MOS®, France). In order to obtain the compounds responsible for aroma in equilibrium in the headspace, each sample was incubated at 37°C during 150 s with agitation (500 rpm) prior to the analysis which was performed in duplicate. Data acquisition in the EN was accomplished by injecting 1 ml of headspace at a rate of 1 ml/s. Chromatographic air (N35) was used as carrying gas. The acquisition time per sample was 120 s, with a frequency of 0.5 s. The recovery period between each data acquisition was 20 min.

## 2.9. Statistical Analysis

Treatments were compared by analysis of variance (ANOVA) using the General Linear Model (GLM) procedure as implemented in the IBM SPSS Statistics software package (Chicago, Illinois). Mean comparisons used the Bonferroni or Tukey test. Principal Component Analysis (PCA) was performed to describe the relationship between varieties and variables. Data from aroma profiles were analyzed by means of Discriminant Factorial Analysis (DFA, Alphasoft v8®).

# 3. Results and Discussion

## 3.1. Dry Matter and Solids Content

Dry matter (DM) and soluble solids (SS) content varied significantly among the onion cultivars evaluated (Table 1). DM ranged from 80 to 242 g·kg<sup>-1</sup>. In average, colored onions had significantly lower DM content (97

$\text{g}\cdot\text{kg}^{-1}$ ) than white onions ( $196 \text{ g}\cdot\text{kg}^{-1}$ ). Fresh market onions like Angaco, Navideña and Valuno had the lowest DM content, while Refinta 20 and Ancasti, both cultivars used for dehydration, had the highest DM. SS content varied from  $77 \text{ g}\cdot\text{kg}^{-1}$  to  $216 \text{ g}\cdot\text{kg}^{-1}$  in year 1 and  $70 \text{ g}\cdot\text{kg}^{-1}$  to  $223 \text{ g}\cdot\text{kg}^{-1}$  in year 2. Strong positive correlations were found between DM and SS in year 1 ( $r = 0.99$ ;  $P < 0.0001$ ) and year 2 ( $r = 1.00$ ;  $P < 0.0001$ ). Comparable DM values were reported previously for fresh-market onions (range  $85 - 105 \text{ g}\cdot\text{kg}^{-1}$ ) and two commercial dehydration cultivars (range =  $186 - 194 \text{ g}\cdot\text{kg}^{-1}$ ) from US [24].

### 3.2. Polyphenols

Significant variation ( $P < 0.05$ ) was found for total polyphenols (TPs) and for individual polyphenol compounds among the onion cultivars in both growing seasons (Table 2). TPs content, expressed on dry weight basis, varied more than three-folds, with ranges of  $2.26 - 7.94$  and  $5.98 - 20.22 \text{ mg}\cdot\text{gAE}\cdot\text{g}^{-1} \text{ dw}$ , in year 1 and year 2, respectively. Yellow onion cultivars Valcatorce, Cobriza, and Valuno had the highest TPs content, whereas white bulb cultivars Refinta20, Ancasti and Antartica had the lowest TPs content regardless of year. However, if TPs values were expressed on fresh weight basis, the differences between yellow and white onions were smaller and often not significant (Table 2), suggesting a dilution effect of polyphenols content in yellow onions due to their higher water bulb content, as compared to white onions (Table 1). Presumably, biosynthesis and accumulation of polyphenols in our yellow onion varieties is much higher than in white varieties, but such differences are partly masked—when expressed on fw basis—due to the higher water content in the formers. In agreement with our results, previous studies have also noted significantly higher TPs content in red versus yellow versus white onion varieties [26] [28].

HPLC analysis of individual phenolic compounds revealed the same relative rank among the onion cultivars as observed for TPs (expressed on dw basis) for quercetin (both total quercetin and the aglicon) and myricetin in both growing seasons (Table 2). However, this relative rank was less concordant and highly influenced by year-for catechins, EGCg and ECg.

Quercetin was the most abundant polyphenol in yellow onions, accounting for 17.1% - 25.1% of TPs, whereas its content was much lower in white onions, representing only 0.57% - 5.8% of the TPs. The overall variation for total quercetin content among the onion cultivars was 60-fold in year 1 and 13-fold in year 2 (Table 2).

A year effect was observed for catechin, quercetin (total and aglicon), ECg and kaempherol showing, in general, higher content for these compounds in year 1, whereas the opposite trend was observed for TPs and EGCg, for which significantly higher values were obtained in year 2 (Table 2). In addition, several cases of year-to-year variation for particular compounds and cultivars clearly indicate “genotype x year” interactions (e.g., catechins in cv. Cobriza and EGCg in Valcatorce).

**Table 2.** Bulb content of total and individual polyphenols in eight onion cultivars.

Cultivar	Total Polyphenols (TPs)		Catechin		EGCg		ECg		Quercetin (aglicon)		Total quercetin		Kaempherol (aglicon)		Total kaempherol		Myricetin			
	1	2*	1*	2	1	2*	1*	2	1*	2	1*	2	1*	2	1*	2	1	2*		
Valcatorce	0.82ab	7.94a	2.09b	20.22a	0.18ab	<0.01d	0.91b	0.028c	2.19c	1.02a	2.61b	1.83a	17.59a	4.88a	0.08cd	0.06b	0.47cd	0.39d	2.46a	3.91a
Cobriza	0.85a	7.79a	2.08b	18.10ab	0.22a	0.73a	1.09ab	6.96a	1.35d	0.98ab	2.63b	1.85a	11.93b	5.57a	0.05cd	0.05b	0.29cd	0.33c	0.86b	0.99b
Valuno	0.76bc	7.91a	1.54c	16.40b	0.23a	0.06bc	1.26a	3.90b	2.20c	0.47cd	5.67a	1.01b	16.71a	6.46a	0.37a	0.04b	1.92b	0.57b	0.62b	0.43c
Angaco	0.51d	5.67b	1.49c	18.41ab	0.17b	0.13b	0.40cd	1.99bc	0.96d	1.11a	1.74bc	0.67bc	6.23c	2.64b	0.20b	<0.01c	0.95c	<0.01	0.44b	0.42c
Navideña	0.57d	6.42b	1.54c	17.45ab	0.14bc	0.03c	0.60c	1.96bc	1.16d	0.12d	1.10cd	0.46c	7.42c	2.44bc	0.09c	0.02c	0.42cd	0.19e	0.85b	0.32cd
Antártica	0.39 e	3.45c	1.37c	8.48d	0.16bc	0.05c	0.30d	1.61bc	2.74b	0.56bc	0.22d	0.42c	0.38d	0.84cd	0.03d	0.02c	0.08d	0.23e	0.14b	0.21d
Ancasti	0.56d	2.26d	2.63a	12.27c	0.09d	<0.01d	0.32d	0.28c	2.24c	0.16cd	0.15d	0.04d	0.30d	0.50d	<0.01d	<0.01c	<0.01e	0.72a	ND	0.16d
Refinta20	0.73c	3.32c	1.45c	5.98d	0.12cd	0.08bc	0.30d	3.57b	5.91a	1.01a	0.06d	0.04d	0.29d	1.80bcd	0.02d	0.09a	2.78a	0.55b	<0.02	ND

Values are means of four replicates, expressed as  $\text{mg}\cdot\text{gAE}\cdot\text{g}^{-1} \text{ fw}$  (left column) and  $\text{mg}\cdot\text{gAE}\cdot\text{g}^{-1} \text{ dw}$  (right column) for TPs, and as  $\mu\text{g}\cdot\text{g}^{-1} \text{ fw}$  for individual polyphenols. Different letters within a column indicate significant differences ( $P < 0.05$ ) among cultivars. Asterisks (\*) denote significantly higher content in year-to-year comparisons for a given compound and all cultivar.

Analysis of polyphenols by year and bulb color is presented in **Table 3**. Cultivars with yellow bulbs had significantly higher mean TPs content ( $12.7 \text{ mg}\cdot\text{g}^{-1}\cdot\text{dw}$ ) than white bulb cultivars ( $5.9 \text{ mg}\cdot\text{g}^{-1}\cdot\text{dw}$ ) when expressed on dry weight basis (averaged data from years 1 and 2). The average TPs content in yellow onions was more than twice the content in white onions in both growing seasons (**Table 3**). Regarding individual phenolic compounds, yellow onions consistently had significantly higher levels of quercetin and myricetin than white onions, whereas for the other polyphenols no clear association was found between compound concentration and bulb color.

The present study characterized, for the first time, bulb polyphenols content in a genetically diverse onion collection from Argentina. These open pollinated varieties represent more than 90% of the total Argentine onion production, of which nearly 35% is exported to other South American countries. In these germplasm, broad variation was observed for both TPs and individual phenolic compounds. Quercetin, a compound belonging to the flavonol sub-type within flavonoids, was clearly the predominant polyphenol in colored onions, while kaempferol and the flavan-3-ols (another type of flavonoids) EGCg and ECg were the major polyphenols in white onions (**Table 3**).

TPs values in this onion collection (**Table 2**) were within the range of values reported previously in most onion germplasms from other geographical origins [25]–[27], but lower than found in one study [28]. For instance, TPs content in ten US onion varieties (eight yellow, one red and one white) ranged from 0.17 to 1.05  $\text{mg}\cdot\text{gAE}\cdot\text{g}^{-1}$  fw (values are highly comparable to our data from year 1), with the red and white varieties inter-ranked with the yellow ones. Another study by Lu *et al.* [25] evaluated four US onion varieties cultivated in different locations, and found decreasing –and significantly different–TPs content in red ( $4.28 \text{ mg}\cdot\text{gAE}\cdot\text{g}^{-1}$  fw), white ( $2.69 \text{ mg}\cdot\text{gAE}\cdot\text{g}^{-1}$  fw) and yellow ( $1.64 \text{ mg}\cdot\text{gAE}\cdot\text{g}^{-1}$  fw) varieties, with values for yellow and white onions being similar to our year 2 data. However, TPs values from the present study were lower than those reported for three Czech cultivars with mean TPs content of 26.4, 65.2, and 108.3  $\text{mg}\cdot\text{gAE}\cdot\text{g}^{-1}$  dw for white, yellow and red onions, respectively [28].

Variation for total phenolics content among onion varieties with different bulb color, as reported herein and in previous studies, is directly related to the differential accumulation of major colored polyphenols in bulbs of these varieties. While quercetin (a yellow polyphenol) is a major contributor to TPs in yellow and red onions, with usually higher content observed in yellow onions, this compound is present in low quantities in white onion bulbs (**Table 2**, and [29]). In addition to quercetin, red onions may accumulate large quantities of red/purple anthocyanins contributing to their higher TPs level, whereas anthocyanins occur at low levels—if at all— in yellow onions, and are missing in white ones [30].

Other phenolic compounds present in onion bulbs in relatively low quantities, such as the Flavan-3-ols catechin, EGCg and ECg, were also quantified in this study. While not quantitatively important in yellow onions, these polyphenols represented a substantial proportion of the TPs in white onions (**Table 2** and **Table 3**). Previous studies have reported undetectable [31] or very low content ( $0.80 \mu\text{g}\cdot\text{EGCg}\cdot\text{g}^{-1}\cdot\text{fw}$  in a sweet onion variety) [32] for these polyphenols. In our onion germplasm the three compounds could be unambiguously detected and quantified in all the varieties.

With the exception of Refinta20 and Ancasti, which are used for dehydration due to their high solids content, the rest of the onion cultivars included in our study are for fresh consumption (**Table 1**). In addition to the former, Antartica, the only fresh consumption white onion variety in our collection, also had high solids content. The nearly two-fold difference in the average solids content observed between white and yellow onions generates a concentration/dilution effect when the data are expressed on fresh weight basis. Although the results expressed on a dry weight basis avoid the dilution effect due to differences in bulb water content, which may be

**Table 3.** Mean polyphenols content by cultivar's bulb color and year.

Year	Bulb color	TPs		Catechin	EGCg	ECg	Total kaempferol	Total quercetin	Myricetin
1	yellow	0.70 <sup>a</sup>	7.2 <sup>*</sup>	0.19 <sup>*</sup>	0.85	1.57	0.81	12.00 <sup>*</sup>	1.15 <sup>*</sup>
	white	0.56	3.0	0.12	0.31	3.67 <sup>*</sup>	1.43 <sup>*</sup>	0.32	0.13
2	yellow	1.74	18.1 <sup>*</sup>	0.07	3.04 <sup>*</sup>	0.77	0.37	4.50 <sup>*</sup>	0.57 <sup>*</sup>
	white	1.81	8.9	0.06	2.00	0.37	0.50	1.00	0.18

<sup>a</sup>Mean values were calculated from all the replicates of yellow- and white-bulb onions regardless of cultivar, and expressed as  $\text{mg}\cdot\text{gAE}\cdot\text{g}^{-1}$  fw (left column) and  $\text{mg}\cdot\text{gAE}\cdot\text{g}^{-1}$  dw (right column) for total polyphenols (TPs), and as  $\mu\text{g}\cdot\text{g}^{-1}$  fw for individual polyphenols. Asterisks indicate significantly ( $P < 0.05$ ) higher content for comparisons within the same year.

more informative for the dehydration or pharmaceutical industries (e.g., in order to establish the content of active compounds in dry onion powder), we presented our data on a fresh weight basis, providing direct information for the fresh-onion consumer which is still the largest onion market worldwide.

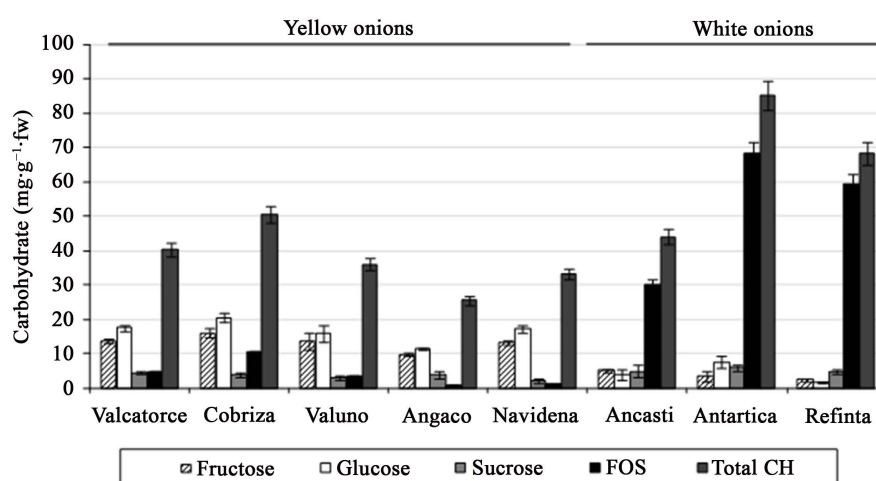
### 3.3. Antioxidant Activity

Significant variation was found for total antioxidant activity (TAA) among the onion cultivars (Electronic Supplementary Material, **Figure 1**). A two-fold difference in TAA was observed between the cultivars with highest (Cobriza) and lowest (Ancasti) TAA. Significant strong positive correlation was found between TPs and TAA, and between quercetin content and TAA ( $r = 0.83$ ;  $P = 0.0001$ ) suggesting that quercetin is a major contributor to onion antioxidant capacity. Cultivars Valcatorce, Cobriza and Valuno, had the highest TPs, quercetin and TAA values. Together, these three cultivars represent valuable materials for both, the fresh-market end consumer and for using them in breeding programs aiming at developing onion varieties with high nutraceutical value. Our results are in agreement with previous studies reporting strong positive associations between TPs and antioxidant activity in other onion germplasm [25] [27]. Additionally, quercetin, the major contributor to TPs content in yellow and red onions, is considered a potent antioxidant [33].

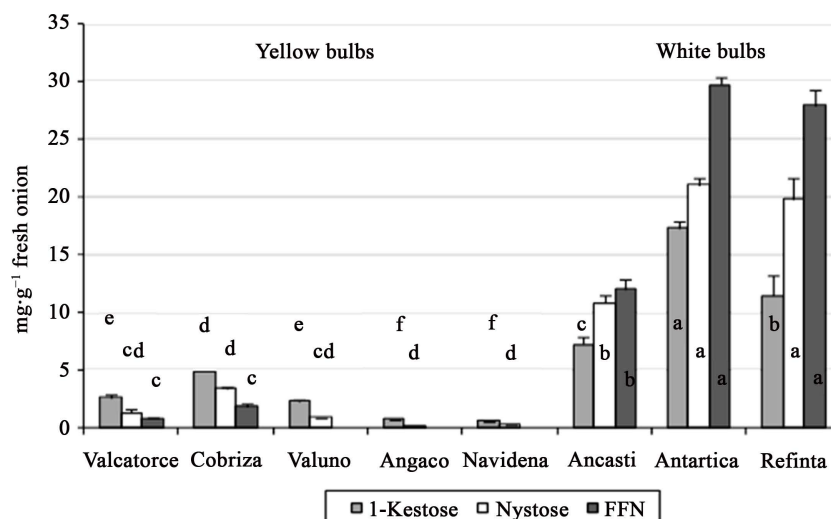
### 3.4. Carbohydrates Content

Substantial and significant variation was found for total carbohydrates and for fructose, glucose, sucrose and FOS among the onion cultivars (**Figure 1**). The content of total non-structural carbohydrates, considered as the sum of free fructose, free glucose, sucrose and total FOS, differed significantly among the onion cultivars, with values ranging from 25.5 (in cv. Angaco) to 85.0  $\text{mg}\cdot\text{g}^{-1}$  fw (Antartica) (**Figure 1**). White onions had in general higher total carbohydrate content than yellow onions and this was mainly due to their strikingly higher FOS levels. The average FOS content in white onions ( $52.5 \text{ mg}\cdot\text{g}^{-1}$  fw) was nearly 8 times higher than the average FOS level in yellow onions ( $6.7 \text{ mg}\cdot\text{g}^{-1}$  fw). Significant and substantial variation was also found for individual FOS compounds 1-kestose, nystose, and fructo-furanosyl-nystose (FFN) (**Figure 2**). The latter (FFN) was the mayor contributor to the total FOS content in white onions, followed by nystose and 1-kestose. Conversely, yellow onion varieties had little or undetectable quantities of FFN, with 1-kestose being the predominant FOS in all cases.

Further analysis of individual carbohydrates revealed significant variation for concentration of reducing sugars (RS) fructose and glucose, and such variation was associated with the bulb color phenotype. In average, RS accounted for only 13% of the total carbohydrates present in white onions, with Refinta20 having the lowest RS content (representing 6% of its total carbohydrates), whereas in yellow onions RS represented 81% of the total



**Figure 1.** Bulb carbohydrates content in onion cultivars. Values for fructose, glucose, sucrose, FOS, and total non-structural carbohydrates (Total CH) are means and SD of four replicates, expressed as  $\text{mg}\cdot\text{g}^{-1}$  fw. Total CH values are the sum of the four individual carbohydrates. The data correspond to onions cultivated in year 2.



**Figure 2.** Bulb fructo-oligo-saccharides (FOS) content in onion cultivars. Values for 1-Kestose, Nystose and Fructo-Furanosyl-Nystose (FFN) are means and SD of four replicates, expressed as mg·g<sup>-1</sup> fw, from onions cultivated in year 2. For each compound, different letters indicate statistical differences ( $P < 0.05$ ) among varieties. FFN was not detected in Valuno, Angaco and Navidena.

carbohydrates, with cv. Cobriza having the highest level of RS. The opposite was observed for sucrose; all the white onions cultivars had higher sucrose content than any yellow cultivar, with a nearly 2-fold difference observed between the mean values for each color class ( $5.8 \pm 1.2$  vs.  $3.1 \pm 0.8$  mg·g<sup>-1</sup> fw).

FOS are polymers of fructose. Free fructose—available for polymerization into FOS—mainly derives from cleavage of the disaccharide sucrose, thus releasing glucose and fructose. Because higher sucrose availability positively correlates with higher fructan biosynthesis [34], several authors have suggested that the supply of carbohydrates in the form of sucrose may play a central role in the control of fructan biosynthesis [34] [35]. In line with these reports, we found positive and significant correlation between sucrose and total FOS content ( $r = 0.80$ ,  $P < 0.01$ ), and between sucrose and individual FOS compounds ( $r > 0.67$ ,  $P < 0.01$ ) for all FOS compounds.

We found broad variation for total FOS content in our onion materials, ranging from 0.8 to 85 mg·g<sup>-1</sup> fw, with higher FOS levels generally observed in white onions. A previous study by Jaime *et al.* [36] reported FOS values for yellow onion cultivars ranging from 23.3 to 141.9 mg·g<sup>-1</sup> dw, that are highly comparable with FOS values obtained for yellow onions in the present study [13.8 - 137.1 mg·g<sup>-1</sup> dw (for comparison purposes, values were expressed on dw basis)].

The broad variation found for FOS concentration in these onion germplasm provides an opportunity to further investigate the genetic basis underlying FOS biosynthesis and accumulation; for instance by means of developing segregating populations for FOS content and genetically mapping quantitative trait loci (QTL) for this trait. From a breeding and end-consumer point of view, the white onion cultivars evaluated in this study are of interest due to their very high level of total and individual FOS (Figure 1 and Figure 2). FOS content may also be important for onion post-harvest storage, since direct associations were found between bulb fructan content and the length of marketable post-harvest storage, suggesting that estimation of fructan content at harvest time may help predict onion shelf life period [36].

### 3.5. Pyruvate Content

Pyruvate level is a predictor of onion total thiosulfinates content [11] and intensity of the pungency sensory perception [37]. Significant variation was found for pyruvate bulb content (Electronic Supplementary Material Table 1) among the onion cultivars, with values ranging from 3.1 (in cv. Angaco) to 6.9 (in cv. Refinta20) mol pyruvate g<sup>-1</sup> fw (mean values for year 1 and year 2 harvests). In average, white onions had significantly ( $P < 0.01$ ) higher pyruvate content than yellow onions. Mean pyruvate levels for both harvests in yellow and white onions were 4.19 and 5.62 mol pyruvate g<sup>-1</sup> fw, respectively. Positive and significant correlation was found between



pyruvate and dry matter content ( $r = 0.71$ ;  $P < 0.0001$ ), suggesting a dilution/concentration effect due to cultivar differences in bulb water content (**Table 1**). In agreement with these results, Galmarini *et al.* [12] reported significant positive phenotypic and genetic correlations between pyruvate levels, DM and onion-induced antiplatelet activity (OIAA) in onion F3 families, which were genetically unrelated to the materials used in our study. Together, these results suggest that the increase in water content associated with low-DM onions may be responsible for diluting solids and organosulfur compounds associated with onion pungency and antiplatelet activity. In addition, they suggest that analyses of DM content, which are relatively simple to perform, can be used effectively for indirectly selecting for onion pungency and OIAA.

### 3.6. Amino Acids

Broad and significant variation was found for total (TAAs) and essential amino acids (EAAs) among the eight onion cultivars (**Table 4**). An 11-fold and 17-fold difference in concentration between the highest and lowest content was found for TAAs and EAAs, respectively. White onions Refinta20, Ancasti and Antartica had the highest TAAs and EAAs content, while yellow cultivars Valcatorce and Navideña had the lowest. Further analysis of individual AAs indicated that glutamic acid (Glu) and aspartic acid (Asp) were the most abundant non-essential AAs in all varieties, whereas leucine (Leu) and lysine (Lys) predominated as EAAs in all cultivars except Refinta20 and Navideña, with Arginine (Arg) prevailing in the latter (Electronic Supplementary Material **Table 2**).

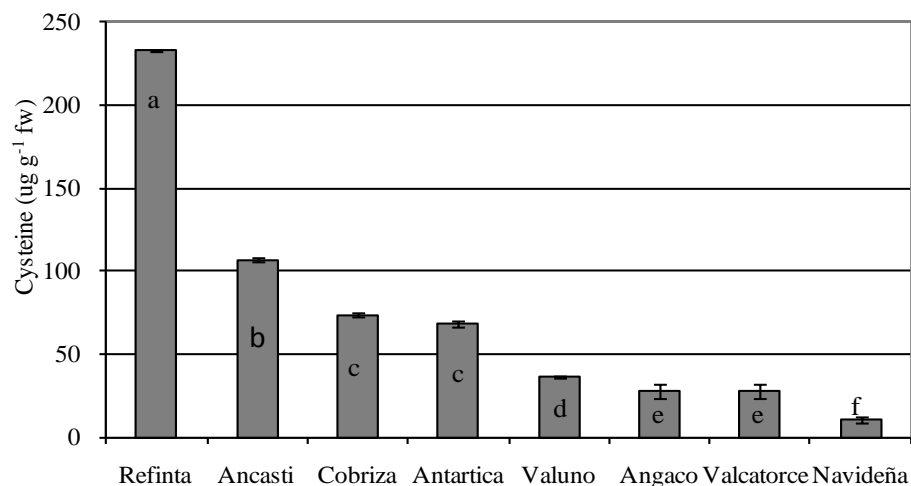
AAs composition in foods is of interest due to the various physiological functions in which they are involved, beyond their role as proteins building blocks. AAs are involved in glucose homeostasis, gastrointestinal health, cell signaling and satiety, among others [17]. In addition, some AAs play a role in the development of flavor. For instance, Cysteine (Cys) is the main sulfur supply for most organic sulfur compounds in *Allium*, including volatile thiosulfinates, therefore contributing to the development of flavor and pungency in these species. We found important differences in bulb Cys content among the onion cultivars (**Figure 3**). Cys content correlated positively with pyruvate ( $r = 0.87$ ,  $P < 0.01$ ), an estimator of total thiosulfinates content, suggesting that indeed Cys is necessary for the production of organosulfur compounds. In addition, both variables Cys and pyruvate were positively correlated with DM ( $r = 0.90$  and  $r = 0.86$ ,  $P < 0.01$ ; for Cys and pyruvate, respectively), reflecting the same dilution/concentration effect noted earlier.

Development of flavor in *Allium* depends on several factors, including the plant's capacity to uptake sulfur from the soil, its reduction to sulfides and the subsequent assimilation into cysteine [38]. Other compounds, such as complex and simple carbohydrates (*i.e.*, sugars), and aromatic and sulphur AAs are also involved in flavor development. Flavor differences among onion cultivars may be explained by several reasons. Cultivars may uptake and accumulate different quantities of sulfur from the soil and/or they may have different efficiencies for incorporating sulfur into cysteine and other flavor precursors (*e.g.*, ACSOs) [39]. Regardless of the specific mechanisms underlying flavor development in *Allium*, Cys content seems to play a central role in this process.

**Table 4.** Total and essential aminoacids in eight onion cultivars.

Cultivar	Total aminoacids	Essential aminoacids
Valcatorce	530f	94f
Cobriza	1140d	193d
Valuno	1030e	212c
Angaco	1050e	187d
Navideña	390g	117e
Ancasti	1590b	243b
Antartica	1310c	210c
Refinta20	4410a	1669a

Values are means of eight replicates. Results are expressed as  $\text{mg}\cdot\text{kg}^{-1}$  fw. Different letters in the same column indicate statistical differences ( $P < 0.05$ ).



**Figure 3.** Bulb cysteine content in onion cultivars. Results are means and SD of four replicates. Different letters indicate statistical difference ( $P < 0.05$ ).

### 3.7. Aroma Profiles

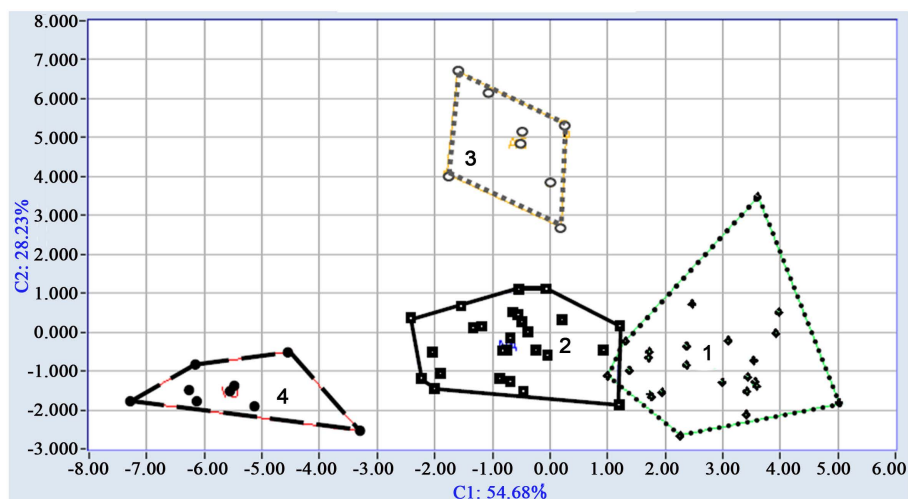
Results from aroma profiles (AP) revealed substantial differences among the onion cultivars when analyzed by means of Discriminant Factorial Analysis (DFA) (Figure 4). Two selected components explained nearly 83% of the total variability. Four distinct groups of cultivars with common aroma profiles could be identified; a group with only white onion cultivars, a second group with three yellow cultivars (Valcatorce, Cobriza and Navidena), and the two remaining groups differentiating samples from the yellow onions Angaco and Valuno.

Significant positive correlation ( $r = 0.65$ ,  $P < 0.01$ ) was found between “electronic nose” (EN) data and pyruvate content. The positive correlation between aroma data and pyruvate suggests that volatile thiosulfinates are responsible—to a large extent—of onion aroma and taste. Thiosulfinates have been shown—directly and indirectly—to be responsible for most of garlic and onion antiplatelet properties [11] [40]. Altogether, these results strongly predict that onions with pungent and intense flavor will have higher antiplatelet activity and, thus, higher functional value for the prevention of cardiovascular disease. They also suggest that it will be difficult to develop mild onion cultivars with high antiplatelet activity.

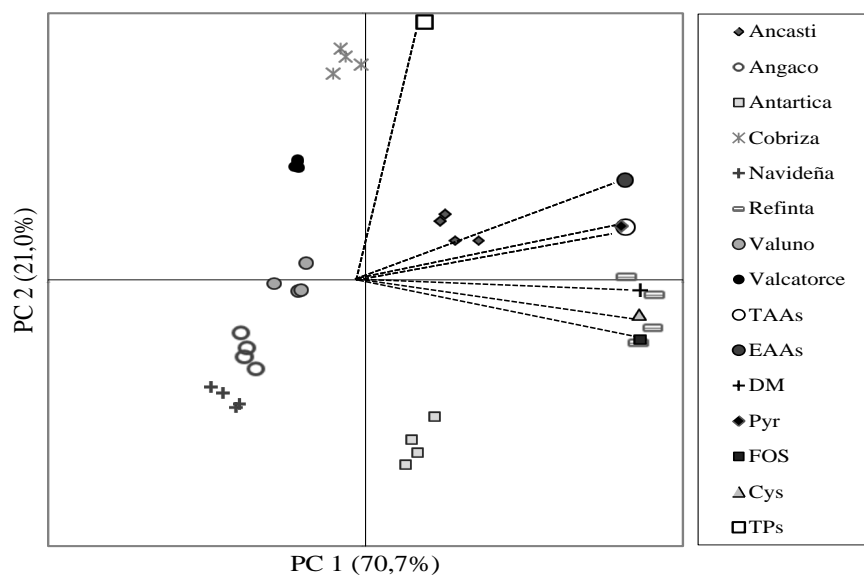
Two previous studies have evaluated onion flavor by means of EN data [41] [42]. These studies examined onion flavor as affected by soil type and nutrient content in a single onion genotype. In the present study, we provide evidence—for the first time—that EN data can effectively be used for discriminating among onions cultivars grown under the same environmental conditions, as well as for characterizing onion germplasm based on their aroma profiles, a relevant trait for fresh onion consumers.

A principal component analysis (PCA) was performed to integrate all the biochemical data from the eight onion cultivars (Figure 5). Positive correlations among variables associated with bioactive compounds (e.g., TPs, FOS and pyruvate) and those related to flavor (e.g., AAs, pyruvate) were found. In agreement with our earlier analysis of aroma profiles, and with previous studies linking pyruvate levels, pungency and antiplatelet activity [12] [13], our PCA results predict that cultivars with intense flavor will have highest functional value. In addition, the concentration/dilution effect observed in onion cultivars due to their differential bulb water content, seems to play an important role in determining the final concentration of both flavor and nutraceutical compounds.

Different target populations demand different health-enhancing foods to match nutritional requirements according to their particular needs. In this context, different onion germplasm may be suitable for this purpose based on their nutraceutical properties. If antioxidant properties are of interest, Cobriza, Valuno and Valcatorce would be the choice of preference for fresh consumption. These cultivars had the highest antioxidant activity and the highest levels of EGCg and quercetin. On the other hand, Refinta20 is a cultivar well suited for dehydration, with high levels of thiosulfinates and FOS as well as with interesting levels of some essential aminoacids (e.g., arginine).



**Figure 4.** Discriminant Factor Analysis (DFA) of aroma profiles from eight onion cultivars. Four distinct groups of cultivars with common aroma profiles could be identified: group 1 with only white onion cultivars; group 2 with three yellow cultivars (Valcatorce, Cobriza and Navidena); and groups 3 and 4 differentiating samples from the yellow onions Angaco and Valuno. C1 and C2: component 1 and 2 respectively (83% recognition) constructed with normalized data. The aroma is a good variable to predict group membership.



**Figure 5.** Principal component analysis (PCA) of compounds associated with onion flavor and nutraceutical properties. PC1, PC2: principal component N°1 and 2 respectively. In two components 91.7% of the total variability is explained. TAAs: total aminoacids; EAAs: essential aminoacids; DM: dry mater; Pyr: pyruvate; FOS: fructo-oligosaccharides; Cys: cysteine; TPs: total polyphenols.

## 4. Conclusions

The detailed characterization of these genetically diverse onion germplasm for several health-enhancing compounds will have a positive impact on breeding programs aiming at increasing onion nutraceutical value. For example, the immediate selection of cultivars with high OSC (e.g., Refinta20) and/or high TAA (e.g., Cobriza INTA) can be used as functional food for preventing chronic illnesses, whereas cultivars with high solids and DM content (e.g., Refinta20) are suitable for the dehydration industry. On the other hand, onions with low OSC content and weak aroma profiles (e.g., Angaco INTA) can be used as mild varieties for fresh consumption.

The broad variation observed for most of the traits suggests that it will be possible to intercross selected genotypes for developing segregating progenies (e.g., F2 families) suitable for genetic mapping of these traits. Such approach would be of great value for detecting markers closely linked to the traits which can be used to assist onion selection and breeding.

The long-day varieties with yellow bulbs Valcatorce INTA, Valuno INTA and Cobriza INTA are rich in phenolic compounds and total antioxidant activity. In contrast, white onion cultivars stand out for their high solids and FOS. By means of analysis of aroma profiles we are able to discriminate between the onion cultivars, and the aroma data correlated with pyruvate content, a predictor of pungency intensity and a strong characteristic for consumers' choices.

The characterization of these cultivars based on their functional compounds content will provide valuable information for the consumer and—because of the widespread use of these materials—will contribute to diversifying onion production and commerce in Argentina and South America.

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## Abbreviations

AAs: amino acids  
AP: aroma profile  
EAAs: essential amino acids  
ECg: epicatechin gallate  
EGCg: epigallocatechin gallate  
EN: electronic-nose  
FOS: fructo-oligosaccharides  
FW: Fresh Weight  
GAE: gallic acid equivalents  
OSC: organosulfur compounds  
TAA: total antioxidant activity  
TAAs: total amino acids  
TPs: total phenols  
TSs: thiosulfinates



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