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Characterization of Six Artichoke Cultivars and Their Suitability for Agro-industrial Processing

Nuria García-Martínez, Pedro Andreo-Martínez, Luis Almela*

Department of Agricultural Chemistry, University of Murcia, Campus de Espinardo, 30100, Murcia, Spain
*Corresponding author: almela@um.es

Abstract The study of artichoke cultivars characteristics is a useful tool for processors, wholesalers and retailers when selecting varieties for fresh marketing or agro-industrial processing. In this work six artichoke cultivars grown in Murcia (Spain) were evaluated to ascertain their optimum commercial use by determining their physical, chemical and biochemical parameters (production yield, shape, size, moisture and color of their heads, respiration rate, phenolic content, enzymatic activities and browning potential). When considering purplish varieties, the high oxidative and physiological stability obtained in Violet de Provence led us to identify this cultivar as the most suitable purplish cultivar for processing; while in white varieties, Blanca de Tudela showed the lowest browning and respiration rates. On the other hand, cultivars with higher browning susceptibility, physiological activity and phenolic content should be marketed preferably for fresh consumption. It was observed that phenolic content, enzymatic activities and browning potential were affected by factors such as genotype, harvest time and head parts. In addition, physical parameters such as morphology, moisture and color of heads, and plant productivity varied according to cultivar. Results demonstrate that there is a high variability on physical, chemical and biochemical characteristics of artichoke genotypes. Results may be used to define the optimum commercial use for different artichoke cultivars.

Keywords: *artichoke, browning susceptibility, enzymatic activity, polyphenol oxidase, respiration rate, phenolic compounds*

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1. Introduction

The artichoke (*Cynara cardunculus* var. *scolymus* (L.) Fiori) is a diploid (2n = 2x = 34), perennial and cross-pollinated plant of the Asteraceae family. The fleshy heads formed prior to flower development are the edible part of the plant [1]. Artichoke is a highly appreciated vegetable in the Mediterranean diet due to its high nutritional and pharmacological values that are linked to its composition, which includes high mineral content (sodium, calcium, potassium, iron, magnesium and phosphorus), vitamins (mainly vitamin C), and high levels of inulin, which has an interesting prebiotic action on intestinal bifidobacteria. Artichoke also accumulates a great number and diversity of phenolic compounds like monophenols (caffeic and coumaric acids) and polyphenols (5-O-caffeoylquinic, 1,5-di-O-caffeoylquinic, 3,4-di-O-caffeoylquinic and 1,3-di-O-caffeoylquinic acids). Other minority phenols include flavonoids like apigenin and luteolin in combined forms (glucosides and rutinosides), and glucosidic and acylated derivatives of anthocyanins such as cyanidin, peonidin and delphinidin, which are responsible for the artichoke color [2-9].

Phenolic compounds are very susceptible to browning through oxidation reaction catalyzed by enzymes polyphenol oxidase (PPO; EC 1.14.18.1) and peroxidase (POD; EC 1.11.1.7). The *o*-quinones thus formed are very reactive and, in a second non-enzymatic step, dark compounds are formed [4,5,7,10].

PPO is the main enzyme responsible for browning in artichokes and its mechanism of action involves two coupled reactions: the hydroxylation of monophenols to *o*-diphenols (cresolase activity), and the oxidation of o-diphenols to *o*-quinones (catecholase activity) [4,11,12,13]. POD also catalyzes the oxidation of *o*-diphenols to their corresponding quinones in the presence of hydrogen peroxide as oxidizing agent [14]. This enzyme is involved to a minor extent in the enzymatic browning reactions because the availability of endogenous H_2O_2 is very limited; however, a possible synergistic effect has been observed between both enzymes (PPO and POD) which would promote the browning reactions [4,10,15,16,17,18].

Enzymatic browning is influenced by many variables dependent on the genotype, such as specific activity of enzymes or quantity and nature of the phenolic compounds [19]. Various authors have shown that there is a direct proportionality between the phenolic content and enzymatic activity with the browning susceptibility in artichoke. Thus, cultivars with an elevated content of phenolic compounds and high enzymatic activity are unsuitable for industrial processing [4,7,11]. In addition,

other parameters not bound to genotype can affect the browning reactions of the artichoke; so, pre-harvest factors such as physiological status, plant part, harvest time or environmental factors (temperature, rainfall, light intensity, atmosphere) can affect the phenolic content of the heads and, therefore, their susceptibility to browning [3,20,21].

Currently, there are over 286 varieties of artichokes grown in the world, originating mostly from Italy, France and Spain, and differentiated by shape (spherical or oval), size, color (green or violet) and the precocity of their heads, among other factors [22].

The most widespread variety of artichoke in Spain is Blanca de Tudela, but nowadays other cultivars such as Violet de Provence, Spinoso Sardo, Camus de Bretagne, Catanese, Green Globe, Thema, Romanesco, Salambo and Calico, among others, are being produced mainly in the geographical area of Murcia. The implementation of new varieties which are appreciated and demanded on the international markets, along with improvements in crop yields, are two good strategies to compete with emerging producing countries. An adequate marketing chain of these varieties requires knowledge of their physical, chemical and biochemical characteristics, and thus assess whether they are suitable for industrial processing (frozen, canned or fresh-cut) or for the fresh market [23].

The purpose of this research was the chemical-physical and biochemical characterization of different cultivars of artichoke in order to define their suitability for fresh marketing or agro-industrial processing. In this regard, and on the basis of the findings of other authors, the selected parameters to carry out the varietal study were morphology of the varieties, agronomic characteristics, moisture, numerical determination of the color, respiration rate and stability against the browning reactions (phenolic substrates, enzymatic activity and browning potential).

2. Materials and Methods

2.1. Experimental Field and Plant Material

Six artichoke cultivars, four with purple heads ("violet cultivars") - Violet de Provence, Thema, Romanesco and Salambo- and two with green heads ("white cultivars") - Calico and Blanca de Tudela- were used. The genotypes were grown in an experimental field located in Torre Pacheco (Murcia, Spain). Soil preparation, fertirrigation guidelines, phytosanitary treatments and other growing practices were as commonly applied by farmers in Southeast of Spain. Note that the planting density and inter and intra-row distances varied depending on the cultivar; from about 10,000 plants/ha and 1x1 m of planting framework for B. Tudela, Thema and V. Provence, to 7,000 plants/ha and 1.5x1.5 m of planting framework for cultivars of larger sizes, like Calico, Romanesco and Salambo.

Sampling was carried out along the annual vegetative cycle (twice per productive month), during the agricultural campaign 2014-2015, once the heads of each cultivar had reached their average commercial size. After harvest, artichokes were transported to the laboratory, stored at 3°C and analyzed within a maximum of 24 h. Vegetal material of seven artichoke heads was used to elaborate phenolic and enzymatic extracts for each sampling and cultivar, and to determine physical parameters (morphology, moisture and color of heads), in order to ensure that the sample was representative. All determinations were carried out in triplicate.

2.2. Physical Characterization

Artichoke heads were harvested and weighed with 15-20 cm of floral stem. The determination of length/diameter ratio (L/D ratio) provides information about the form of the artichoke heads and is a characteristic trait of each variety; this ratio varies between 0.9 to 1.1 in spherical/subspherical varieties and L/D ratio > 1.2 in long shaped cultivars [3].

The production yield of the six cultivars was determined by calculating the total amount (kg) of capitula produced per plant along the whole vegetative cycle. Results were the mean value of 45 plants per cultivar.

The moisture content in artichoke bracts (outer and inner) was determined by drying the samples to a constant weight in an oven at 103 ± 1°C.

2.3. Respiration Rate of the Artichoke Heads

The respiration rate (RR) of the heads during the vegetative cycle (October-May) was determined at two temperatures, 4 ± 0.5°C and 20 ± 0.5°C, using a closed system consisting of glass jars (5,000 mL) with a septum in the lid for the inner atmosphere sampling. Raw artichoke heads were put into closed jars, and CO_2 production rates were analyzed by extracting of 1 mL of the inner atmosphere into a gas chromatograph Trace GC (Thermo Finnigan, Rodano, Italy) equipped with a thermal conductivity detector and a CTR1 column (Alltech Assoc., Deerfield, USA). Helium was used as gas carrier at a flow rate of 60 mL min^{-1}; temperatures for injector, oven and detector were set at 35°C. A commercial standard mix of gases (O_2 24%, CO_2 2% and N_2 74%) (Alltech) was used for calibration.

CO_2 production was analyzed at intervals of 2 h for a period of 72 h. Three jars were analyzed for each set time and cultivar, by triplicate injection. RR was calculated from the slope of the fitted linear equation of CO_2 production according to the equation described by Fonseca et al. [24].

2.4. Color Determinations

The color of outer bracts (non-edible fraction) and inner bracts (edible tender fraction) was determined by reflectance spectroscopy according to L*a*b* color space using a reflectance spectrophotometer (CM-508I, Konica Minolta Spain, Madrid, Spain). Standard illuminant C (color temperature 6774 K) and 2º viewing angle were used. Besides the coordinates L*, a*, b*, and for a more easy artichoke color classification in a bidimensional space, chroma ($C^*=[(a^*)^2+(b^*)^2]^{1/2}$), chroma differences ($\Delta C^*= [(C^*_2-C^*_1)]$) and color differences ($\Delta E^*= [(\Delta L^*)^2+(\Delta a^*)^2+(\Delta b^*)^2]^{1/2}$) were calculated. In this way, the color of the genotypes can be classified in terms of lightness and chroma differences.

Color was measured in triplicate at four points around each bud, with 84 measures for each sampling.

2.5. Phenolic Content

Total polyphenols were determined spectrophotometrically by two methods: the polyphenols index I_{280} and Folin-Ciocalteau Index (IFC). For phenolic extraction, samples of 3 grams of outer or inner bracts were triturated separately in a homogenizer Polytron PT 10-35 (Kinematica GMBH, Eschbach, Deutschland) in a phosphate buffer solution 0.1 M (pH 7.2) for 2 min. The mixture was filtered, brought to 100 mL and then centrifuged at 20000×g for 30 min at 5°C (Sorvall Model RC-5, Waltham, USA). The supernatant was used for I_{280} and IFC determinations.

The I_{280} expresses total content of phenolic compounds on the basis of the strong absorbance of the benzene ring of the phenolic compounds [25]. For determination, the bract extracts were diluted with water (1:25) and the absorbance measured directly at 280 nm (Genesys 6, Thermo Co., USA). I_{280} values were given taking into consideration the dilution factor and the weight of plant material used.

The IFC is based on the reduction of Folin-Ciocalteu reagent (FCR) by polyphenols. For the artichoke samples, the IFC assay was run by means a technical modification of Singleton and Rossi [26]: 4 mL of extract, 50 mL of distilled water, 5 mL of FCR and 20 mL of sodium carbonate solution (200 g L^{-1}), added strictly in this order, were put in a 100 mL volumetric flask and brought to 100 mL with distilled water. The solution was stirred and allowed to react for 30 min in darkness, whereupon the absorbance was read at 750 nm. IFC was performed taking into consideration the dilution factor and the weight of the bracts used to prepare the extracts.

2.6. PPO and POD Extraction and Assay

Extracts for PPO and POD activities determination were prepared by crushing 10 g of artichoke bracts (outer or inner) in an ice-water bath for 3 min in a homogenizer (Polytron GMBH, Eschbach, Deutschland) with 60 mL of phosphate buffer solution 0.1 M (pH 7), TRITON X-100 (10 g kg^{-1}) (BDH Chemicals, UK) and 0.5 g of insoluble polyvinylpyrrolidone (PVP) (Sigma-Aldrich Química, Madrid, Spain). The homogenate was filtered and centrifuged at 20000×g at 5°C for 30 min. The supernatant fractions were used to determine PPO and POD activities.

PPO activity on artichoke bracts was determined spectrophotometrically according to the method of Gauillard et al. [27] based on the stoichiometric reaction of L-cysteine with o-quinones produced during the enzymatic oxidation of phenols, forming a stable adduct with a maximum of absorbance at 300nm. The assay was carried out with the subsequent reaction mixture: 20 µL of enzymatic extract, 4-methylcatechol (20 mM) as substrate and L-cysteine (10 mM) (Sigma-Aldrich Química, Madrid, Spain), until achieving the desired volume (3 mL) with 100 mM phosphate buffer (pH 7.2). The oxidation of the substrate was assessed by reading the absorbance increase at 300 nm. PPO activity was calculated by Beer's law and expressed as units (U) of specific enzyme activity (U/mg protein), using a ε value of 2460 M^{-1} cm^{-1} at 300 nm for the adduct formed [27], with one unit of PPO activity (U) being defined as the amount of enzyme that produces 1 micromole of product per minute.

POD activity assay on artichoke bracts was carried out by spectrophotometry, according to the method of Ferrer et al. [28], based on the H_2O_2 oxidation of 4-methoxy-α-naphthol (4-MN) to form a deep-blue product with a maximum of absorbance at 600 nm. The assay mixture contained 20 µL of enzymatic extract, 4-MN (0.2 mM) (Sigma-Aldrich Química, Madrid, Spain) and H_2O_2 (2 mM) bringing to desired volume (3 mL) with 50 mM TRIS-HCl buffer (pH 7.5). POD activity was determined by measuring the amount of product produced. Enzyme activity (U/mg protein) was calculated from the linear portion of the absorbance curve at 600 nm, using a ε value of 21000 M^{-1} cm^{-1} for the blue-product formed [28,29].

The total protein content was determined in all enzymatic extracts according to the colorimetric method described by Bradford [30]. Values were obtained from a calibration standard curve using bovine serum albumin (BSA) (Sigma-Aldrich Química, Madrid, Spain) at 595 nm.

2.7. Browning Potential (BP)

The browning potential for outer or inner bracts was estimated according to the modified method described by Omuaru et al. [31] based on Walter and Purcell [32] method. The BP, or degree of darkening, was measured as the absorbance difference measured at 450 nm between the extract for determining phenol content and another extract prepared similarly but with the addition of 2 mL of solution β-mercaptoethanol (BME) 100 g L^{-1} as inhibitor of enzymatic browning. The absorbance was read after two hours of incubation. Weight and dilution factors of the samples were taken into consideration.

2.8. Statistical Analysis

The effect of artichoke cultivars, harvest time, type of bract and their interaction on chemical-physical and biochemical parameters was tested by variance analysis multifactor for independent samples (ANOVA) and Tukey's test using the SYSTAT program version 13.0. A similar analysis was made to evaluate the effect of cultivars and storage temperature on respiration rate. In addition, a Pearson's correlation matrix was applied in order to study the strength of the relationship between oxidative stability parameters. A linear regression analysis between PPO and POD was carried out. A level of $p \leq 0.05$ was considered as a significance level.

3. Results and Discussion

3.1. Physical and Agronomic Characteristics of the Artichoke Genotypes

3.1.1. Morphological Characteristics and Crop Yield

The morphological characteristics of the six artichoke genotypes along with the crop yield are shown in Table 1. Size, shape and color showed high variability depending of the genotype. B. Tudela and Thema were the cultivars

of lowest fresh weight, while Calico and Salambo were the heaviest. Romanesco, Calico and Salambo had spherical/sub-spherical heads (L/D ratio ≤ 1.1) while B. Tudela, Thema and V. Provence had cylindrical/conical heads (L/D ratio > 1.2).

The highest yields were achieved with Calico, Romanesco and Thema, exceeding 4 kg of artichoke heads per plant. B. Tudela and V. Provence had significantly lower yields, around 3 kg per plant, and Salambo showed an intermediate behavior.

The average moisture content in the inner bracts (870 g kg^{-1}) was significantly higher than in the outer bracts (830 g kg^{-1}) for every cultivar and harvest date (p of 0.000). This is because the outer bracts are exposed to intense sunlight. Moisture values of artichoke with respect to harvest time showed significant differences (p of 0.000). Artichokes collected during the spring months were less humid than those harvested in the colder months (fall-winter). B. Tudela, Calico and Romanesco showed higher moisture content than Thema, V. Provence and Salambo in both the outer bracts and inner bracts (data not shown) (p of 0.047), although these differences were not detectable using Tukey's test because it is a conservative statistical tool which shows no significant difference if the number of cases is not high enough.

All these features are very important when selecting varieties for marketing fresh artichoke or industrial processing (canned, frozen or minimally processed product). For example, large varieties with a spherical shape, such as Salambo and Calico, are greatly appreciated by the French market for their fresh consumption for the preparation of stuffed artichokes. In the fresh-cut processing of artichoke, the moisture of the hearts is a parameter to consider because it is related to the tenderness of the edible part of the artichoke. In our study, B. Tudela, Calico and Romanesco were the most tender cultivars.

3.1.2. Numerical Determination of the Color

The color of external bracts ranged from green to deep purple with a wide range of different color hues and the color of internal bracts varied from yellow to yellow with purple shades, depending on the genotype. Significant differences were found in CIELAB parameters (L*a*b*) (Table 2) depending on the cultivar ($p \leq 0.05$) and type of bracts (outer or inner) ($p \leq 0.05$). On the outer bracts, B. Tudela and Calico showed high lightness (L* values above 50), green hues (negative values a*) and absence of purple shades (markedly positive b* values); by contrast, Romanesco, Thema, Salambo and V. Provence were cultivars with darker external bracts (L*< 50), absence of green hues (a*>0) and presence of bluish shades (low b* values). V. Provence showed some green shades and had a higher variability in a* and b* values with regard to the studied harvests.

Only small color differences between the productions of fall-winter and spring for each cultivar were found considering that a ΔE of about three units or lower is difficult to appreciate for the human eye. If the lightness and chroma differences for the outer bracts of each cultivar are plotted against those of B. Tudela (typically green), cultivars can be grouped as green or violet, with little differences between harvest periods (Figure 1A).

With respect to inner bracts (Table 2), B. Tudela and Calico were the varieties of lightest color, with yellow-greenish tone (high L* and b* values and low a* values).

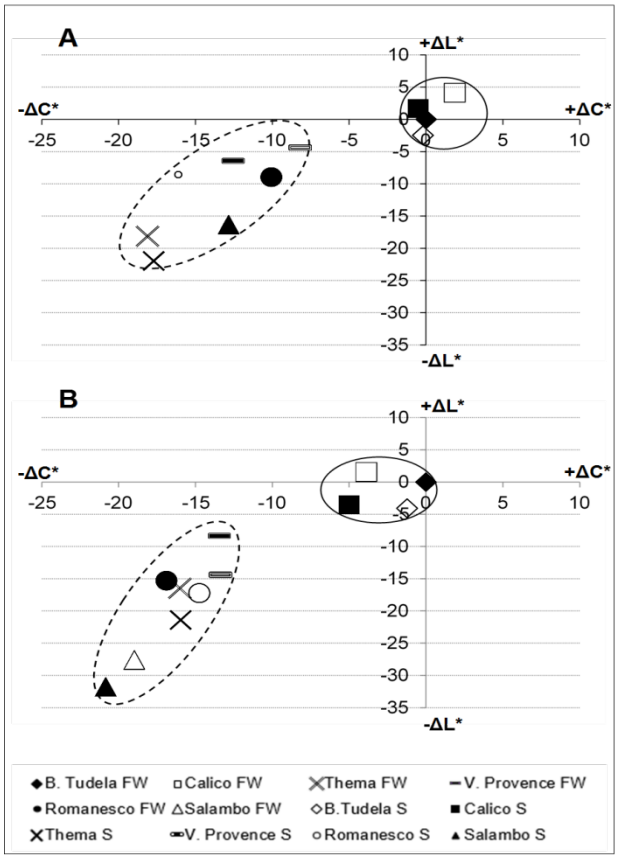

Figure 1. Classification of the color of six artichoke cultivars in terms of lightness (ΔL*) and chroma differences (ΔC*), taking into account type of bracts, outer bracts (A) and inner bracts (B), and harvest time (S: spring; FW: fall-winter)

Table 1. Morphological and Agronomic Characteristics of Six Cultivars of Artichoke

Variety	Fresh weight (g)	L/D ratio	Crop yield (kg/plant)	Outer bracts color	Inner bracts Color	Other characteristics
B.Tudela	164±16[a]	1.38±0.10[a]	3.12±0.21[a]	Green	Yellow	Spine-free, compact bracts
Calico	470±45[b]	0.85±0,06[b]	4.23±0.46[b]	Green	Yellow	Spine-free, compact and closed leaves
Thema	203±46[a]	1.50±0.05[a]	4.80±0.34[b]	Deep purple	Yellow with purple shades	Small spines and tendency to open
V.Provence	233±42[a]	2.04±0.11[d]	2.92±0.26[a]	Green with purple shades	Yellow with purple shades	Spine-free
Romanesco	237±24[a]	1.14±0.05[c]	4.57±0.33[b]	Purple	Yellow with purple shades	Spine-free
Salambo	400±55[b]	0.88±0.06[b]	3.84±0.50[ab]	Deep purple with green shades	Yellow with purple shades	Spine-free, compact and closed leaves

Table 2. Color Parameters for Outer and Inner bracts of the Six Artichoke Cultivars at Different Harvest Times.

Variety	Harvest time	Outer bracts				Inner bracts			
		L*	a*	b*	ΔE*	L*	a*	b*	ΔE*
B.Tudela	Fall-Winter	52.01±2.67	-6.48±0.77	23.33±2.00	0	64.49±2.71	-8.29±0.66	32.98±1.99	0
	Spring	54.49±2.35	-6.34±0.50	23.57±1.55	2.50	68.56±3.52	-7.60±1.11	34.41±2.37	4.37
Calico	Fall-Winter	56.10±2.06	-6.29±1.03	25.32±1.18	4.55	66.02±1.59	-7.43±0.48	29.19±2.76	4.18
	Spring	56.16±2.77	-6.24±0.71	23.08±2.00	4.16	65.01±3.46	-7.75±0.85	29.23±2.79	6.28
Thema	Fall-Winter	33.87±1.28	3.32±0.90	5.11±1.07	27.51	48.02±6.46	9.16±2.68	15.48±1.38	29.70
	Spring	32.53±2.98	3.46±1.95	5.72±3.39	29.95	47.16±5.80	10.31±4.05	16.29±5.16	33.27
V.Provence	Fall-Winter	45.56±2.79	3.04±0.80	11.27±2.79	16.66	56.13±3.52	-0.85±2.13	20.55±3.06	16.73
	Spring	50.07±3.42	3.53±2.40	16.24±2.28	13.29	54.16±1.80	5.65±2.07	21.14±4.63	23.64
Romanesco	Fall-Winter	43.05±3.47	7.03±0.70	12.28±2.94	19.62	49.19±4.21	8.37±1.80	14.93±3.45	28.94
	Spring	45.93±3.11	4.23±0.82	7.12±1.69	21.34	51.34±5.49	6.82±1.76	19.32±3.32	27.06
Salambo	Fall-Winter	35.74±1.68	7.93±1.31	7.91±2.52	26.65	36.89±3.58	12.89±1.79	7.73±1.84	42.99
	Spring	38.17±3.54	7.83±1.32	8.51±2.24	26.34	36.79±5.45	12.39±1.55	7.34±1.45	46.28

The other varieties showed hearts of color yellow with purple shades. The violet cultivars showed high color differences (ΔE*) with respect to B. Tudela.

The plot of lightness and chroma differences also grouped the artichoke heads with respect to the color of inner bracts, which were clearer and less saturated for the green cultivar (Figure 1B).

Color is an appearance variable taken into account by consumers to accept a food [33]. The purplish varieties are suitable for consumption on the Italian and French markets where this color is appreciated; while in Spain midsize varieties with yellow-green hearts without purple shades like B. Tudela are preferred.

3.2. Respiration Rate of Artichoke Heads

In general, fruits and vegetables characterized by a great physiological and biochemical stability are appreciated by processors and retailers, due to their longer shelf life and, consequently, duration at the point of sale. One way of measuring the metabolic activity of plant is to obtain the respiratory rate which is inversely correlated with postharvest durability [34]. Artichoke is classified as one of the vegetables with higher respiration rates, leading to a quick loss of organoleptic quality, so making it a highly perishable commodity [35].

Table 3. Respiration Rate (mL CO_2 kg^{-1} h^{-1}) of the Artichoke Heads for the Cultivars Studied.

Variety	RR at 4 °C	RR at 20 °C
B.Tudela	23.56±2.57[a]	88.12±4.34[a]
Calico	41.85±2,90[b]	119.08±5.08[b]
Thema	54.35±4.35[c]	147.34±6.49[c]
V.Provence	26.55±3.62[a]	91.04±3.53[a]
Romanesco	60.14±3.30[d]	162.39±6.67[d]
Salambo	32.42±2.33[e]	102.21±4.40[e]

Different letters within each column indicate statistically significant differences between means ($p \leq 0.05$).

Table 3 shows the RR values for artichoke heads of the six cultivars studied. It is observed that there are significant differences between most of cultivars, with Romanesco the variety with the highest RR value, followed by Thema, Calico and Salambo with intermediate behavior. B. Tudela and V. Provence, with similar values, had the lowest RR. Although B. Tudela artichoke is often classified as a vegetable with an extremely high respiration rate and rapid deterioration [36], compared to the majority of studied varieties it can be said that it is the most physiologically stable cultivar, with a shelf life similar to V. Provence.

As expected, low storage temperature reduced the RR of all artichoke cultivars significantly (p of 0.000), values which agree with those of Kader [35] and Suslow and Cantwell [37], who demonstrated that exposure to high temperatures increased the RR and ethylene production and, therefore, reduced the shelf life of artichokes.

3.3. Influence of Genotype, Harvest Time and Head Parts on the Oxidative Stability of Artichokes

3.3.1. Phenolic Content

The results obtained for total polyphenols content (I_{280} and IFC) in the different artichoke varieties according to the type of bracts and harvest time are shown in Figure 2.

The statistical analysis ANOVA of both indexes confirmed that the average total phenolic content was significantly lower in outer bracts than inner bracts ($p \leq 0.05$). The preferential accumulation of phenolic compounds in the edible part (inner bracts) was also confirmed by other authors, such as Fratianni et al. [38], Gil-Izquierdo et al. [6], Lombardo et al. [3], Negro et al. [39] and Pandino et al. [40].

Phenolic indexes were also affected by artichoke cultivar ($p \leq 0.05$). This relation was also found by Cabezas-Serrano et al. [7], Cefola et al. [4], Fratianni et al. [38], Lombardo et al. [3] and Negro et al. [39]. Thus, for I_{280} index, Thema was the genotype that showed the highest average value, regardless of the type of bracts or harvest time (185.96), followed by B. Tudela (161.76); while Salambo showed the lowest average value (93.51). Calico, Romanesco and V. Provence had an intermediate behavior. According to the IFC index, the maximum value was obtained by the Romanesco variety (193.16) followed by Thema (173.11); the lowest average value was obtained by Salambo (102.38). Both indexes had a similar

behavior, except for Romanesco, where the IFC average value was markedly higher than I_{280}. This fact may be because the Folin-Ciocalteu reagent can also react with other non-phenolic compounds [41]. So the use of both indexes was useful to explain the possible presence of other interfering compounds of reducing character (mainly amino acids and proteins) in the artichoke extracts.

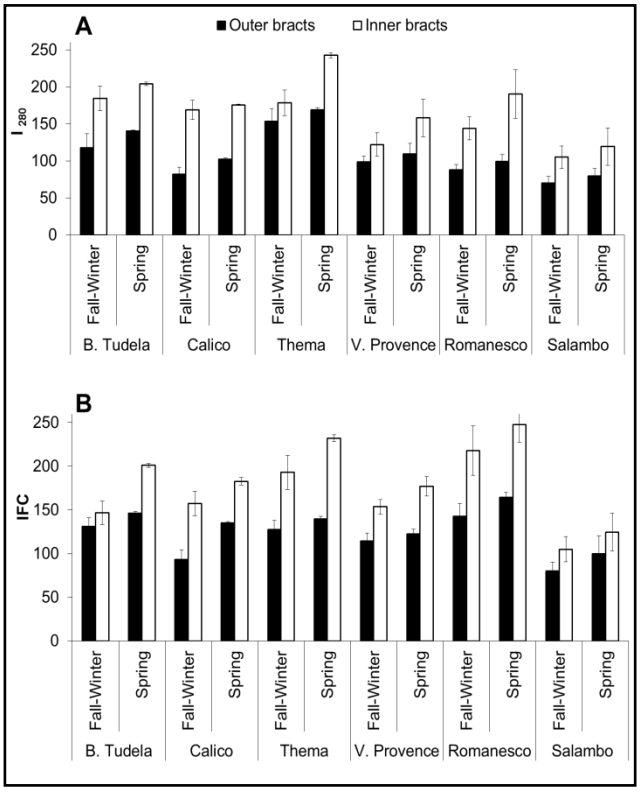

Figure 2. Polyphenols index (A) and Folin-Ciocalteau Index (B) in six artichoke cultivars according to type of bracts and harvest time

However, to our knowledge, the usefulness of the I_{280} has not been tested in artichoke extracts as in the case of other food matrices [42,43,44]. The high positive correlation found between IFC and I_{280} for the six varieties together in all the harvest times (r of 0.796, p of 0.000) verified the usefulness of both indexes for estimating the total phenolic content in artichoke extracts. Therefore, I_{280} and IFC can be used equally to estimate total phenolic content in artichoke. Besides, the I_{280} measurement has some advantages over IFC; it is simpler, quicker, cheaper and without pre-treatment or centrifugation and filtration steps.

According to Lombardo et al. [3], artichoke cultivars of lower polyphenol content will go to the food industry, while varieties rich in these compounds would be more adequate for the fresh market [4]. So, taking into account our results, the genotype Thema, with high phenolic content, would be interesting for fresh consumption given the beneficial health effects of these compounds. Conversely, genotypes with low phenolic content, as Salambo, would be more stable against browning reactions, so suitable for industrial handling.

The variation of phenolic content in relation to harvest time (spring, and fall-winter), regardless of genotype or type of bracts (Figure 2), shows that phenolic content (measured as I_{280} and IFC) was influenced by climatic conditions ($p \leq 0.05$). Artichokes harvested in spring had higher phenolic content than those harvested in fall-winter, with lower temperatures. These data agree with Lombardo et al. [3] and Todaro et al. [11], who found this same trend in Romanesco and V. Provence and Thema, respectively.

3.2.2. Determination of Enzymatic Activity

Enzymatic activities (PPO and POD) in the different artichoke genotypes, according to the type of bracts and harvest time, were analyzed to evaluate which were most susceptible to browning during handling. As shown in Table 4, PPO activity in inner bracts was significantly higher than in outer bracts (p of 0.000), agreeing with Lattanzio et al. [5], who found higher PPO activity values in artichoke hearts and inner bracts than in outer bracts. This is due not only to the presence of this enzyme in inner bracts, but also the high content of phenolic compounds capable of being oxidized. Conversely, the lowest POD activity was obtained in this same type of bracts. Although the polyphenol content was higher in inner bracts, POD activity may likely be limited by two main factors: the high affinity of PPO for phenolic substrates and the low H_2O_2 level in the internal tissue [15,45]. Thus, the highest POD activity in the outer bracts could be explained by the lowest PPO activity in these bracts. These results highlight that the PPO enzyme was mainly responsible for browning in artichoke.

In addition to the differences in the enzymatic activities associated to the bracts type, the results also show that variety and harvest time had influence on PPO and POD activities (p values ≤ 0.05). Fig. 3 shows the results of a single comparison procedure (Tukey's test) for the mean values of all PPO and POD activities for each cultivar, regardless of bract type or harvest time. Romanesco had the highest PPO activity value followed by Thema, Salambo and Calico with an intermediate value, while B. Tudela and V. Provence showed the lowest values. POD activity followed the same trend as PPO in all the cultivars.

Our results agree with Cefola et al. [4] and Cabezas-Serrano et al. [7] who found that enzymatic activities (PPO and POD) in artichoke were affected by the artichoke genotype. Furthermore, a relation was observed between enzymatic activity and respiratory intensity (r of 0.952) that can be related with different biotic stress undergone by artichokes. This means that the increase in enzymatic activity can be induced by high ethylene or CO_2 levels [4,46].

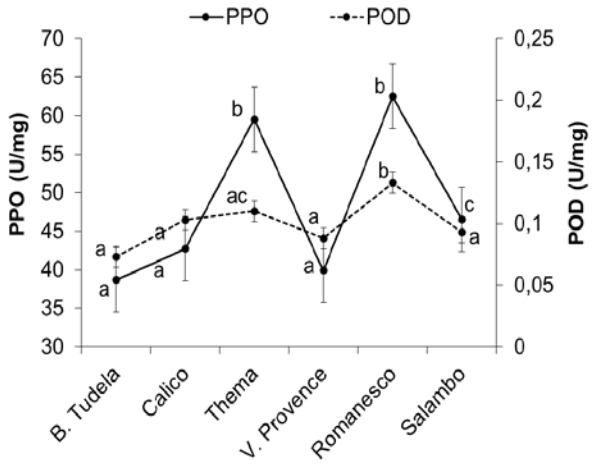

Figure 3. Main effects of artichoke cultivars on PPO and POD activities regardless of type of bracts and harvest time (different letter denotes a significant difference by Tukey's test)

Table 4. PPO and POD Activities (U mg^{-1} protein) of Six Cultivars of Artichoke According Type of Bracts and Harvest Time

		Outer bracts		Inner bracts	
Variety	Harvest time	PPO	POD	PPO	POD
B.Tudela	Fall-Winter	23.00±3.45	0.09±0.02	40.44±3.18	0.04±0.01
	Spring	38.82±3.11	0.12±0.03	52.39±3.96	0.06±0.01
Calico	Fall-Winter	20.66±2.23	0.07±0.01	53.97±3.71	0.03±0.01
	Spring	41.06±3.61	0.14±0.03	55.28±4.68	0.14±0.03
Thema	Fall-Winter	35.64±3.36	0.09±0.02	41.88±3.09	0.08±0.01
	Spring	37.89±2.64	0.14±0.02	122.57±6.19	0.13±0.02
V.Provence	Fall-Winter	36.33±1.13	0.08±0.01	39.97±2.90	0.08±0.01
	Spring	37.58±2.14	0.10±0.01	45.78±2.74	0.09±0.02
Romanesco	Fall-Winter	46.13±2.22	0.14±0.02	48.69±2.82	0.12±0.02
	Spring	63.87±2.77	0.14±0.03	91.17±4.11	0.13±0.03
Salambo	Fall-Winter	38.56±4.15	0.11±0.02	41.54±3.78	0.03±0.01
	Spring	52.21±5.17	0.16±0.01	53.87±3.23	0.07±0.01

As with the phenolic compounds, PPO activity in artichoke was significantly influenced by climatic conditions, with the highest average amount recovered in spring, with about 57.71 U/mg, and the lowest in fall-winter, with about 38.90 U/mg. These differences were caused by the direct relation between phenolic content and PPO enzyme noted above. POD activity followed the same trend as PPO according to the harvest time, with the highest value for spring (0.119 U/mg) and the lowest for fall-winter harvest (0.081 U/mg).

A regression analysis between PPO and POD activities in outer and inner bracts was carried out to examine a possible synergistic effect between both enzymes during browning reactions of artichoke. In outer bracts, the two enzymes were correlated with a r of 0.826, R^2 of 0.683 and a p of 0.002, supporting the hypothesis of Dawson [47] that both enzymes work in tandem during browning. This enzymatic correlation was corroborated by other authors such as Richard-Forget and Gauillard [15] and Subramanian et al. [48] in the case of other vegetable products; and by Cefola et al. [4] for artichoke cultivars. For inner bracts, no significant linear regression was found (r of 0.505, R^2 of 0.255 of and p of 0.053). The lack of a linear relationship between both enzymes for inner bracts may be explained by the low POD activity in these bracts, as a consequence of the high affinity of PPO for phenol substrates (Table 4).

3.2.3. Evaluation of Browning Potential

Browning processes in artichoke heads can be caused by enzymatic and non-enzymatic reactions involving phenols [49]. In healthy tissues where the cell compartmentalization is maintained and phenols and PPO enzymes are located in separated cell structures, browning may be attributed to non- enzymatic reactions. Conversely, when vegetable tissues are mechanically damaged, enzymatic browning gains importance [5,7]. In the latter case, the different phenolic content and the extent of enzymatic activity may explain the different suitability for minimally processing of the artichoke cultivars. Therefore, to evaluate which cultivars are most susceptible to enzymatic browning during handling and cutting, BP index was analyzed (Figure 4).

Significant differences between cultivars, type of bracts and harvest time on BP index were found (p values ≤ 0.05). The inner bracts of artichoke cultivars had higher susceptibility to browning than the outer bracts. Consequently, it can be stated that PPO enzyme is the main agent responsible for enzymatic browning because there was a higher PPO activity in inner bracts, while POD activity in these bracts was low.

The variation of the BP index in relation to harvest time was also evaluated and a higher activity was found in spring (BP index of 6.81), while in fall-winter harvests it was lower (BP index of 4.11). This confirms that harvest time considerably affects the phenolic content, enzymatic activity and therefore, the susceptibility of browning.

Browning potential also varied among the tested cultivars, with Calico, Romanesco and Thema showing the highest mean values (6.70, 6.60 and 6.36, respectively), followed by Salambo with an intermediate behavior (4.81); while B. Tudela and V. Provence displayed the lowest values (4.60 and 3.65, respectively). In addition, Pearson's correlation analysis showed that BP index was influenced by PPO activity and phenolic content in a similar proportion (r values for PPO, IFC and I_{280} of 0.781, 0.760 and 0.722, respectively). These results agreed with Cabezas-Serrano et al. [7] and Cefola et al. [4], who also found that phenolic content and enzymatic activity were directly correlated to the browning rate in fresh-cut artichoke heads.

Considering the BP values along with PPO activities and phenolic content, we can conclude that in purplish varieties, Romanesco and Thema were the genotypes of lower stability against the browning reactions (high BP) due to their high phenolic content and higher PPO activity. Conversely, V. Provence had the lowest browning susceptibility (low BP), a consequence of its low PPO activity. Salambo showed an intermediate sensitivity to browning (midrange PPO activity). With regard to "white" varieties, B. Tudela had higher oxidative stability than Calico, despite its high phenolic content, a circumstance that can be attributed to its lower PPO activity.

All these parameters contribute to define the optimum commercial use for the artichoke cultivars. So, varieties with higher enzymatic activity, phenolic content and browning index should preferably be marketed for fresh consumption while cultivars with lower oxidative parameters would be the most suitable for industrial processing [4]. Likewise, the suitability of the varieties V. Provence and B. Tudela for processing was confirmed.

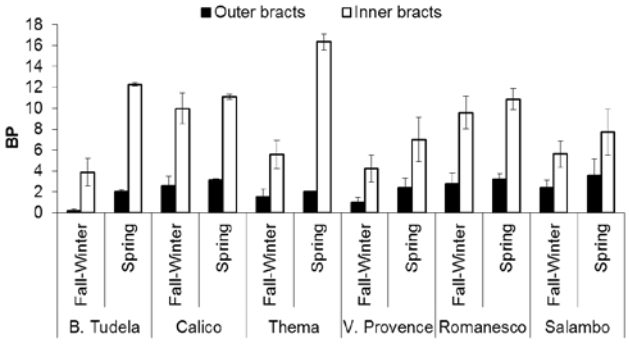

Figure 4. Browning Potential in six artichoke cultivars according type of bracts and harvest time.

4. Conclusions

The physical, chemical, physiological and biochemical characterization of six artichoke cultivars in this work contribute to define their optimum commercial use (fresh consumption or industrial processing). Taking into account our results, V. Provence and B. Tudela are the most suitable cultivars for processing due to their high oxidative stability, lower respiration rates and adequate size. Processed hearts of V. Provence are suitable for consumption in the Italian and French markets, where their purple color is appreciated. However processed formats (fresh-cut, frozen or canned) of B. Tudela are preferred in the Spanish market, due to the absence of purple shades which could be confused by the consumer with symptoms of product degradation. On the other hand, cultivars with higher susceptibility of browning (high enzymatic activity and phenolic content) and metabolic activity should preferably be marketed for fresh consumption. In addition, this study may be useful for the fresh-cut industry, providing information about post-cutting browning of artichokes and helping to select suitable raw materials for processing, treatment solutions, storage conditions and packaging materials.

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Statement of Competing Interests

The authors have no competing interests.

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