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Fruit chemical composition of hazelnut cultivars grown in Portugal

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Abstract

Chemical composition (crude protein, crude fat, starch, neutral detergent fibre – NDF and free α -amino acids) of six hazelnut cultivars (Butler, Ennis, Fertile de Coutard, Grossal, Merveille de Bollwiller and Segorbe) was investigated. Genotype significantly affected fruit nutritive value. Crude protein ranged from 12-17 g 100 g⁻¹ dry weight (dw) in cultivar Ennis and Merveille de Bollwiller, respectively; crude fat was 50-62 g 100 g⁻¹ dw in cvs. Fertile de Coutard and Butler; starch varied from 1.0 to 2.4 g 100 g⁻¹ dw in cvs. Segorbe and Butler; and NDF was 8-14 g 100 g⁻¹ dw in cvs. Merveille de Bollwiller and Ennis. Total free α -amino acids content ranged from 144 mg 100 g⁻¹ dw (cv. Segorbe) to 413 mg 100 g⁻¹ dw (cv. Butler). The essential amino acids content varied between 23 mg 100 g⁻¹ dw (cv. Butler) to 55 mg 100 g⁻¹ dw (cv. Merveille de Bollwiller). Alanine was the main amino acid found (62% of total amino acids) and methionine was the lowest (0.3%). Based on the available data on the phytochemical content of hazelnuts, including the data presented in this study, there is a high likelihood that this fruit will provide positive health benefits.

Key words: Crude fat, crude protein, free α -amino acids, neutral detergent fibre, proximate analysis, starch

Introduction

In recent years, nuts have become more important in human nutrition because of their potential health benefits. Epidemiological studies, in Framingham Massachusetts, USA, have shown that the frequency of nut intake was correlated with a risk reduction of coronary heart disease, atherosclerosis and some types of cancer by up to 50% (Alpan *et al.*, 1997; Richardson, 1997; Brehme, 2002). Moreover, Salas-Salvadó and Megias (2005) considered nuts as a natural functional healthy food. The recognition by the U.S. FDA that nuts must be regarded as “heart-healthy” foods gives a great input for the increase of the consumption of these fruits.

Consumers have become interested in food composition beyond the data usually available in standard composition tables (Souci *et al.*, 1994; Holland *et al.*, 1998). There is also increasing worldwide demand for non-meat protein sources with balanced amino acid profiles. As interest in nuts has been increased, it is important to evaluate the composition of these fruits commonly grown in each country. While it is obvious that hazelnuts have a positive role in human nutrition, it will not be easy to recognize which components have the more significant effects (Savage, 2001). However, Fraser *et al.* (1992) suggested that the health benefits of nuts are due to the synergistic effect between its constituents and enhanced complex biochemical interrelationships working together. Studies carried out by Shahidi *et al.* (2007) suggest that hazelnut and its byproducts, green leafy covers, hard shells and tree leaf, could potentially be considered as an excellent source of natural antioxidants. Most of the studies on the composition of hazelnut kernel have mainly focused on lipid content with little data on amino acid composition, starch, fibre and ash. According

to Alasalvar *et al.* (2003), the good nutritional value of hazelnut is its amount of fibre that has an important protective effect against intestinal disorders, cholesterol and hypertension, among other effects. Gu *et al.* (2004) showed that hazelnut kernel contain significant concentration of proanthocyanidins that are known to have positive health effects.

Amino acids are important because they are the precursors of secondary plant metabolites and are involved in the production of compounds which directly or indirectly affect human health (Gomes and Rosa, 2000). Moreover, humans cannot synthesize ten amino acids, and these must be provided by the diet (Anderson *et al.*, 1998). Free amino acids, are essential nonvolatile compounds involved in the overall taste and flavor of many foods having a considerable influence on the sensory characteristics of fruits, bitter, sour and sweet taste, affecting both quality and nutritional value (Fuke and Konosu, 1991).

Several studies indicated that the nut composition of hazelnut is affected by cultivar, harvest year, soil, irrigation and method of cultivation (Parcerisa *et al.*, 1993; Parcerisa *et al.*, 1994; Parcerisa *et al.*, 1995; Amaral *et al.*, 2006).

Therefore, a study was performed to evaluate the fruit quality of six hazelnut cultivars in field grown conditions in Portugal in relation to crude protein, crude fat, starch, neutral detergent fibre and free α -amino acids for better knowledge of their composition *vis-a-vis* nutritional significance.

Materials and methods

Plant material and growth conditions: The study was carried out on 20-year-old plants of hazelnut (*Corylus avellana* L.) in an experimental plot near Vila Real, Northeast Portugal (41° 19' N

and 7° 44' W; altitude 470 m above sea level) in 2005. The climate is characterized as a transition from Csb to Csa (mesothermic climate with a partially dry summer) of Köpen. A plot of 75 plants representing eleven cultivars planted at 5 x 3 m spacing on a Typic Dystrochrept silt loam soil and left unpruned was used for the study. The orchard was fertilized and periodically drip-irrigated (Santos *et al.*, 1998). Nuts from six hazelnut cultivars: Butler, Ennis, Fertile de Coutard, Grossal, Merveille de Bollwiller and Segorbe were hand-picked from the ground at the beginning of the harvest in September, and kept unshelled in a refrigerator (2 °C) until analyses were carried out.

Physical parameters: Yield per tree was recorded, and individual fruit and kernel weight estimated from 3 samples of 100 fruits. Yield per unit (kg m⁻³) was calculated. Canopy volume (*v*) was calculated for a prolate spheroid, a plant taller than wide, by the formula $v = 4/3\pi ab^2$, where *a* = 1/2 of the tree canopy height and *b* = 1/2 of the tree canopy width (Lagerstedt and Painter, 1973).

Proximate analysis: Moisture content was determined using the Official Analytical Chemists Methods (AOAC, 1995). Representative samples of each cultivar were removed from the shell and the kernel was finely chopped, and ash content determined by incineration at 550 °C for 3 h in accordance with the AOAC method (1995). Crude protein (nitrogen x 6.25) was evaluated using the Kjeldahl procedure with selenium as a catalyst (AOAC, 1995). Crude fat was measured by extraction with petroleum ether in a Tecator Soxtec System (model HT1043) according to AOAC (1995). Starch was determined by enzymatic hydrolysis of starch to glucose as described by Salomonson *et al.* (1984). Neutral detergent fibre (NDF) was evaluated after extraction with the neutral detergent solution hydrolysis according to the procedures described by Van Soest *et al.* (1991).

Free α -amino acids: The extraction and purification of free α -amino acids were performed according to Gomes and Rosa (2000). Powdered freeze-dried tissues were extracted twice with boiling methanol (90%) for 2 min under continuous homogenisation, centrifuged for 2 min at 6.25 g, and the supernatant poured into a 10 mL volumetric flask. This step was repeated twice using methanol (70%). Combined supernatants were made up to a final volume of 10 mL with methanol (70%) and kept at -18°C until analysis. Subsequently 2 mL of each extract was evaporated and resuspended in 2 mL of 0.1 M HCl. Mini-columns of 1 mL (Chromabond from Macherey-Nagel) were connected to a solid phase extraction vacuum system (Gilson) and eluted with 0.5 mL of 0.1 M HCl before being filled up to 2 cm with a cation exchange resin, Dowex (H⁺) 50WX8-499 (Sigma-Aldrich Chemicals, St Louis, MO, USA). The amino acids were loaded onto the columns and washed with 5 mL of 0.1 M HCl. Free α -amino acids were eluted with 4 x 2.5 mL of 7 M NH₃ pa (Merck, Darmstadt, Germany). After evaporation, the residue was resuspended in 0.3 mL of distilled water, filtered (Spartan 13, 0.2 μ m) and kept in vials at -18 °C until analysis. Amino acids were determined by HPLC using C18 column (Waters, Spherisorb S30DS, id 4.6 mm) 150 mm length and a

UV/VIS detector set at 340 nm, after precolumn derivatisation with o-phthalaldehyde/2-mercaptoethanol. The mobile phase was made of two solvents: A – 350 mM Na₂HPO₄ · 2H₂O and 250 mM propionic acid (1:1), acetonitrile and Milli-Q water (40:8:52); B – acetonitrile / methanol / water (30:30:40). With these solvents, a gradient was set (Table 1). Identification and quantification of detected amino acids were done against external standards after adjustment through regression lines.

Statistical analysis: Data analyses were performed as analysis of variance using the Super ANOVA software (1.1, Abacus Concepts Inc., 1991). Mean separations were made using Fisher's Protected LSD Test (*P* = 0.05), designed to allow all possible linear combinations of group means to be tested. All determinations were performed in triplicate.

Results and discussion

Physical parameters: The physical parameters (fruit and kernel weight, percent kernel and yield) were additionally measured to better characterize the fruits of the six cultivars that showed important visual differences. Physical parameters varied significantly among cultivars (*P* < 0.01) (Table 2). Cv. Ennis presented the heaviest nuts, 64% higher than cv. Segorbe, which presented the lightest ones. The kernel weight followed the same trend, *i.e.*, highest kernel weight was observed in cv. Ennis (1.8 g) and the cv. Segorbe (1.1 g) had the lowest. These values were similar to the average data of the six cultivars recorded during fifteen years (Silva *et al.*, 2005). The percent kernel was high in cvs. Segorbe, Butler, Ennis and Grossal (~45%), and low in cv. Merveille de Bollwiller (39%). Cv. Butler had the highest yield (Table 2).

Proximate analysis: Regarding chemical composition, crude protein, crude fat, starch and neutral detergent fibre (NDF) varied significantly among cultivars (Table 3). Crude protein ranged between 12.3 g 100 g⁻¹ (cv. Ennis) and 17.1 g 100 g⁻¹ (cv. Merveille de Bollwiller). Hazelnuts, like other nuts, contain high levels of crude protein but few reports are available in literature (Alasalvar *et al.*, 2003). These values were comparable with the levels recorded for six cultivars grown in Tarragona which ranged from 12 to 18 g 100 g⁻¹ (Bonvehí, 1995), and slightly lower than the values obtained for Butler, Ennis and Fertile de Coutard grown in New Zealand that showed values of 18, 17 and 15 g 100 g⁻¹, respectively (Savage and McNeil, 1998). Also, Alasalvar *et al.* (2003) reported that cv. Tombul contains 15 g 100 g⁻¹ of protein. However, some cvs. like Yassi and Yuvarlak showed values as low as 7 g 100 g⁻¹ (Ayfer *et al.*, 1997).

Generally, crude fat content of the samples was around 50% (50 to 61 g 100 g⁻¹ dw in cvs. Butler and Fertile de Coutard, respectively) and was the predominant component of hazelnuts (Table 3). According to Richardson (1997) the oil content varied between 57% dw in cv. Merveille de Bollwiller and 65% dw in cvs. Tombul, Casina and Negret. Fat makes up 60-70% of the kernel, which is responsible for the high source of energy

Table 1. HPLC gradient for free α -amino acid analysis

Time (min)	0.0	9.5	11.0	13.6	20.4	23.4	25.4	32.0	34.0	37.0
Flow (mL min ⁻¹)	1.3	1.3	1.3	1.3	1.3	1.3	0.8	0.8	1.3	1.3
Solvent B (%)	0.0	11.0	12.0	20.0	45.0	50.0	60.0	100.0	0.0	0.0

Table 2. Physical parameters of the fruits of the six hazelnut cultivars

Cultivar	Fruit weight (g)	Kernel weight (g)	Percent kernel (%)	Yield (kg m ⁻³)
Butler	3.55 ± 0.4 c	1.61 ± 0.1 c	45.41 ± 1.2 c	0.15 ± 0.0 c
Ennis	3.86 ± 0.2 d	1.76 ± 0.3 d	45.53 ± 3.1 c	0.05 ± 0.0 a
Fértil de Coutard	3.36 ± 0.2 c	1.45 ± 0.2 b	42.80 ± 3.2 b	0.09 ± 0.0 b
Grossal	2.44 ± 0.2 a	1.12 ± 0.1 a	45.85 ± 2.9 c	0.08 ± 0.0 b
Merveille de Bollwiller	2.81 ± 0.0 b	1.09 ± 0.0 a	38.57 ± 2.5 a	0.14 ± 0.0 c
Segorbe	2.35 ± 0.1 a	1.08 ± 0.1 a	46.09 ± 3.1 c	0.08 ± 0.0 b
<i>P</i>	< 0.001	< 0.01	< 0.01	< 0.01

Different letters within one column denote statistically significant differences ($P < 0.05$) by ANOVA and Fisher's LSD test. Values are average of three individual samples ± standard deviation.

Table 3. Proximate analysis of the fruits of the six hazelnut cultivars

Cultivar	Crude protein (g 100 g ⁻¹ dw)	Crude fat (g 100 g ⁻¹ dw)	Starch (g 100 g ⁻¹ dw)	NDF (g 100 g ⁻¹ dw)	Moisture (%)	Ash (g 100 g ⁻¹ dw)
Butler	14.53 ± 0.6 c	49.90 ± 1.5 a	2.38 ± 0.2 c	12.07 ± 1.3 d	4.03 ± 0.1 c	2.82 ± 0.2 a
Ennis	12.30 ± 0.3 a	54.44 ± 1.6 b	1.90 ± 0.2 b	14.33 ± 0.9 e	6.59 ± 0.2 a	2.93 ± 0.5 a
Fertile de Coutard	13.59 ± 0.5 b	61.15 ± 3.1 d	2.00 ± 0.8 bc	9.60 ± 0.9 b	6.30 ± 0.1 b	2.55 ± 0.5 a
Grossal	12.52 ± 0.2 a	55.00 ± 2.5 c	1.14 ± 0.2 a	11.10 ± 0.9 c	5.52 ± 0.2 a	2.38 ± 0.2 a
Merveille de Bollwiller	17.08 ± 0.9 d	56.18 ± 2.3 c	2.16 ± 0.3 c	8.05 ± 2.0 a	4.58 ± 0.2 d	3.30 ± 0.3 b
Segorbe	14.68 ± 0.9 c	53.75 ± 1.7 b	1.00 ± 0.0 a	9.96 ± 1.9 b	4.88 ± 0.2 c	2.67 ± 0.5 a
<i>P</i>	< 0.001	< 0.001	< 0.01	< 0.001	< 0.001	< 0.01

Different letters within one column denote statistically significant differences ($P < 0.05$) by ANOVA and Fisher's LSD test. Values are average of three individual samples ± standard deviation.

(Parcerisa *et al.*, 1993; Bota *et al.*, 1997; Pala and Ünal, 1997), where approximately 80% of the calories of nuts are derived from the fat (Salas-Salvadó and Megias, 2005). However, according to Richardson (1997), nuts are low in saturated fatty acids and rich in monounsaturated and polyunsaturated fatty acids and have no cholesterol which is beneficial in reducing the risk of circulatory and coronary diseases.

As referred before, differences among the hazelnut cultivars were observed based on the starch content (Table 3), but all cultivars showed relatively low values of the total starch. Moreover, cv. Butler had two times more starch than cv. Segorbe (2.4 and 1.0 g 100 g⁻¹ dw, respectively). Savage and McNeil (1998) also reported similar values of the total starch (1.3 to 2.7 g 100 g⁻¹ dw) in the kernels of six cultivars grown in New Zealand. The low starch content (little transformation of starch into sugar during storage) is associated with low state of hydration and when storage conditions are good, is responsible for the long storage period of hazelnuts.

The lowest NDF content (8 g 100 g⁻¹ dw) was observed in cv. Merveille de Bollwiller, whilst cv. Ennis showed the highest (14 g 100 g⁻¹ dw) (Table 3). These cultivars had higher values than those reported in other hazelnut studies with different cultivars (Lintas and Cappeloni, 1992). Savage and McNeil (1998) and Megias-Rangil *et al.* (2004) indicated one of the human benefits of hazelnuts is their fibre content. Alasalvar *et al.* (2003) assumed that eating ~200 g of Tömbül hazelnuts per day is adequate to supply 100% of the total fibre requirement for adults. Although, cv. Ennis nuts seem to be the best for the preparation of fibre-based foods, more data are needed to confirm this.

Ash and moisture were also determined and these parameters varied among cultivars (Table 3). The average ash content was found to be 2.77%, parallel to the results of Pala *et al.* (1996) and Köksal *et al.* (2006). The minimum and maximum values, based on this parameter, ranged between 2.4 g 100 g⁻¹ dw (cv. Grossal) and 3.3 g 100 g⁻¹ dw (cv. Merveille de Bollwiller). Hazelnuts had

very low moisture content (lower than 7%), which is an advantage for adequate storage.

Free α -amino acids: Total free α -amino acids and essential amino acids were significantly ($P = 0.001$) different among cultivars (Table 4). Total amino acid contents ranged from 144 to 413 mg 100 g⁻¹ dw in cvs. Segorbe and Butler, respectively. These results are different from those obtained by Silva *et al.* (2005) in the years 2001 and 2002, which emphasises the dependence between the total amino acid and weather. Alasalvar *et al.* (2003) considered that the content of amino acids in hazelnuts varies according to cultivars, growing seasons, environmental factors and maturity. Essential amino acids content ranged from 22 to 55 mg 100 g⁻¹ dw in cvs. Butler and Merveille de Bollwiller, respectively, indicating that hazelnuts are a good source of these compounds (Table 4).

Table 4. Amino acid content (mg 100 g⁻¹ dw) in the fruits of the six hazelnut cultivars

Cultivar	Total essential amino acids	Total free α -amino acids
Butler	22.23 ± 0.2 a	413.46 ± 11.1 e
Ennis	48.94 ± 1.2 d	237.91 ± 10.2 c
Fértil de Coutard	23.13 ± 2.1 a	390.12 ± 15.3 e
Grossal	27.65 ± 0.5 bc	185.26 ± 10.9 b
Merveille de Bollwiller	55.20 ± 1.9 e	330.65 ± 12.6 d
Segorbe	30.91 ± 0.9 c	144.17 ± 10.2 a
<i>P</i>	< 0.001	< 0.001

Different letters within one column denote statistically significant differences ($P < 0.05$) by ANOVA and Fisher's LSD test.

Table 5 depicts content of 16 amino acids identified in the 6 hazelnut cultivars, namely, L-alanine (Ala), L-asparagine (Asn), L-aspartic acid (Asp), glycine (Gly), L-glutamic acid (Glu), L-glutamine (Gln), L-serine (Ser), and the essential amino acids: L-arginine (Arg), L-histidine (His), L-isoleucine (Ile), L-leucine (Leu), L-methionine (Met), L-threonine (Thr), L-phenylalanine (Phe), L-tyrosine (Tyr) and L-valine (Val). Specifically, only the

Table 5. Free α -amino acid content (mg 100 g⁻¹ dw) from the fruits of the six hazelnut cultivars

Cultivar	Ala	Asn	Asp	Gly	Glu	Gln	Ser	Arg	His	Ile	Leu	Met	Thr	Phe	Tyr	Val
Butler	315.9±9.9e	11.37±2.5bc	3.91±0.2a	3.04±0.0b	8.37±0.6a	29.92±1.7c	6.25±0.0c	7.40±2.5a	3.50±0.0ab	2.60±0.0b	1.76±0.0a	0.71±0.0a	3.68±0.0b	4.42±0.2bc	4.48±0.2a	6.18±0.1c
Ennis	86.6±4.7b	12.28±0.0c	15.49±1.8b	5.62±0.6c	24.04±1.7b	18.65±0.3b	5.41±0.5bc	15.00±0.3b	3.52±0.0b	4.81±0.6c	14.07±0.8d	0.71±0.0a	17.62±2.0d	4.56±0.3bc	5.88±0.3b	3.66±0.3b
F. Coutard	311.2±4.3e	8.43±0.1ab	4.38±0.6a	3.14±0.6b	7.29±2.7a	14.19±0.0a	5.37±0.1bc	7.77±0.3a	3.51±0.0ab	1.93±0.3ab	4.79±0.8b	0.71±0.0a	5.87±0.4c	3.10±0.6a	5.22±0.4ab	3.28±0.8b
Grossal	108.6±8.3c	7.84±0.0ab	2.13±0.1a	0.75±0.0a	9.20±0.3a	13.96±0.0a	4.71±0.0b	5.54±0.3a	3.45±0.0a	1.67±0.0a	12.91±0.6d	0.71±0.0a	0.01±0.0a	5.43±0.0c	4.90±0.0a	3.49±0.1b
M. Bollwiller	142.1±3.6d	13.23±0.5c	24.08±2.9c	7.77±0.5d	30.50±3.8b	17.31±0.0b	8.02±0.5d	23.74±2.1c	3.47±0.0ab	4.47±0.3c	7.99±0.9c	0.71±0.0a	22.94±0.4e	4.33±0.3b	8.68±0.3c	11.30±0.8d
Segorbe	62.3±0.1a	6.40±0.0a	3.88±1.5a	3.44±0.1b	10.83±3.1a	14.19±0.0a	2.13±0.0a	5.47±0.2a	3.46±0.0ab	1.42±0.0a	14.05±0.1d	0.71±0.0a	6.99±1.2c	2.61±0.1a	4.60±0.2a	1.69±0.1a
P	<0.001	<0.01	<0.01	<0.001	<0.01	<0.01	<0.01	<0.01	NS	<0.01	<0.01	NS	<0.001	<0.01	<0.01	<0.01

Different letters within one column denote statistically significant differences ($P < 0.05$) by ANOVA and Fisher's LSD test. Values are average of three individual samples \pm standard deviation.

amino acids, histidine and methionine were not affected by the cultivar. As in previous studies (Rennie, 1995; Savage and McNeil, 1998; Alasalvar *et al.*, 2003; Köksal *et al.*, 2006) we also did not detect any tryptophan, even though some nutritional databases report this amino acid in hazelnuts (Souci, 1994; Holland *et al.*, 1998).

In all hazelnut cultivars studied Ala was the most common non-essential amino acid, which was significantly different ($P < 0.001$) among the cultivars, and represented 62% of total free α -amino acids. Mean Ala concentration varied from 62 to 316 mg 100 g⁻¹ dw in cvs Segorbe and Butler, respectively. Apart from an important source of energy, Ala, a non-polar amino acid, is responsible for an increase in immune responses and takes part in the metabolism of sugars and organic acids (Rennie, 1995). Other important amino acids included Gln that varied from 13.96 mg 100 g⁻¹ dw in cv. Grossal to 29.92 mg 100 g⁻¹ dw in cv. Butler, followed by Glu that varied between 7.29 mg 100 g⁻¹ dw in cv. Fertile de Coutard and 30.5 mg 100 g⁻¹ dw in cv. Merveille de Bollwiller. A group of seven amino acids, five of them essential, had values lower than 10 mg 100 g⁻¹ dw: Met, His, Ser, Gly, Phe and Ile. Specifically, Met concentration was the lowest among the amino acids (0.3% of total free α -amino acids) determined (Table 5). Köksal *et al.* (2006) also considered Met the most insignificant amino acid. In general, the fruits of cv. Butler (American origin) had the highest amino acid content and was two times greater than the fruits of cvs. Grossal and Segorbe (Spanish origin). However, the values obtained in diverse studies were different for the amount and the relative proportion of each amino acid (Alasalvar *et al.*, 2003; Köksal *et al.*, 2006).

Our data confirm that hazelnuts are a rich source of a number of important nutrients that can have a very positive effect on human health. The composition of hazelnut kernels, particularly total and individual free α -amino acids, crude protein, crude fat, starch and neutral detergent fibre are strongly affected by cultivar. The major amino acid found was alanine, representing 60% of the total free α -amino acids, and methionine was the lowest (1.5% of the total free α -amino acids).

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