Morphological, mechanical and antioxidant properties of Portuguese almond cultivars

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Abstract The aim of this study was to evaluate morphological (of fruit and kernel), mechanical (namely shell rupture force) and antioxidant properties (including phenolics and flavonoid content) of five Portuguese almond cultivars, comparing them with two commercial cultivars (Glorieta and Ferragnès). Of the analyzed traits, nut and kernel dimensions varied substantially and were used to describe cultivars. However, some traditional cultivars recorded similar (Pegarinhos), or even higher (Amendoão, Casanova and Refêgo) nut and kernel weight than commercial cultivars. Furthermore, shelling percentage of traditional cultivar (Bonita) was higher than commercial cultivars. Rupture force necessary to break fruits of all traditional cultivars was higher than commercial ones, and was correlated to nut weight cultivars. The phenolics, flavonoids content and antioxidants were higher for Casanova. Parameters like high kernel weight, low percentages of double kernels or losses during shelling and considerable higher phenolics and flavonoids content may be considered by industry during selection of almond.

Keywords Almond · *Prunus dulcis* · Traditional cultivars · Morphological characterization · Antioxidant activity · Mechanical traits

Introduction

Apparently, the almond tree [*Prunus dulcis* (Mill.) D. A. Webb] was originated from one or more wild plant species from deserts and lower mountain slopes of central and southwestern Asia. Almond orchards are spread inside the Mediterranean basin, and, in Portugal, notably excellent edaphic and climatic conditions in the regions of Trás-os-Montes and Algarve are found (Cordeiro and Monteiro 2001). In recent years, the Portuguese area occupied with almond orchards has changed considerably (37,900 ha in 2007, 26,800 in 2010, and presently around 30,150 ha), with production following the same trend. The main reason for the increase of the area of production is related to the use of foreign commercial cultivars, including the French Ferragnès, Ferraduel and Ferrastar cvs., and the Spanish Marcona, Guara, Masbovera, Francolí and Glorieta cvs. (Cabrita et al. 2014). These cultivars have been well characterized, either in their country of origin, but also in subsequent works. This has allowed a good level of knowledge, regarding their behaviour, both agronomical and productive, in several other areas of production. The introduction of new foreign commercial cultivars, joined to the absence or reduced amount of scientific work regarding traditional Portuguese cultivars (Cordeiro and Monteiro 2001; Barreira et al. 2008) contributed to a significant decrease of interest in those ancient Portuguese cultivars. In fact, traditional cultivars are associated to low performances, in most situations, not for their intrinsic characteristics, but mainly due to other factors. Of these, it must

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be refereed the installation of almond orchards on marginal soils in unsuitable areas for growing and the use of out-of-date or inappropriate crop management practices. Besides, and as referred before, there is a lack of studies regarding the fruits of these cultivars, considering morphological, but also biochemical parameters. However, it should be considered that these cultivars can be used for genetic improvement, by introducing new genes or alleles (Colic et al. 2012), since they possess characteristics of interest. Some of these characteristics include productivity, adaptableness to different agro-climatical and agro-pedological conditions, tolerance or resistance to diseases (Antonucci et al. 2012). However, other traits are also of great importance, like those related to qualitative, organoleptic and nutritional traits. The traditional methods for the characterization of a given cultivar are mostly based in morphological traits, as they are able to allow a fast and simple appraisal of those characteristics (Colic et al. 2012). Furthermore, almond has a significant content in natural antioxidants, due to the presence of various biologically active compounds, such as phenolic compounds, including flavonoids, phenolic acids, tannins, and vitamin E (Rao 2012). Hence, in this work, five traditional Portuguese cultivars (Amendoão, Bonita, Casanova, Pegarinhos and Refêgo) and two commercial cultivars (Glorieta and Ferragnès) were studied, regarding antioxidant content and capacity, morphological traits (including nut and kernel dimensions, shelling percentage, and colour characteristics), but also concerning mechanical properties, namely the rupture force, an important parameter for industrial processing.

Materials and methods

Samples

Almond samples were obtained directly from producers located at the Northeastern Portugal. Portuguese almond cultivars included Amendoão and Pegarinhos, collected from the municipality of Murça, and Bonita, Casanova, Pegarinhos, and Refêgo, collected from the municipality of Torre de Moncorvo. Two commercial cultivars, Ferragnès and Glorieta, were also studied, and obtained at a producer from the municipality of Alfândega da Fé.

Morphological measurements

From each cultivar, 50 representative fruits were selected, and nut and kernel length, width, and thickness were measured using a digital caliper and expressed in millimetres. Nut weight and kernel weight were scale-weighed and expressed in grams. Several indexes were calculated:

length to width ratio— L/W , length to thickness ratio— L/T , width to thickness ratio— W/L , volume— $V = \frac{\pi}{6} (LWT)$ (Mohsenin 1980). The indices related to form, I_1 and I_2 (Valverde et al. 2006), were calculated as: $I_1 = \frac{T}{L} \times 100$ and $I_2 = \frac{W}{L} \times 100$. Shelling of the nuts were performed using a Texture Analyser, Stable Micro Systems TA.HD.-Plus, combined with a 75 mm plate and a 250 kg load cell. Results were expressed as Newton (N) and they represented the mean value of maximum force applied to disrupt the shell of each almond.

Colour parameters

Shell and kernel colour was evaluated using a MINOLTA CM-2300d colorimeter, with the results expressed in the CIELAB mode, evaluating the trichromatic coordinates L^* , a^* and b^* . The coordinate L^* refers to the lightness of the sample (from 0, representing black, to 100, representing white), while a^* is the coordinate that reflects the degree of approximation to the colour red, while positive, or green when negative. The coordinate b^* describes yellow, when positive, and blue when negative. Chroma (C^*) and Hue (H°) are calculated from the following formulas (McGuire 1992): $C^* = \left[(a^*)^2 + (b^*)^2 \right]^{1/2}$ and $H^\circ = \arctang(b^*/a^*)$.

The C^* is a measurement of chromaticity, which denotes the purity or saturation of the colour and the H° expresses the colour nuance (red–purple: 0° , yellow: 90° , bluish-green: 180° , and blue: 270°) (McGuire 1992).

Total phenolics, flavonoids, and antioxidant activity

Extraction was performed as previously described by Barreira et al. (2008), with minor modifications. Previous to extraction, fruits were air-dried to reduce moisture to the minimum. Kernels were grounded finely, and 1 g was extracted with 10 mL of methanol, with constant stirring, for 60 min, at room temperature. Extracts were filtered using a $0.45 \mu\text{m}$ syringe filter and used for antioxidant assays. All extractions were done in triplicate and readings done in duplicates.

Total phenolics

The determination of total phenolics was performed using the methodology described by Singleton and Rossi (1965) and quantification was achieved by spectrophotometrical readings at 765 nm, using gallic acid as standard.

Flavonoid contents

Flavonoid content in the extracts was determined by the colorimetric method described by Jia et al. (1999) with

some modifications. Extract (250 μL) was mixed with 1.25 mL of distilled water and 75 μL of a 5% NaNO_2 solution. After 5 min, 150 μL of a 10% $\text{AlCl}_3 \cdot \text{H}_2\text{O}$ solution was added. After 6 min, 500 μL of 1 M NaOH and 275 μL of distilled water were added to the mixture. The solution was well mixed and the intensity of pink colour was measured at 510 nm. The standard curve was calculated using (+)-catechin and the results were expressed as μg of (+)-catechin equivalents (CEs) per g of extract.

2,2-diphenyl-1-picrylhydrazyl: DPPH radical scavenging activity

To determine the DPPH radical scavenging assay was performed by the method described by Oyaizu (1986). Three hundred μL of sample extract were added to 2.7 mL of DPPH methanol solution (6×10^{-5} mol/L) and incubated for 1 h at room temperature in the dark. The absorbance was measured at 515 nm against a blank.

2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid): ABTS discoloration assay

The $\text{ABTS}^{+\cdot}$ discoloration assay was performed to evaluate the radical scavenging ability of crude extracts by the method of Re et al. (1999) with minor changes. $\text{ABTS}^{+\cdot}$ was generated by adding 88 μL of $\text{K}_2\text{S}_2\text{O}_8$ (140 mmol/L) to 5 mL of ABTS 7 mmol/L and incubated in the dark at room temperature for 12–16 h. This stock solution of $\text{ABTS}^{+\cdot}$ was diluted with ethanol to give an absorbance of 0.70 ± 0.02 at 734 nm. 20 μL of extract or standard was mixed with 2 mL diluted $\text{ABTS}^{+\cdot}$ solution and after 6 min of reaction, absorbance value was measured at 734 nm with UV–vis spectrophotometer.

Ferric reducing/antioxidant power: FRAP assay

The antioxidant capacity of the almond extracts was also estimated by the FRAP (ferric reducing/antioxidant power) assay, in which the antioxidants present in the sample reduce the $\text{Fe(III)/tripirydyltriazine}$ (TPTZ) complex to the blue ferrous form with an increase in absorbance at 593 nm. The FRAP assay was adapted from the method developed by Benzie and Strain (1996). FRAP reagent was prepared by mixing 10 volumes of 300 mM acetate buffer, pH 3.6, with 1 volume of 10 mM TPTZ in 40 mM hydrochloric acid and with 1 volume of 20 mM ferric chloride. A reagent blank reading was taken at 593 nm ($A_{\text{reagent blank}}$). Extracts or standard (0.2 mL) were added to this FRAP reagent (2 mL), and were incubated for 50 min. The absorbance was read (A_{sample}). Sample blank reading, using extract or standard (50 μL) and acetate buffer (2150 μL), was taken too ($A_{\text{sample blank}}$). The difference between A_{sample} and $A_{\text{sample blank}}$ was calculated as A_{1sample} .

The FRAP value was calculated according to the change in absorbance of A_{1sample} against $A_{\text{reagent blank}}$. Results for DPPH, ABTS and FRAP assays were expressed as mmol of Trolox equivalent (TE) per kilogram of sample, by means of a dose-response curve for Trolox (0–1000 μM).

Statistical analysis

Differences among means were determined by analysis of variance (ANOVA), using SPSS (Statistical Package for Social Sciences) software, version 19.0 (IBM Corporation, New York, U.S.A.) software. The fulfilment of the ANOVA requirements, namely the normal distribution of the residuals and the homogeneity of variance, were evaluated by means of the Kolmogorov–Smirnov with Lilliefors correction (if $n > 50$) or the Shapiro–Wilk's test (if $n < 50$), and the Levene's tests, respectively. Pearson correlations and regression analysis were also calculated using SPSS, but, in the latter situation, only those presenting a R^2 value > 0.5 are presented.

Results and discussion

Morphological and mechanical measurements

Morphological characteristics of almond cultivars are presented in Tables 1 and 2. All traits were considerably diverse which parameters that clearly define different cultivars. The weight of nut and kernel, as well as to the shelling percentage are considered important traits by producers. Nut weight varied from 2.66 ± 0.46 g in Bonita to 13.22 ± 1.67 g in the Amendoão (Table 1). The average nut weight for Ferragnès and Glorieta was 3.70 ± 0.77 g and 4.89 ± 0.91 g, respectively. The weight recorded were comparable to results reported for commercial cultivars (Arslan and Vursavus 2008). The values for Ferragnès were statistically similar to the one recorded for Bonita, while Glorieta presented results similar to both accessions of Pegarinhos cv. Cultivars Casanova and Refêgo showed higher values of nut weight, namely, 6.74 ± 1.02 and 7.50 ± 1.11 g. From breeding point of view, one of the most important traits is the kernel weight above 1 g, although some commercial cultivars showed kernel weight below than 1.0 g (Askin et al. 2007). Bonita and Pegarinhos—Moncorvo presented kernel weights lower than 1 g (Table 2). Highest values were recorded for Amendoão (1.92 ± 0.24 g) against the lowest for Bonita (0.69 ± 0.10 g). For Ferragnès and Glorieta, intermediate values (and statistically similar between them) were recorded (1.10 ± 0.21 and 1.09 ± 0.16 g). Kernels of Casanova presented a similar weight to those recorded for Ferragnès, while Pegarinhos—Murça presented similar

Table 1 Nut dimensions of different almond cultivars

	Nut weight (g)	Nut length (mm)	Nut width (mm)	Nut thickness (mm)	Nut L/W	Nut L/T	Nut W/T	Nut I ₁	Nut I ₂	Nut volume (cm ³)	Shelling percentage (%)	Shell thickness (mm)
<i>Anendoño</i>												
Mean	13.22a	46.17a	32.60a	24.93a	1.42d	1.86e	1.31e	54.79b	71.72c	19.72a	14.45f	6.45a
Min– máx	9.96–17.7244	24.39–52.53	30.26–36.07	20.81–24.93	0.74–1.60	1.04–2.32	0.89–1.45	43.08–96.15	62.64–135.42	9.89–30.10	5.21–17.93	4.46–8.02
SD/cv	1.670.13	4.86/0.11	1.31/0.04	2.17/0.09	0.14/0.10	0.23/0.12	0.09/0.07	9.27/0.17	11.77/0.16	3.31/0.17	2.08/0.14	0.78/0.12
<i>Bonita</i>												
Mean	2.66e	21.81f	17.13g	14.26ef	1.27f	1.53f	1.20f	65.54a	78.57a	2.82e	26.33b	2.68f
Min– máx	1.77–3.59	18.27–24.54	14.01–19.71	11.79–20.34	1.21–1.38	1.01–1.67	0.79–1.30	59.91–99.61	72.56–82.98	1.58–3.95	38.72–15.03	1.93–3.71
SD/cv	0.46/0.17	1.53/0.07	1.25/0.07	1.19/0.08	0.04/0.03	0.10/0.06	0.07/0.06	5.58/0.09	2.39/0.03	0.54/0.19	3.57/0.14	0.33/0.12
<i>Casanova</i>												
Mean	6.74b	36.13de	26.77c	16.25d	1.36e	2.23bc1.	1.65a	45.14d	74.30bc	8.25c	17.98e	3.74c
Min– máx	5.17–9.29	28.92–41.44	20.47–36.56	14.31–18.66	0.96–1.89	0.68–2.63	1.12–2.44	37.98–59.68	52.81–104.25	6.07–12.64	11.17–22.49	2.93–4.57
SD/cv	1.02/0.15	2.51/0.07	2.75/0.10	0.88/0.05	0.13/0.10	0.16/0.07	0.21/0.12	3.37/0.07	8.08/0.11	1.29/0.16	2.22/0.12	0.38/0.10
<i>Ferragnès</i>												
Mean	3.69d	34.83e	21.53e	14.71e	1.62bc	2.37b	1.47cd	42.30e	61.88d	5.83d	29.70a	3.01e
Min– máx	1.78–5.52	31.03–40.63	18.10–25.31	12.97–16.92	1.47–1.93	2.01–2.69	1.29–1.68	37.19–49.80	51.89–68.05	3.93–8.54	18.38–36.72	2.19–3.83
SD/cv	0.77/0.21	2.19/0.06	1.51/0.07	0.99/0.07	0.09/0.05	0.14/0.06	0.08/0.06	2.60/0.06	3.26/0.05	1.03/0.18	2.97/0.10	0.35/0.12
<i>Glorieta</i>												
Mean	4.89c	39.33b	24.03d	16.97c	1.64b	2.33b	1.42d	43.31de	61.18d	8.51c	22.55c	3.77c
Min– máx	3.33–7.09	34.17–47.72	20.42–28.18	14.78–25.27	1.51–1.97	1.58–2.86	1.04–1.64	34.97–63.16	50.73–66.39	5.73–13.46	16.81–27.94	3.04–4.91
SD/cv	0.91/0.19	3.36/0.09	1.91/0.08	1.60/0.09	0.08/0.05	0.19/0.08	0.10/0.07	4.01/0.09	2.73/0.04	1.92/0.22	2.76/0.12	0.47/0.12
<i>Pegarinhos (Moncorvo)</i>												
Mean	5.06c	37.97bc	20.63f	13.90f	1.84a	2.74a	1.49bc	36.76f	54.41e	5.79d	17.08e	3.30d
Min– máx	2.99–7.34	32.10–44.98	17.20–25.18	11.50–16.26	1.61–2.05	2.27–3.12	1.29–1.70	32.01–44.09	48.86–62.13	3.45–9.08	13.32–19.63	2.33–4.21
SD/cv	1.08/0.21	3.44/0.09	1.86/0.09	1.06/0.08	0.10/0.05	0.21/0.08	0.10/0.07	2.95/0.08	2.93/0.05	1.33/0.23	1.53/0.09	0.38/0.13
<i>Pegarinhos (Murça)</i>												
Mean	5.19c	34.59e	21.94e	14.87e	1.58c	2.34b	1.48bc	43.05de	63.50d	5.93d	20.20d	3.33d
Min– máx	3.70–6.88	31.01–39.36	19.99–24.63	11.18–18.40	1.38–1.78	1.89–3.08	1.25–1.94	32.50–53.04	56.22–72.50	4.38–7.85	15.80–23.09	2.72–4.20
SD/cv	0.74/0.14	1.93/0.06	1.12/0.05	1.12/0.08	0.08/0.05	0.19/0.08	0.11/0.07	3.45/0.08	3.23/0.05	0.86/0.14	1.57/0.08	0.37/0.11

Table 1 continued

	Nut weight (g)	Nut length (mm)	Nut width (mm)	Nut thickness (mm)	Nut L/W	Nut L/T	Nut W/T	Nut I_1	Nut I_2	Nut volume (cm ³)	Shelling percentage (%)	Shell thickness (mm)
<i>Refêgo</i>												
Mean	7.5b	36.91cd	27.73b	18.08b	1.33e	2.05d	1.54b	49.09c	75.23b	9.76b	19.33d	4.45b
Min– máx	5.20–10.30	31.35–42.21	22.43–32.74	16.13–21.03	1.15–1.52	1.58–2.37	1.26–1.65	42.24–63.15	65.85–86.74	6.43–14.02	14.11–22.36	3.37–5.60
SD/cv	1.11/0.15	2.39/0.06	1.74/0.06	1.11/0.06	0.07/0.05	0.13/0.06	0.08/0.05	3.32/0.07	3.73/0.05	1.58/0.16	1.76/0.09	0.47/0.11
P value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Each value is expressed as mean $\pm$ standard deviation (n = 50). SD—standard deviation; cv, coefficient of variation. L—length, W—width, T—thickness; $I_1 = \frac{L}{W} \times 100$ and $I_2 = \frac{W}{T} \times 100$

kernel weights as Glorieta. Other morphological traits evaluated include several indexes. The average values of I_1 and I_2 indexes indicated four different shape classifications: for Ferragnès, Glorieta and Pegarinhos—Moncorvo and Murça fruits were flat, long and narrow ($I_1 < 50$ and $I_2 < 70$). Casanova and Refêgo were considered flat and slightly elongated ($I_1 < 50$ and I_2 —70–80). Fruits from Amendoão cv. presented medium thickness and were slightly-elongated (I_1 —50–60 and I_2 —70–80), being the fruits of Bonita classified as round and slightly-elongated ($I_1 > 60$ and I_2 —70–80) (Yaghini et al. 2013; Table 1). Using a similar classification, almond kernels can be classified as flat and round, as shown by Amendoão, Pegarinhos—Murça and Refêgo cvs. ($I_1 < 30$ and $I_2 > 60$). The classification of flat and slightly elongated, was recorded for Casanova, Ferragnès, and Glorieta ($I_1 < 30$ and I_2 —50–60), while kernel from Bonita must be classified as globular and round ($I_1 > 40$ and I_2 —50–60). Finally, the Pegarinhos—Moncorvo kernel was classified as flat, long and narrow ($I_1 < 30$ and $I_2 < 50$) (Table 2). Nut L/W ratio ranged from 1.27 ± 0.04 for Bonita to 1.84 ± 0.10 for Pegarinhos—Moncorvo cv., with L/T ratio following the same trend (1.53 ± 0.10 and 2.74 ± 0.21). The W/L ratio was lower in fruits from Bonita (1.20 ± 0.07), but higher for Casanova (1.65 ± 0.21). For kernel indexes, L/W ratio varied from 1.46 ± 0.08 recorded in Refêgo cv., to 2.16 ± 0.15 in Pegarinhos—Moncorvo. L/T ratio was lower in kernel from Bonita (2.28 ± 0.19). This index was higher in kernels from Pegarinhos—Moncorvo (4.91 ± 0.56), while W/T ratio ranged from 1.41 ± 0.22 to 2.71 ± 0.21 , in kernel from Bonita and Amendoão, respectively. Another important trait was the percentage of double kernels, as they were ranked in lower categories of almond, reducing the profit potential of almond growers (Ledbetter and Palmquist 2006). Moreover, they were usually deformed kernels making the shelling process, size selection and blanching more difficult (Socias i Company et al. 2008). In all cultivars, the presence of double kernels was low (Table 2), with a maximum of three fruits presenting double kernels recorded for Bonita, Casanova and Pegarinhos—Moncorvo cvs. Only two double kernels were found in Refêgo, one in Pegarinhos—Murça, while in Amendoão, Ferragnès and Glorieta no double kernels were found. Regarding the shelling percentage (Table 1), significantly higher values were recorded for the commercial cultivar Ferragnès ($29.70 \pm 2.97\%$). The shelling of the traditional Bonita fruits resulted in the second highest percentage ($26.33 \pm 3.57\%$) while the other commercial cultivar (Glorieta) showed the third best shelling percentage ($22.55 \pm 2.76\%$). It should also be pointed out that fruits of Amendoão, presented higher weights and dimensions, and lower shelling percentage ($14.45 \pm 2.08\%$) was

Table 2 Kernel dimensions in different almond cultivars

	Kernel weight (g)	Kernel length (mm)	Kernel width (mm)	Kernel thickness (mm)	Kernel L/W	Kernel L/T	Kernel W/T	Kernel I ₁	Kernel I ₂	Kernel volume (cm ³)	% Internal volume occupied by kernel	Double (n)/damaged (%) kernels
<i>Amendoão</i>												
Mean	1.92a	29.98a	18.37a	6.81cd	1.63b	4.42b	2.71a	22.87d	61.58a	1.97a	50.45c	0/12
Min–máx	1.28–2.61	21.16–33.74	15.88–20.25	5.91–8.20	1.13–1.87	2.94–5.20	2.20–3.20	19.23–34.03	53.50–88.28	1.41–2.69	30.3–95.53	
SD/cv	0.24/0.13	2.27/0.08	0.96/0.05	0.50/0.0	0.12/0.07	0.43/0.10	0.21/0.08	2.63/0.11	5.30/0.09	0.28/0.14	13.64/0.27	
<i>Bonita</i>												
Mean	0.69f	17.25e	10.65f	7.59a	1.90a	2.28e	1.41e	44.08a	61.69a	0.74f	82.03a	3/4
Min–máx	0.47–0.98	14.28–20.33	10.94–12.33	6.34–9.57	1.98–1.48	2.84–1.91	1.20–1.92	35.20–53.36	58.90–67.71	0.60–1.22	41.02–99.74	
SD/cv	0.10/0.14	1.18/0.07	1.58/0.15	0.63/0.08	2.18/1.14	0.19/0.08	0.22/0.16	3.51/0.08	8.29/0.13	0.17/0.23	16.76/0.20	
<i>Casanova</i>												
Mean	1.21c	26.71b	15.61c	6.60de	1.71ab	4.06c	2.37b	24.83cd	58.57ab	1.44c	59.02b	3/23
Min–máx	0.76–1.46	20.85–29.59	13.38–16.80	5.83–7.55	1.42–1.91	2.84–4.96	1.94–2.68	21.58–35.25	52.25–70.60	1.06–1.72	38.06–93.84	
SD/cv	0.16/0.13	1.64/0.06	0.73/0.05	0.43/0.06	0.09/0.06	0.35/0.09	0.16/0.07	2.42/0.10	3.34/0.06	0.17/0.12	13.41/0.23	
<i>Ferragnès</i>												
Mean	1.10d	24.88d	13.23d	6.98bc	1.89ab	3.59d	1.91d	28.10b	53.32bc	1.21d	59.36b	0/8
Min–máx	0.72–1.57	21.41–29.37	10.28–14.97	5.54–8.78	1.49–2.43	2.95–4.47	1.43–2.47	22.36–33.89	41.07–67.10	0.86–1.77	39.87–8326	
SD/cv	0.21/0.19	1.79/0.07	0.94/0.07	0.69/0.10	0.14/0.07	0.32/0.09	0.22/0.12	2.47/0.09	3.88/0.07	0.23/0.19	10.34/0.17	
<i>Glorieta</i>												
Mean	1.09d	26.48bc	13.64d	6.39e	1.94ab	4.17c	2.15c	24.24cd	51.57bc	1.21d	48.27c	0/38
Min–máx	0.56–1.38	22.83–29.43	11.80–15.37	5.19–7.63	1.74–2.20	3.37–4.93	1.73–2.53	20.29–29.70	45.45–57.47	0.83–1.53	20.59–70.13	
SD/cv	0.16/0.15	1.63/0.06	0.85/0.06	0.47/0.07	0.09/0.05	0.41/0.10	0.22/0.10	2.45/0.10	2.48/0.05	0.15/0.12	9.2/0.19	
<i>Pegarinhos (Moncorvo)</i>												
Mean	0.84e	26.9b	12.46e	5.51f	2.16a	4.91a	2.27b	20.64de	46.41c	0.98e	62.02b	3/14
Min–máx	0.54–1.26	21.72–31.10	10.58–14.84	4.60–6.62	1.79–2.55	3.32–6.42	1.68–2.89	15.59–30.15	39.28–55.76	0.632–1.51	42.46–90.88	
SD/cv	0.18/0.22	2.30/0.09	1.12/0.09	0.49/0.09	0.15/0.07	0.56/0.11	0.24/0.11	2.57/0.12	3.28/0.07	0.19/0.20	10.66/0.17	
<i>Pegarinhos (Murça)</i>												
Mean	1.04d	25.71cd	13.31d	6.48e	1.93ab	3.99c	2.07c	25.26cd	51.85bc	1.16d	65.57b	1/8
Min–máx	0.67–1.34	22.72–28.64	11.71–14.90	5.45–8.21	1.74–2.18	3.30–4.81	1.65–2.47	20.78–30.31	45.97–57.54	0.79–1.58	45.81–92.74	
SD/cv	0.13/0.12	1.39/0.05	0.63/0.05	0.60/0.09	0.09/0.05	0.36/0.09	0.19/0.09	2.26/0.09	2.43/0.05	0.17/0.15	12.99/0.20	

Table 2 continued

	Kernel weight (g)	Kernel length (mm)	Kernel width (mm)	Kernel thickness (mm)	Kernel L/W	Kernel L/T	Kernel W/T	Kernel I_1	Kernel I_2	Kernel volume (cm ³)	% Internal volume occupied by kernel	Double (n)/damaged (%) kernels
<i>Refêgo</i>												
Mean	1.45b	24.87d	17.01b	7.20b	1.46b	3.47d	2.37b	29.05b	68.46a	1.59b	64.7b	2/12
Min–max	1.04–1.75	21.80–28.60	14.09–19.63	5.72–8.26	1.26–1.74	2.66–4.26	1.93–2.81	23.50–37.61	57.45–79.15	1.11–1.97	42.40–96.76	
SD/cv	0.18/0.12	1.37/0.06	0.98/0.06	0.52/0.07	0.08/0.05	0.30/0.09	0.20/0.09	2.63/0.09	3.68/0.05	0.20/0.13	10.99/0.17	
P value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.002	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	

Each value is expressed as mean $\pm$ standard deviation (n = 50). SD—standard deviation; cv, coefficient of variation. L—length, W—width, T—thickness; $I_1 = \frac{L}{W} \times 100$ and $I_2 = \frac{W}{T} \times 100$

recorded. This parameter can be looked at as a quantitative measure of shell hardness (Kodad and Socias i Company 2008). A general classification indicates 10–30% of shelling percentage for very hard shells, 30–50% for hard shells, and 50–70% for soft shells (Socias i Company et al. 2008). Using this classification, all of the studied cultivars are classified as very-hard shelled. Shelling percentage is inversely related to shell hardness (Kodad et al. 2007). In the present work, a similar negative correlation was found between shelling percentage and the rupture force needed to break the shell (-0.623 , $p < 0.01$). Additionally, a significant positive correlation was also found between the internal volume occupied by the almond kernel and shelling percentage (0.419 , $p < 0.01$) and rupture force (0.363 , $p < 0.01$). This link between lower empty space in the almond cavity and shelling percentage (and consequently to the rupture force) has been previously described and even used as a trait for selection of genotypes (Kodad et al. 2014). The presence of damaged kernels after shelling of almonds is a key factor, regarding the value of the production. In the present work, similar percentages of damaged kernels were found for almost all cultivars (Table 2), ranging from 4% in Bonita cv., to 14% in Pegarinhos—Murça, with in-between values for Amendoão (12%), Ferragnès (8%), Pegarinhos—Moncorvo (8%) and Refêgo (12%). Casanova and Glorieta cvs. presented the highest values for damaged kernels, reaching as much as 23% in Casanova and 38% in Glorieta. No clear relationship between morphological characteristics and the high number of damaged kernels was found in this work. However, it should be pointed out that these two cultivars presented statistically similar values of nut thickness, L/T ratio and nut volume. Shell thickness also presented considerable variations, when comparing cultivars (Table 1). This parameter ranged from 6.45 ± 0.78 mm, in Amendoão cv. to 2.67 ± 0.33 mm in Bonita cv.. Values for shell thickness recorded for the commercial cultivars were of 3.01 ± 0.35 mm in Ferragnès cv. and 3.77 ± 0.47 mm in fruit of Glorieta cv. whilst Pegarinhos—Moncorvo (3.29 ± 0.38 mm) and Casanova (3.74 ± 0.38 mm) cvs. showed statistically similar values. With the exception of the values recorded for Amendoão cv., all other values may be considered similar to those previously recorded in other cultivars (Askin et al. 2007; Altuntas et al. 2010). The evaluation of this specific parameter is of great importance owing to its relationship to several other characteristics. There have been found clear relationships between shell thickness and fatty acid composition of almond kernels (Askin et al. 2007). In addition, it can also influence the mechanical properties of almonds, including the required power to crack the almond (Altuntas et al. 2010). In the present study, rupture force ranged from 417.02 ± 105.06 N for Ferragnès cv., to 1194.53 ± 346.44 N recorded for Amendoão cv. (Figure 1). It should also be pointed out that

the other commercial cultivar—Glorieta, recorded the second lower rupture force (672.92 ± 142.67 N). The values obtained for the remaining traditional Portuguese cultivars were always above 800 N (832.98 ± 196.27 N for Pegarinhos—Murça, 898.62 ± 241.36 N for Refêgo, 944.19 ± 170.62 N for Bonita, 1061.11 ± 181.29 N for Pegarinhos—Moncorvo and 1184.30 ± 257.82 N for Casanova). For most of the tested cultivars, and as far as we were able to find, these are the first recorded data for rupture force. Previous data are available regarding Ferragnès cv. (Arslan and Vursavus 2008), with results showing similar values to the ones recorded in the present work. Four other cultivars—Gulcan 101-23, Nonpareil, Picantili, and Drake had also recorded values for rupture force similar to those found for Ferragnès cv. (Aktas et al. 2007; Arslan and Vursavus 2008). Other authors, analysing different almond accessions, recorded considerable variations in the rupture force, from a minimum of 227.9 ± 53.4 N, in Monterey cv., to 874.4 ± 60.4 N, in Y113-20 accession, without significant influence of the sampling year (Ledbetter 2008). Besides these considerable differences in the rupture force, it is also noticeable the large variation of the data in some of the studied cultivars. Indeed, and especially for Amendoão, Casanova and Refêgo cvs., range of the rupture force is substantial. From a processing point of view, this is a negative characteristic, since the setting of a standard force for breaking the shell may lead to undesirable consequences. From the problems that may occur, it must be referred the presence of unshelled fruits, if lower force than necessary is used or the

damaging of kernels, if higher force is applied. The rupture force needed to break the fruits was found to be correlated to several morphological traits, although varying for each cultivar, with no clear trend found (Table 3). For Amendoão cv., no correlations were found, while for Bonita cv. only the nut weight appears to correlate to the rupture force. In fact, nut weight was more often found to be correlated with the rupture force, with this relationship being found in five of the studied cultivars (the exceptions were Amendoão and Refêgo). Nut thickness, as well as nut and kernel volume were also found to correlate with rupture force, in half of the studied cultivars. Some other morphological parameters were only correlated to rupture force in three or less than three cultivars. As referred before, shell thickness has been linked to higher rupture force. However, in this study, this was only true for fruits of Casanova cv., where a positive correlation was found between shell thickness and the force applied to promote the rupture of the shell. Shell hardness has been inversely related to shelling percentage, and although no apparent effect on kernel quality exists, it may reduce the number of undamaged kernels after shelling (Kodad et al. 2014). Another important feature of almond dimensions, either for processing but also for marketing, is the homogeneity of the fruits, evaluated by the coefficient of variation. The nut weight showed a coefficient of variation as high as 0.36, in Amendoão fruits, with the minimum found for fruits of Pegarinhos—Murça cv. (0.14). The studied commercial cultivars, Ferragnès and Glorieta, presented values of coefficient of variation from 0.19 to 0.21, while the other

Fig. 1 Rupture force of shell of different almond cultivars. Each value is expressed as mean $\pm$ standard error ($n = 50$). Different letters mean significant differences between the almond cultivars

Table 3 Correlations between rupture force (N) and nut and kernel parameters recorded in different almond cultivars

	Amendoão	Bonita	Casanova	Ferragnès	Glorieta	Pegarinhos (Moncorvo)	Pegarinhos (Murça)	Refêgo
Nut weight (g)	0.069	0.308*	0.495**	0.344*	0.610**	0.442**	0.346*	0.061
Nut length (mm)	– 0.136	0.261	0.407*	– 0.004	0.423**	0.278	0.196	– 0.113
Nut width (mm)	0.056	0.195	0.052	0.067	0.415**	0.316*	0.248	– 0.275
Nut thickness (mm)	– 0.010	0.083	0.422*	0.251	0.304*	0.459**	0.304*	0.037
Nut L/W	– 0.161	0.133	0.262	– 0.088	0.068	– 0.094	– 0.024	0.200
Nut L/T	– 0.100	0.159	0.064	– 0.270	0.067	– 0.146	– 0.024	– 0.152
Nut W/T	0.069	0.100	– 0.141	– 0.209	0.035	– 0.099	– 0.132	– 0.359*
Nut I ₁	0.181	– 0.136	– 0.095	0.287*	– 0.061	0.150	0.133	0.151
Nut I ₂	0.218	– 0.135	– 0.203	0.092	– 0.043	0.097	0.044	– 0.194
Nut volume (mm ³)	– 0.062	0.197	0.362**	0.105	0.440**	0.387**	0.319*	– 0.138
Kernel weight (g)	0.007	0.093	0.259	0.105	0.398**	0.441**	0.273	– 0.082
Kernel length	– 0.126	0.202	0.482**	0.176	0.281*	0.226	0.239	0.066
Kernel width (mm)	0.038	0.151	0.164	– 0.141	0.422**	0.296	0.197	– 0.160
Kernel thickness (mm)	0.083	– 0.071	0.018	0.285*	0.010	0.369*	0.210	– 0.031
Kernel L/W	– 0.164	– 0.033	0.407**	0.308*	– 0.185	– 0.113	0.076	0.240
Kernel L/T	– 0.152	0.251	0.318*	– 0.175	0.163	– 0.112	– 0.035	0.081
Kernel W/T	– 0.35	0.184	0.092	– 0.341*	0.242	– 0.052	– 0.074	– 0.068
Kernel I ₁	0.132	– 0.240	– 0.311*	0.172	– 0.178	0.110	0.061	– 0.051
Kernel I ₂	0.133	0.066	– 0.400**	– 0.352*	0.169	0.093	– 0.083	– 0.235
Kernel volume (mm ³)	0.014	0.092	0.327*	0.153	0.366**	0.398*	0.309*	– 0.055
Shell thickness (mm)	0.123	0.223	0.292*	0.129	0.284	0.301	0.165	0.091
% Internal volume occupied by kernel	0.235	0.092	– 0.004	0.230	– 0.295*	– 0.058	– 0.034	0.297*

* and ** value indicates significant at $P < 0.05$ and $P < 0.01$, respectively

cultivars showed values equal or below than these ones. Considering the coefficient of variation of the kernel weight, differences between cultivars are less marked, with values ranging from 0.12 to 0.15, with the exception of Ferragnès (0.19) and Pegarinhos—Moncorvo (0.22). Shelling percentage also presented low coefficient of variation, ranging from 0.08 in Pegarinhos—Murça cv. to 0.09 in Pegarinhos—Moncorvo and Refêgo cvs.

The mean values of the colour coordinates (L^* , a^* , b^*) and the colour attributes (Chroma and Hue angle) for almond shell and kernel of the studied cultivars are presented in Table 4. Significant differences were observed in all of the parameters, with values of lightness (L^*) of shells ranging from 52.28 ± 3.13 for Casanova cv. (similar to those recorded in Glorietta) to 62.31 ± 2.71 in Amendoão cv. (statistically similar to Pegarinhos—Murça). Colour coordinate a^* ranged from 10.99 ± 0.77 in Amendoão cv. (with similar values found for Bonita) to 14.28 ± 1.77 in Glorietta cv.. The b^* coordinate and the Chroma (C^*) attribute presented a similar trend, with the highest values recorded for fruits of Pegarinhos—Murça cv. (31.01 ± 1.38), while the lowest values were found in Bonita (27.79 ± 0.88) fruits.

Values of Hue angle were higher in fruits from Bonita and Pegarinhos—Murça cvs., while Glorietta recorded the lowest values. Although previous results concerning the colour attributes of almond shells are scarce, the values obtained are not quite different to those found by Valverde et al. (2006), who recorded values of 66.30 ± 6.04 , 40.59 ± 5.30 and 72.34 ± 3.46 for lightness, Chroma and Hue, respectively. Regarding the colour coordinates and attributes of almond kernel, higher values in all parameters were recorded for kernels from Pegarinhos—Moncorvo cv., with L^* , a^* and H° statistically similar to the Bonita, Ferragnès and Casanova cvs. The lowest values for L^* and H° were found in kernel from Ferragnès cvs., while those of Refêgo had the lowest values of a^* , b^* and C^* . Previous works regarding colour parameters of kernels in other almond cultivars, showed diverse values. Valverde et al. (2006) reported lightness, Chroma and Hue values of 57.55 ± 7.16 , 48.49 ± 3.41 and 68.28 ± 3.86 , respectively, for Guara Ledbetter and Sisteron (2010) indicated L^* of 2.66 ± 4.66 , Chroma of 38.74 ± 2.84 and Hue of 69.43 ± 2.61 , for Nonpareil cv., and Agila and Barringer (2012) registered a L^* of 58.8 and C^* of 9.1.

Table 4 Colour coordinates (L^* , a^* , b^*) and the colour attributes (Chroma and Hue) of shell of different almond cultivars

Cultivar	L^*			a^*			b^*			Chroma		Hue	
	Shell	Kernel		Shell	Kernel		Shell	Kernel		Shell	Kernel	Shell	Kernel
Amendoão	62.31 ± 2.17a	40.78 ± 3.07bc		10.99 ± 0.77e	14.83 ± 2.02bc		28.91 ± 1.46c	23.44 ± 3.54ef		30.93 ± 1.54d	27.74 ± 4.02d	69.18 ± 1.07a	57.60 ± 1.40d
Bonita	55.90 ± 1.79b	45.08 ± 2.18a		11.21 ± 0.66e	15.44 ± 1.41b		27.79 ± 0.88d	27.58 ± 2.34b		29.97 ± 0.98e	31.61 ± 2.67b	68.05 ± 0.98b	60.77 ± 1.03b
Casanova	52.28 ± 3.13d	42.01 ± 2.99b		13.37 ± 1.01b	14.12 ± 1.82c		29.79 ± 2.43c	26.51 ± 3.38bc		32.09 ± 2.55c	30.04 ± 3.77bc	65.33 ± 1.18c	61.95 ± 1.42a
Ferragnès	55.63 ± 2.61b	38.80 ± 6.55d		12.55 ± 1.24c	16.93 ± 3.17a		28.06 ± 1.73d	26.06 ± 5.36c		30.75 ± 1.88de	31.09 ± 6.12b	65.90 ± 1.83c	56.81 ± 2.54e
Glorieta	53.26 ± 2.71cd	39.72 ± 2.44cd		14.28 ± 1.77a	15.28 ± 2.22b		29.06 ± 1.84c	24.49 ± 3.24de		32.36 ± 2.07bc	28.89 ± 3.81cd	63.82 ± 2.65d	58.07 ± 1.98d
Pegarinhos (Moncorvo)	55.07 ± 2.63b	45.85 ± 3.47a		12.15 ± 1.06cd	17.30 ± 1.27a		30.49 ± 1.94ab	32.89 ± 3.02a		32.83 ± 2.07abc	37.17 ± 3.16a	68.28 ± 1.37b	62.20 ± 1.36a
Pegarinhos (Murça)	61.40 ± 1.62a	39.10 ± 2.64d		11.74 ± 0.76d	15.19 ± 1.17b		31.01 ± 1.38a	25.90 ± 1.92cd		33.16 ± 1.49a	30.04 ± 2.16bc	69.26 ± 0.84a	59.59 ± 1.20c
Refêgo	53.75 ± 2.29c	40.75 ± 2.72bc		13.47 ± 0.86b	12.94 ± 1.43d		30.03 ± 1.34b	22.64 ± 2.23f		32.92 ± 1.44ab	26.08 ± 2.57e	65.85 ± 1.19c	60.24 ± 1.43bc
P value	< 0.0001	< 0.0001		< 0.0001	< 0.0001		< 0.0001	< 0.0001		< 0.0001	< 0.0001	< 0.0001	< 0.0001

Each value is expressed as mean ± standard deviation (n = 50). In each column different letters mean significant differences between the almond cultivars

Total phenolics, flavonoids, and antioxidant activity

Considering the amount of phenolic compounds, flavonoids and the evaluation of antioxidant activity, significant differences were observed among the studied cultivars (Table 5). The content of phenolic compounds ranged from 0.32 ± 0.04 mg GAE/g fresh weight in Bonita (although statistically similar to fruits of Amendoão, Glorieta, Pegarinhos–Murça and Refêgo cvs.) to 3.47 ± 0.17 mg GAE/g fresh weight found in Casanova kernels. These values are higher than the results reported by Milbury et al. (2006) who detected a range of total phenolics from 126.8 to 240.8 mg/100 g fresh weight, depending on the studied cultivar. Similar values were also found by Kornsteiner et al. (2006). Some of these studies showed that phenolics of almond kernel were mainly present in the skin, with Milbury et al. (2006) indicating that an average value of 60% of almond phenolics were present in the skin, while Kornsteiner et al. (2006) referred as much as 80%. Milbury et al. (2006) made an attempt to justify the differences among total phenolic content of almond cultivars, indicating that they may be linked to the skins, while kernel presented similar concentrations of these compounds. In what concerns flavonoid content, the detected variation among cultivars was small. Nevertheless, fruits of Casanova presented the highest amount of flavonoids (2.82 ± 0.11 µg CE/g fresh weight) while the lowest content was recorder in Glorieta (1.61 ± 0.16 µg CE/g fresh weight, statistically similar to the value recorded in fruits from Bonita cv.). However, our results reveal higher amounts of phenolics and flavonoids recorded in traditional cultivars, rather than in the commercial ones, which is the opposite of the previous work from Barreira et al. (2008). To provide a clear overview of the antioxidant activity of almonds, three different methods were used (ABTS, DPPH and FRAP). Results for ABTS and FRAP showed a clear increase on antioxidant activity provided from extracts from Casanova kernels (19.24 ± 0.83 and 9.16 ± 1.36 µg Trolox/g fresh weight, respectively for ABTS and FRAP). These values were considerably higher than values obtained for Bonita (1.26 ± 0.16 and 1.88 ± 0.16 µg Trolox/g fresh weight). For the DPPH assay, higher values of antioxidant activity were recorded for Ferragnès (2.21 ± 0.04 µg Trolox/g fresh weight, similar to Casanova and Pegarinhos–Moncorvo cvs.), while lower values were found in Amendoão (similar to results recorded for Bonita). The antioxidant activity, measured by different methodologies, in almond and in other nuts, had been previously correlated to the content in phenolic compounds (Pereira et al. 2008), a relationship also found in the present work. For all the three methodologies, a clear positive correlation was found between phenolic contents and antioxidant activity. The DPPH radical scavenging activity showed the lower correlation to the phenolic contents ($R^2 = 0.57$),

Table 5 Total phenolics and flavonoids contents and antioxidant activity in different almond cultivars

Cultivar	Phenolics (mg GAE/g FW)	Flavonoids (μ g CE/g FW)	ABTS (μ g Trolox/g FW)	DPPH (μ g Trolox/g FW)	FRAP (μ g Trolox/g FW)
Amendoão	0.45 $\pm$ 0.09c	1.99 $\pm$ 0.09cd	1.29 $\pm$ 0.16d	0.78 $\pm$ 0.08e	1.88 $\pm$ 0.16d
Bonita	0.32 $\pm$ 0.40c	1.89 $\pm$ 0.13de	1.49 $\pm$ 0.07d	0.94 $\pm$ 0.11de	1.95 $\pm$ 0.44d
Casanova	3.47 $\pm$ 0.17a	2.82 $\pm$ 0.11a	19.24 $\pm$ 0.83a	2.14 $\pm$ 0.05ab	9.16 $\pm$ 1.36a
Ferragnès	1.88 $\pm$ 0.52b	1.98 $\pm$ 0.25cd	7.00 $\pm$ 0.63bc	2.21 $\pm$ 0.04a	4.04 $\pm$ 0.32b
Glorieta	0.40 $\pm$ 0.05c	1.61 $\pm$ 0.16e	2.44 $\pm$ 0.24d	1.22 $\pm$ 0.16c	2.24 $\pm$ 0.15d
Pegarinhos (Moncorvo)	1.95 $\pm$ 0.37b	2.25 $\pm$ 0.18bc	7.87 $\pm$ 1.36b	2.16 $\pm$ 0.01a	4.44 $\pm$ 0.61b
Pegarinhos (Murça)	0.78 $\pm$ 0.11c	2.07 $\pm$ 0.26cd	5.36 $\pm$ 0.60c	1.99 $\pm$ 0.06b	3.68 $\pm$ 0.21bc
Refêgo	0.42 $\pm$ 0.13c	2.42 $\pm$ 0.17b	1.70 $\pm$ 0.17d	0.97 $\pm$ 0.09d	2.72 $\pm$ 0.35cd
P value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Each value is expressed as mean $\pm$ standard deviation (n = 6). In each column different letters mean significant differences between the almond cultivars

while ABTS and FRAP showed higher values for correlation coefficients ($R^2 = 0.84$ and $R^2 = 0.85$, respectively for FRAP and ABTS). Concerning flavonoids, the strongest correlation (data not showed) was observed for DPPH ($R^2 = 0.51$), while for ABTS and FRAP the variance of data regarding antioxidant activity explained by flavonoid content was lower ($R^2 = 0.41$ and $R^2 = 0.12$, respectively). The relationship between ABTS, DPPH and FRAP, and the amount of phenolic content is expressed by the equations $y = 4.787x + 0.2089$, $y = 0.4194x + 1.0444$ and $y = 1.947x + 1.408$, respectively.

Conclusion

The present work reported the characteristics of five Portuguese almond cultivars, and can be used as a stepping-stone to further studies focusing on selected traits of these cultivars. Casanova and Pegarinhos—Moncorvo, showed considerable higher content of phenolic compounds and flavonoids that resulted in their higher antioxidant activity. Further characteristics of interest include the high kernel weight of some cultivars, like Amendoão or Casanova, or the low percentages of double kernels or losses at shelling, with similar antioxidant, morphological or mechanical traits, like Pegarinhos or Refêgo, when compared to commercial cultivars. The results showed that the Portuguese traditional cultivars has potential to be used by the almond industry, if properly selected accordingly to their characteristics, and may be further used by breeding programs.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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