M182:
Determination of sugars in chestnut (*Castanea sativa* Mill.) cultivars from Portugal Northeast region by HPLC-RI

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Sugar profile; *Castanea sativa* Mill.; HPLC-RI

SUMMARY
Chestnut (*Castanea sativa* Mill.) is one of the most important cultivated fruits in Portugal. It has a relevant place at the socioeconomic level, reaching an annual production of more than 30,000 tons. The main production area is located in Trás-os-Montes region (84% of Portuguese production) (*1*). Hence, the assessment of the commercial quality of these fruits is an essential activity. Carbohydrates are the major components in chestnut, especially starch and sucrose. This disaccharide is one of the most important parameters in the assessment of chestnut quality, once sugar content and composition can be modified by several conditions, like storage temperature, relative humidity, harvest time, oxygen level or packaging (*2*). In this study, sugars profile of four chestnut cultivars [*Aveleira* (A), *Boa Ventura* (B), *Judia* (J) and *Longal* (L)], all belonging to the *Castanha da Terra Fria* PDO (Protected Designation of Origin) were determined. Samples were previously peeled off, dried, finely chopped and defatted in a Soxhlet apparatus using petroleum ether. Sugars where then extracted in a thermostatized bath, with a hydroalcoholic solution (80%; v/v). Sugar contents were measured using high-performance liquid chromatography (HPLC), by means of a refractive index detector (RI). The chromatographic separation was achieved using a Eurospher 100-5 NH_2 column using an isocratic elution with acetonitrile/water (70:30, v/v) at a flow rate of 1.0 mL/min. The four chestnut cultivars showed a relevant heterogeneity (A: fructose (F) = 0.72±0.07, glucose (G) = 1.10±0.10, sucrose (S) = 22.05±1.48; B: F = 5.18±0.39, G = 6.63±0.49, S = 4.03±0.30; J: F = 0.62±0.05, G = 1.02±0.06, S = 23.30±0.83; L: F = 1.81±0.12, G = 2.69±0.23, S = 9.56±0.91). To evaluate if the differences verified in each individual profile may contribute as an authenticity discrimination factor, these results were screened through a discriminant analysis using SPSS 16.0 software.

INTRODUCTION
According to FAO, chestnut worldwide production is estimated in 1.1 million tons. Europe is responsible for about 12% of worldwide production, with relevance for Italy and Portugal, corresponding to 4% and 3%, respectively. Trás-os-Montes region represent 75.8% of Portuguese chestnut crops and 84.9% of chestnut orchards area (23,338 ha). In 1994, three protected

designation of origin (PDO) called "*Castanha da Terra Fria*", "*Castanha dos Soutos da Lapa*" and "*Castanha da Padrela*" were created (*3*).

Unlike most nuts, chestnuts are low in fat but present a high content in carbohydrates with a high prevalence of starch, followed by sucrose (*4*). Together with sucrose, glucose and fructose are present in significant amounts and may contribute for the identification of a specific chestnut cultivar. Discriminant function analysis (DA) was done to determine which variables discriminate between the three naturally occurring groups.

MATERIALS AND METHODS

Fat extraction

Crude lipidic fraction was removed from finely chopped chestnuts (≈50 g with anhydrous sodium sulfate) extracted with light petroleum ether (bp 40-60 °C) during 16 h in a Universal extraction system B-811 (Büchi, Switzerland); the residual solvent was removed by flushing with nitrogen.

Sugar extraction

Dried and defatted powder (2.0 g) was extracted with 10 ml of 80% aqueous ethanol at 70 °C for 30 min. The resulting suspension was centrifuged at 5000 rpm for 15 min. The supernatant was concentrated at 40 °C under reduced pressure and dissolved in water to a final volume of 10 ml.

HPLC analysis

Free sugars profiles were determined by high performance liquid chromatography coupled to a refraction index detector (HPLC-RI). Soluble sugars were determined at 35 °C. The HPLC system was equipped with a Knauer Smartline 2300 RI detector and with a Eurospher 100-5 NH_2 column (4.6 × 250 mm, 5 mm, Knauer). The mobile phase was acetonitrile/deionized water, 7:3 (v/v) at a flow rate of 1 ml/min. The results are expressed in g/100 g of dried weight, calculated by internal normalization of the chromatographic peak area. Sugar identification was made by comparing the relative retention times of sample peaks with standards. The sugar standards used for identification were purchased from Sigma Chemical Co. (St. Louis, USA): L-(+)-arabinose, D-(-)-fructose, D-(+)-galactose, D-(+)-glucose anhydrous, lactose-1-hydrate, maltose-1-hydrate, D-(+)-mannitol, D-(+)-mannose, D-(+)-melezitose, D-(+)-melibiose-monohydrate, L-(+)-rhamnose-monohydrate, D-(+)-sucrose, D-(+)-trehalose and D-(+)-xylose.

Statistical analysis

Sugar extraction was performed in duplicate and each sample was injected twice in HPLC-RI. The results are expressed as mean values ± standard deviation. The differences between different extracts were analyzed using one-way analysis of variance followed by Tukey's honestly significant difference post hoc test with $\alpha = 0.05$, coupled with Welch's statistic. Discriminant function analysis was done following stepwise method (*5*).

RESULTS AND DISCUSSIONS

The four chestnut cultivars showed a relevant heterogeneity (Table 1). Nevertheless, all of them presented fructose, glucose and sucrose. Sucrose was the main sugar in *Aveleira* (22.05±1.48), *Judia* (23.30±0.83) and *Longal* (9.56±0.91), while glucose was prevalent in *Boa Ventura* (6.63±0.49) (**Table 1**).

Table 1. Sugar profile of the assayed cultivars. In each column and for each cultivar, different letters mean significant differences ($p<0.05$).

Cultivar		Fructose	Glucose	Sucrose
Aveleira	Tree 1	0.72±0.05	1.14±0.06	24.17±0.17
	Tree 2	0.64±0.00	1.03±0.00	23.24±0.15
	Tree 3	0.70±0.02	1.08±0.02	20.88±0.13
	Tree 4	0.68±0.01	0.99±0.00	20.91±0.28
	Tree 5	0.84±0.03	1.25±0.01	21.05±0.46
	$\bar{x}$	**0.72±0.07 c**	**1.10±0.10 c**	**22.05±1.48 b**
Boa Ventura	Tree 1	5.11±0.06	6.38±0.09	4.07±0.22
	Tree 2	5.32±0.09	6.79±0.03	3.71±0.06
	Tree 3	5.28±0.10	6.81±0.07	3.87±0.05
	Tree 4	4.84±0.06	6.21±0.09	4.02±0.04
	Tree 5	4.88±0.05	6.24±0.07	4.21±0.11
	$\bar{x}$	**5.18±0.39 a**	**6.63±0.49 a**	**4.03±0.30 d**
Judia	Tree 1	0.63±0.07	1.05±0.09	22.96±1.80
	Tree 2	0.68±0.03	1.09±0.02	23.67±0.10
	Tree 3	0.57±0.01	0.96±0.00	23.44±1.06
	Tree 4	0.59±0.02	0.97±0.02	22.68±0.29
	Tree 5	0.62±0.00	1.03±0.01	23.76±0.08
	$\bar{x}$	**0.62±0.05 c**	**1.02±0.06 c**	**23.30±0.83 a**
Longal	Tree 1	1.76±0.16	2.84±0.26	9.50±1.30
	Tree 2	1.76±0.00	2.56±0.00	8.67±0.03
	Tree 3	1.92±0.15	2.96±0.22	9.20±0.83
	Tree 4	1.92±0.03	2.66±0.06	10.78±0.38
	Tree 5	1.69±0.02	2.45±0.05	9.64±0.20
	$\bar{x}$	**1.81±0.12 b**	**2.69±0.23 b**	**9.56±0.91 c**

In the DA, the two defined functions explained 100% of the observed variance (**Figure 2**).

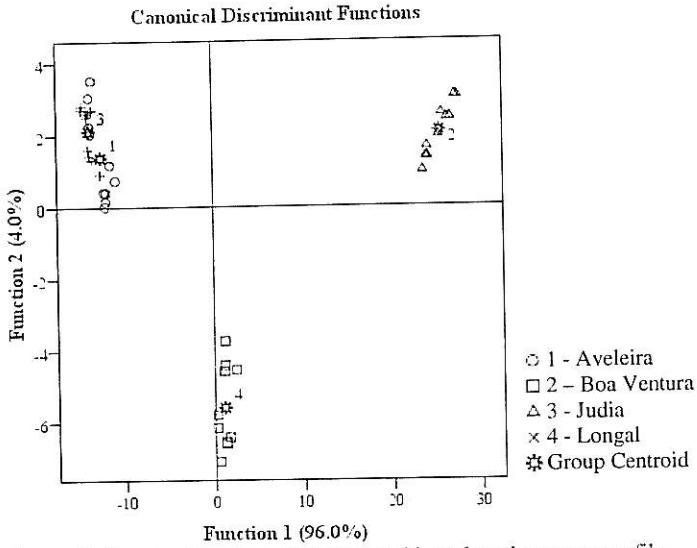

Figure 2. Canonical analysis of chestnut cultivars based on sugar profile.

The first DA function separates primarily *Boa Ventura* and *Longal* from *Aveleira* and *Judia* (means of the canonical variance (MCV): *Aveleira* = -12.573, *Boa Ventura* = 25.386, *Judia* = -13.865 and *Longal* = 1.052), and revealed to be more powerfully correlated with fructose and glucose. The second DA function confirmed the separation of *Longal* and helped to separate *Aveleira* and *Judia*, but not with the desirable clearness (MCV: *Aveleira* = 1.382, *Boa Ventura* = 2.078, *Judia* = 2.127 and *Longal* = -5.587) and showed to be more correlated with sucrose.

CONCLUSION

Some studies have already been applied to sugar composition in chestnut, however, and as far as we know, this is the first study about *Aveleira*, *Boa Ventura* and *Judia* sugar composition; even so the results obtained for *Longal* are in agreement with other reported work (6).

These results are potentially useful from the commercial point of view, because sugar profile proved to be an effective discriminant factor. It's a fact that *Aveleira* was not clustered clearly in a separate group, but this is the cultivar with less productive significance and has also a different phonological cycle, so its separation was not the main objective.

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