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Chemical composition and characterisation of seeds from two varieties (pure and hybrid) of *Aesculus hippocastanum*

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Abstract

Investigations have been conducted on some samples of naturally desiccated horse-chestnuts (*Aesculus hippocastanum*), representative of the two most common mediterranean varieties: the *pure* species (AHP, giving white flowers), and a *hybrid* (AHH, giving pink flowers). Different experimental techniques have been used to gain more information on morphological structure and chemical composition of these complex matrices. Surface analysis by SEM showed no differences in such floured samples (wild type), while thermal behaviour (DSC) outlines some significant differences between them. Chemical composition reveals some differences in residual moisture (AHP = 6.97%; AHH = 6.59%), proteins (AHP = 2.64%; AHH = 1.82%), lipids (AHP = 4.13%; AHH = 5.10%), glucides (AHP = 15.2%; AHH = 14.3%), and ashes (AHP = 2.51%; AHH = 2.19%). Most likely, these characters modulate other undifferentiated chemical parameters, such as cold water solubility (CWS:AHP = 53.9%; AHH = 48.6%), and total inorganic soluble salts (TISS: AHP = 2.18%; AHH = 1.92%). Principal component analysis was applied to differentiate the two horse-chestnuts varieties. In particular, the first principal component effectively distinguish and discriminates AHH and AHP samples in two well-separated categories, giving, at the same time, some information on the influence of the whole set of chemical compositional parameters.

Keywords: Horse-chestnut; Chemical composition; Elemental analysis; PCA

1. Introduction

It is an evidence that the popularity of herbal medicine, as for natural or industrial formulations of nutritional supplements, is at its top, from both the points of view of consumers and researchers. With such a huge interest, the scientific role of researchers is particularly devoted to acquire ever more deeper information about composition, structures and activity effects, in relation to physiological benefits and metabolic processes of these products.

A close examination of the recent literature, indicates some interests about horse-chestnut products. Therefore, it has been a discover for us the healthy benefits for human consumption of various products obtained and derived from *Aesculus hippocastanum* L. which are described in the specific literature (Wei et al., 2005; Oda et al., 2000; Yang et al., 1999; Deli et al., 1998).

Some different specialties, containing bioactive principles from horse-chestnut (tree bark, bud, flowers and other parts, may be safely used for specific targets) are now commercially available in the world (Deli, Matus, and Toth, 2000). So, among others, we appraise that some saponin constituents, also named escins, extracted from *Aesculus hippocastanum* L. seeds, have shown satisfactory evidence

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for a significant clinical activity in some specific therapies. The same is valid for oil extracted from the seeds, that gives some other benefits on therapeutic treatments. Furthermore, it has been proven by many clinical trials that these natural extracts are effective adjuvants in compression therapy as an alternative medical treatment (Sirtori, 2001), in addition to an ethanol absorption-inhibitory effect and hypoglycemic activity (Yoshikawa et al., 1996). Moreover, it has been described in the literature by Konoshima and Lee (1986) the cytotoxic and antitumoral activity of some sapogenols extracted from *Aesculus hippocastanum* L., in particular hippocaesculin and barringtogenol-C.

In addition to these information, we mention that Fant, Vranken, and Borremans (1999) report that antimicrobial protein 1 (Ah-AMP1), isolated from horse-chestnuts and whose structure has been resolved *via* ^{1}H NMR techniques, is a very effective plant defence in that it inhibits growth of a broad range of fungine species. Of course, this knowledge may imply a possible technological transferability, by use of natural products into the integrated biological struggle to preserve vegetation and a lot of foodstuffs as well. It is well understood that natural antibacterials, antimicrobials, antifungins and any other inhibitor of damaging species, are generally active at moderate dosages and concentrations, associated to an excellent tolerability for upper organisms.

The substitution of synthetic phytotherapeutics by natural products with therapeutic activity in many industrial formulations, can provide a very consistent advantages and benefits, first of all the biocompatibility and the ambient preservation.

Even though the relevant importance of these products have been stressed by a lot of papers, it seems to us that very few studies appeared on chemical composition of horse-chestnut seeds. To this respect, we are obliged to mention that Italian (Farmacopea Italiana, 2000) and German (DAB, 1997) pharmacopeiae report only few information about the grade quality of horse-chestnut seeds, which is the most widely used starting material required for manufacturing the above specified native dry extracts. These pharmacopeiae establish that the dry extracts, from the dried seeds of *Aesculus hippocastanum* L., must contain not less than 3.0% of triterpene glycosides, calculated as anhydrous escin, with reference to the dried drug. Furthermore, botanical identity of starting materials must be confirmed using thin-layer chromatography (TLC) as well as macroscopic and microscopic examinations of seeds and dry extracts (DAB, 1997; Farmacopea Italiana, 2000). The typical drug-to-extract ratio for this native dry extract, will fall within the range of 5.0–8.0:1 (w/w), depending on the chemical composition of the starting material and the subsequent yield of soluble extractive.

Horse-chestnut seeds, as all others seeds, are natural products whose chemical composition is a very complex matrix. They contain a lot of different molecules and analytes, the majority of which are polysaccharides (both starches and non-starches), proteins, lipids, mineral salts and many minor components among others, with homogeneous or localised distributions in different districts, all strongly interacting with each other in a synergistic way to form very complex structures. On the other hand, seeds are the natural farmers that guarantee the continuity and the propagation of biodiversity in the vegetable planet. Probably for these reasons, such complex structures are very far from being detailed at microscopic level, even if many efforts by research workers engaged from different areas (biologists and biotechnologists, bioengineers, chemists, pharmacologists, and so far) are in progress.

Therefore, the aims of this work were to study some characters of chemical composition and the relevant classification of seeds from two most wide spread varieties of *Aesculus hippocastanum* L., such as pure species, with white flowers (AHP), and a common hybrid species with pink flowers (AHH). Both these common *Hippocastanum* trees are growing in the mediterranean regions, and their luxurious summer flowering is very showy. From this study we would like to ascertain the presence of some distinctive characters that, on some extent, could be important for their adaptation to industrial and applicative processes.

Given the multivariate nature of the obtained data, principal component analysis (PCA) has been successfully carried out on all the chemical parameters computed for both different types of *Hippocastanum* trees. In fact, PCA, by extracting the relevant information and displaying it in a simple and easy-to-interpret manner, is able to discriminate the AHH from AHP samples, and to give information among some differences in the composition of the two classes.

2. Materials and methods

2.1. Samples treatments

Thirteen seeds, from each of the 5 + 5 selected *Aesculus hippocastanum* L. (AHP and AHH) trees, were collected as spiny fruits from trees in our University Campus of Modena city. The seeds were cleaned, weighted and stored for three months in a dark and dry place at room temperature, to ensure that the most natural desiccation process take place.

The amount of seeds (10–15 pieces) from each tree, necessary for the analytical determinations, were randomly selected. The seeds were then accurately hand-peeled up to remove the cellulosic and wooden hull, and the thin red-brown endosperm that covers the straw-coloured cotyledons. The chips were kept in a virgin polyethylene pouches to avoid contaminations and ground in a agate mortar to obtain a flour, that was packed in plastic pouches and stored in a refrigerator at 4 °C until use. The yield of the flour was about 25–30% on the total mass of fresh fruits. All samples were produced in the year 2004.

Residual moisture content was determined by dehydration in an electric oven at 101 °C, following the AOAC method, Ref. 14.003 (AOAC, 1984), until constant weight.

Glucose and fructose contents were determined, after drying and hydroalcoholic extraction (80/20 v/v), by HPLC (Beckman System Gold). The system consists of an isocratic single pump (mod. 116), injection valve (mod. 210A) equipped with a 20-μL injection loop (provided with a magnetic sensor to automatic start), Refractive index detector (mod. 156), UV–Vis detector (mod. 166), analogical to digital interface (mod. 406), and an Eppendorf TC-50 thermostat for HPLC column. The system was managed by system gold chromatographic software, version 3.0, also supplied by Beckman. A Bio-Rad HPX-87H Aminex® column (length 30 cm, diameter 7.8 mm), was used for all the chromatograhic determinations. As far as mobile phase is concerned, given the kind of the used HPLC column and following the manufacturer technical literature suggestions (HPLC, 1996a, 1996b), a H_2SO_4 0.05 M 92% – acetonitrile 8% solution was used (Cocchi, Lambertini, Manzini, Marchetti, & Ulrici, 2002). The separation was conducted by isocratic technique with a flux of 0.6 mL min^{-1}. Both sugars were detected by the refractive index detector, fructose also by UV device at 210 nm (Cocchi, Ferrari, Manzini, Marchetti, & Sighinolfi, 2007).

In addition to elemental analysis, the total nitrogen content was measured for each sample, by adopting a standard procedure (Kjeldahl method), by using a Gerhardt Kjeldatherm system equipped with a distillation unit, model Gerhardt Vapodest. Lacking specific literature targets, and taking advantage from the analogy with other natural products such as chestnuts, the percentages of nitrogen were transformed into protein content by multiplying by a conversion factor of 4.86, as reported by De La Montana Miguelez, Miguez Bernardez, and Garcia Queijeiro (2004) for Galicia chestnuts.

Lipid content was assessed by applying the AOAC method, Ref. 14.028 (AOAC, 1984). The lipidic fraction was then processed by a standard procedure *via* gas chromatographic analysis. GC determinations were performed on a HP-5890 gas-chromatograph provided with a flame ionisation detector (FID). A total non-polar capillary column (PDMS as stationary phase, column Alltech AT-1, length 60 m, internal diameter 0.25 mm, film thickness 0.20 μm) was used. Helium was used as carrier gas (flow = 1.35 mL min^{-1} evaluated at 150 °C). The split injection mode was used with a split rate 1:30. Flow rates of H_2 and air to the FID were 30 and 300 mL min^{-1}, respectively. The FID and injector temperature were setted at 280 and 230 °C, respectively. The column temperature was held at 180 °C for 10 min.

Cold water solubility (CWS, %) was determined at 100 °C by the method of Eastman and Moore (1984), slightly modified and adapted to this complex matrix. A 100 ml (1%) suspension of flour sample was taken in a close vessel, shaked thoroughly for 3 h on a rotatory shaker immersed in a thermostatic bath maintained at the selected temperature (±0.01 °C). After the required equilibration time, the suspension was centrifuged at 1200*g* for 10 min. A 25 ml aliquots (three replicates for each sample) of the supernatant was taken in a pre-weighted Pt crucible and dried in an air oven at 105 °C for 4 h. The cold water solubility (as swollen and solvent-cleavage material, non-sedimented microgranules) was calculated as: CWS% = (grams of solid in supernatant × 4/grams of sample) × 100.

The dried material obtained as CWS, has been successively treated to obtain total inorganic soluble salts (TISS) content, by incineration at 550 °C, up to a constant weight (AOAC methods, Ref. 14.006) (AOAC, 1984).

According to the AOAC methods (Ref. 14.006) for analogous matrices, ashes have been evaluated by incineration of native flour samples of about 1 g in Pt crucibles at 550 °C, up to the final constant weight (AOAC, 1984).

Elemental analyses (N, C, H, S) were performed by using an analyser CE Instruments (Carlo Erba, Milan), mod. EA 1110.

2.2. Scanning electron microscopy

Scanning electron micrographs of powder samples were obtained with a Philips XL-30 scanning electron microscope, operating at 25 kV, and with a magnification of 5000×. The powder samples were sprinkled and deposited on Al stubs, then coated and sputtered with Au vapour deposition (thin layer of about 5 nm).

2.3. Differential scanning calorimetry

Thermal properties of native flour samples were investigated using a Setaram Labsys TG–DTA/DSC instrument, equipped with a thermal analysis data station. Samples (about 20–30 mg) were weighted into a 100 μl capacity Pt crucible, and processed on a thermal scansion (5 °C/min up to 450 °C, and then 10 °C/min up to 700 °C), working under inert atmosphere (N_2).

3. Results and discussion

The results obtained by investigating the chemical composition of the two different horse-chestnut varieties samples are shown in Table 1. We mention that these data represent the mean values of the five samples (one for each tree of the two groups: AHP and AHH) and their standard deviation (s_5), while each sample/tree has been tested in some replicates (3, 4 or 5) as specified in Table 1, depending on the investigated parameter.

Moisture content in fresh seeds was determined and gave the following results: 50.8% and 50.1% for AHP and AHH samples (on the basis of dry matter as a final step), respectively.

Residual moisture content ranged between 6.21% and 7.44%, considering all samples and all replicates. We observe that AHP samples (average: 6.97%) are slightly above the average value of the AHH samples (6.59%). As a probable rationalization of this evidence, there can be a possible correlation between a greater humidity content

Table 1
Chemical composition data[a] determined on horse-chestnut samples of different botanical origins from Modena city

	AHP (pure, white flowers)	AHH (hybrid, pink flowers)
Moisture % (fresh seeds)	50.8 ± 0.6	50.1 ± 0.6
Moisture % (residual)	6.97 ± 0.35	6.59 ± 0.24
Proteins % (d.s.)	2.64 ± 0.42	1.82 ± 0.34
Lipids % (d.s.)	4.13 ± 0.36	5.10 ± 0.50
Glucids % (d.s.)	15.2 ± 0.66	14.3 ± 0.80
Ashes % (d.s.)	2.51 ± 0.12	2.19 ± 0.09
CWS % (d.s.)	53.9 ± 0.79	48.6 ± 0.65
TISS % (d.s.)	2.18 ± 0.10	1.92 ± 0.11
Elemental analysis		
N %	0.90 ± 0.10	1.39 ± 0.11
C %	43.02 ± 0.27	43.19 ± 0.22
H %	5.45 ± 0.12	6.05 ± 0.15
S %	0.13 ± 0.06	0.11 ± 0.06

(d.s.) = dry sample.
[a] Uncertainties are here expressed as standard deviation of five samples (s_5) pertaining to the same group and representative of each variety, and each sample-tree was tested at least in two or three (CWS, TISS) replicates.

and the sensible greater content of ashes. In fact, it is well understood that mineral salts are very hydrophilic species. In addition, the residual humidity should guarantee an adequate conservation and a relevant stability of these naturally desiccated products for some time next.

The cold water solubility (CWS) of these native flour samples (wild type) represents an undifferentiated mass of hydrophilic organic molecules and other inorganic species mainly swollen, non-sedimented granules as soluble material. This property should mainly depend on the starch source and his granular structure. CWS data differ significantly for AHP (53.9%) and AHH (48.6%) samples. Probably, and as an hypothesis, the lower solubility of AHH samples could be mainly attributed to some concomitant factors, two of them in particular: (i) the lower content of hydrophilic species; (ii) the more rigid structure of the starch granules related to a less swelling ability. Unfortunately, SEM analyses showed no significant morphological differences on the AHP and AHH samples.

As an evidence, TISS (total inorganic soluble salts) values are perfectly coherent to the ashes contents (Table 1). However, we observe that TISS on AHP samples represents the 86.8% of the ashes, while the level slightly increases for AHH samples (87.7%).

3.1. Scanning electron microscopy

The granular structure of native flour obtained by the two varieties of horse-chestnut seeds showed no significant variations in size and shape viewed by SEM. Fig. 1 reports the relevant image of AHP sample, being quite similar to AHH sample too. The granule size ranged between 5 and 20 μm for small and 20–50 μm for large granules in these grounded materials.

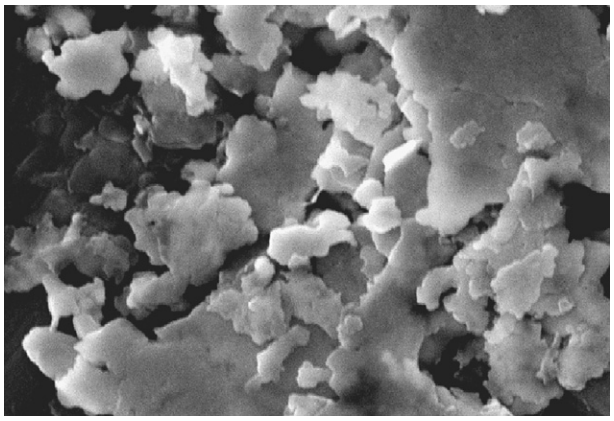

Fig. 1. SEM image of flour sample (AHP, wild type) of horse chestnuts (×5000).

The variation in the size and shape in biosynthetized starched materials may be due to the different biological origins (Svegmark and Hermansson, 1993). The morphology of starch-based granules mainly depends on the biochemistry of the chloroplast and amyloplast, as well as on the general physiology of the plant (Badenhuizen, 1969). The apparent identical morphology of the flour seeds probably masks other micro-specificities and do not reflect an analogous swelling and shrinkage behaviour in cold water.

3.2. Differential scanning calorimetry

As an example, Fig. 2 shows the trend of DSC curves for two native samples of *Aesculus hippocastanum* L., pure (AHP) and hybrid (AHH) species, respectively, whereas Table 2 summarizes the most important and delining features evicted from the trends. At first sight, we observe that

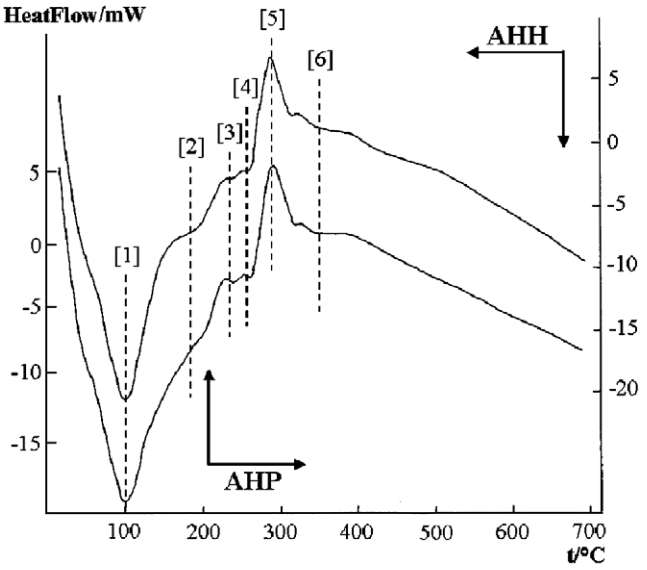

Fig. 2. DSC profiles for two samples of horse-chestnut floured (AHP, AHH).

Table 2
DSC thermal properties of two different horse-chestnut wild-type flours

Peak	Sample AHP				Sample AHH			
	T_o	T_p	T_c	ΔH_p (J/g)	T_o	T_p	T_c	ΔH_p (J/g)
[1]	53.2	103.6	179.0	118.6	63.8	103.3	170.2	115.3
[2]	186.4	206.1	219.4	2.0	180.3	195.8	220.5	3.4
[3]	236.7	245.7	259.7	1.4	238.2	243.5	261.4	0.9
[4]	261.3	268.0	272.6	0.4	262.0	266.7	270.9	0.3
[5]	272.9	298.1	323.5	−41.1	271.5	296.1	325.2	−37.9
[6]	327.9	335.5	345.1	−0.9	325.9	333.6	344.8	−0.9

T_o, onset temperature; T_p, peak temperature; T_c, conclusion temperature; ΔH_p, peak enthalpy.

these curves show many peaks, due to different transformation processes involving different composition fractions (lipids, proteins, glucosides, inorganic species, among other) present in these natural products, taking place at various times along the thermal scansion.

However, in spite of the structural complexity of the samples, we observe that these curves appear well matching in general, but some consistent differences comes out after a closer inspection, and some rationalisation can be given as follows.

[peak 1] – The first broad endothermic peak probably subtend different processes involving contemporarily some different individual chemical species, such as: moisture loss (together with most volatile organic compounds), gelatinisation of native starches (which probably differ to some extent for the amylose – amylopectin ratios into different AHP and AHH samples), mobilization and vehiculation of lipids and proteins into granular structures of starches.

[peak 2] – The following small endothermal peaks (T_p: AHP = 206.1 °C; AHH = 195.8 °C) should be related to lipid fractions of the samples. It is well understood in the literature that lipids mobilization is strongly favoured by increasing temperature (Kulp and Ponte, 2000). These thermal motions involve both distension and elongation of branched and wrapped structures of mono-, di- and triglycerides chains into starches granules. Amylose should be the component mainly involved for these processes, being the internal molecular helix cavity channel particularly suited to trap the lipid units according to a host–guest dynamic mechanism (Colonna, Buleon, and Mercier, 1987). Furthermore, the greater enthalpic effect observed for the AHH sample (3.4 J/g) can be related to the greater lipids content (5.1%) with respect to the AHP sample (4.1% lipid fraction, Table 1), with a reduced thermal effect (2.0 J/g).

[peaks 3,4] – The couple of endothermic peaks at $T_p = 245.7$ °C and $T_p = 268.0$ °C for the AHP sample (243.5 and 266.7 °C for the AHH sample) is probably due to the total denaturation processes involving the proteins fractions. The most significant differences are emphasized by the different enthalpic effects, in coherence with the protein content of the samples of various origins (see Table 1).

[peak 5] – The exothermic peak at $T_p = 298.1$ °C and 296.1 °C for AHP and AHH samples, respectively, probably subtend the recrystallisation involving starch fractions.

[peak 6] – Finally, analogous recrystallization process should involve the fibre fraction (α- and β-glucans, cellulosic fibres, among others) at $T_p = 335.5$ and 333.6 °C for the two varieties, as demonstrated by the same behaviour observed by processing two samples of episperm seeds. Obviously, these components are present in very small quantities in horse-chestnut flour seeds, and their structures are more rigid than that of starches fractions, resulting into a less significant DSC exothermic peak for these samples.

After these peaks, the samples under thermal stress only show the progressive decomposition (endothermic) processes that take place up to the final monitored scansion temperature (700 °C).

3.3. Lipids analysis

Lipids represent a significant fraction on these original products (4–5% of the dry mass), which probably exert some important effects in nature, in relation with the main macromolecular component of flour seeds such as starch, and the other macrocomponents such as glucides and proteins. Unfortunately, the literature reports very scarce information on these topics and, when present, limitedly to some qualitative aspects. Some fatty acids, the most important of which are oleic, linolenic, palmitic, and stearic acids, have been identified in lipid fractions by Leung and Foster (1996). A systematic investigation on our AHP and AHH samples lead to the results summarized in Table 3, and permit us to discriminate between them. In this study, 17 fatty acids have been identified working in GC techniques. Surprisingly, we observe that the group of the main components is differently distributed into the two sample families: the oleic acid prevails in AHH (49.7%, respect to 43.2% in AHP), while linoleic acid strongly prevails in AHP (35.2%, respect to 23.0% in AHH). Linolenic acid takes advantage in AHH (7.5%, over 5.9% in AHP), and the same occurs for stearic acid (2.9% towards 0.8% in AHP) with a marked difference in the content, that amounts to about 75%.

All other components seem to be present in more or less equivalent ratios for both samples groups. To our limited knowledge, very few information is given in the literature about starch–lipids, proteins–lipids and lipids–lipids interactions, and about the ways by which these hydrophobic

Table 3
Fatty acids content on horse-chestnut samples of different botanical origin from Modena city[a]

Acid	AHP (white flowers)	AHH (pink flowers)
Myristic (C14:0)	0.6 ± 0.3	0.6 ± 0.2
Myristoleic (C14:1)	0.2 ± 0.1	0.4 ± 0.1
Pentadecanoic (C15:0)	0.1 ± 0.1	0.2 ± 0.1
Pentadecenoic (C15:1)	0.1 ± 0.1	0.1 ± 0.1
Palmitic (C16:0)	7.1 ± 0.5	6.8 ± 0.6
Palmitoleic (C16:1)	0.7 ± 0.3	0.9 ± 0.3
Heptadecanoic (C17:0)	0.1 ± 0.1	0.4 ± 0.2
Heptadecenoic (C17:1)	0.1 ± 0.1	0.3 ± 0.1
Stearic (C18:0)	0.8 ± 0.2	2.9 ± 0.3
Oleic (C18:1)	43.2 ± 1.1	49.7 ± 1.3
Linoleic (C18:2)	35.2 ± 1.2	23.0 ± 0.8
Linolelaidic (C18:2)	2.2 ± 0.4	2.1 ± 0.2
Linolenic (C18:3)	5.9 ± 0.5	7.5 ± 0.5
Arachidic (C20:0)	0.2 ± 0.1	0.5 ± 0.2
Behenic (C22:0)	0.1 ± 0.1	0.3 ± 0.1
Erucic (C22:1)	2.9 ± 0.3	4.1 ± 0.2

[a] Uncertainties are here expressed as standard deviation of five samples (s_5).

compounds exert their effects inside these globular – prevailing starches structures. In addition, the precise role of lipids is not well defined and understood (other than hydrophobic micelles protection and regulation, and anti-freezing agents in nature) due to the complexity of lipid structures and the relevant location in the seeds.

Such a complexity is a challenging problem to manipulate lipids in these native matrices without changing their organization and associations with other components. Therefore, it will be a future research target to closely investigate the complex nature of these lipid fractions.

3.4. Glucides analysis

The values obtained for total glucides content in these natural products was in the range 14.3% (AHH)–15.2% (AHP). As previously mentioned in the experimental section, our analysis methodology permit us to determine the two most important monosaccharides (and apparently, the only species in these matrices), such as glucose and fructose. However, we observe that while glucose concentration is almost the same on the two sample groups (6.8% for AHP and 6.9% for AHH), fructose content is well differentiated (8.4% for AHP and 7.4% for AHH).

Actually, we are unable do determine the presence of other sugars, such as the most common sucrose (the disaccharide based on glucose and fructose condensation). Nevertheless, we are obliged to mention that, generally, edible chestnut seeds mainly contain sucrose (about 10–30%), and only few traces of free monosaccharides (about 0.1–0.5%) (De La Montana Miguelez et al., 2004).

On the other hand, free monosaccharides content is rather poor in starches-based matrices, such as seeds of various botanical origins (Kulp and Ponte, 2000). Probably, horse-chestnuts also should preferably contain sucrose rather than glucose and fructose monosaccharides. These aspects also will be focused on a further research trial.

For completeness, our experimental data about the composition of common Mediterranean horse-chestnuts, can be compared with some literature values reported by Parmar and Kaushal (1982), even though they worked with *Aesculus indica* seeds from fresh fruits. *Aesculus indica* is a very common botanical species and largely diffused in Himalayan forests, also pertaining to *Hippocastanaceae* family, with some distinctive features and characters from each others.

Probably, this comparison can result in a limited usefulness because of different uses of AHP or AHH and *Aesculus indica* seeds, being the latter foodstuff still largely consumed by local populations of Himachal Pradesh region. Starch of seeds (about 40% on fresh fruits, with a ratio amylopectin:amylose ≅3:1) was recommended as famine food for extending bread flour (or other uses), after removal of bitter characters.

The saponins present in these seeds render them very bitter, disagreable and unedible for humans. On the contrary, they can be consumed for human uses after removing the bitterness of grounded flour, by soaking it in water for about 12 h. The bitter components get dissolved in water and removed when the water is decanted. The remaining slurry can be destined for cuisine manipulations, and is generally taken as a non-cereal-starch food. As reported by Parmar and Kaushal (1982), the *Aesculus indica* seeds, which constitute the edible portion of the fresh fruits, contain about 50.5% moisture. The total sugars content is 5.58% (11.0% on dry matter basis), combining the reducing (4.59%) and non-reducing sugars (0.94%), respectively. The protein and mineral contents are 0.388% and 1.934%, respectively, on fresh samples, that become 0.768% (proteins) and 3.83% (minerals) on dry samples. Therefore, we can assert that *Aesculus indica* seeds are less proteinic and less glucidic with respect to AHP and AHH seeds samples here tested.

4. Statistical analysis

4.1. Principal component analysis (PCA)

To gain an overview of the existing relationships among the two horse-chestnuts families, and to investigate on the possible influence of the compositional chemical characteristics in the discrimination of the seeds from the two varieties of trees, principal component analysis (PCA) (Wold, 1978) has been carried out on data set consisted of a matrix of 10×12 dimension. The five analysed samples for each of the two horse-chestnuts categories (AHP and AHH), i.e. five seeds samples $\times 2$ different varieties of trees, have been reported on the rows of the matrix, while the columns contain the mean values of each of the measured variable, i.e. the N, C, H, S percentages, TISS and CWS values, the percentages of humidity, moisture, ashes, lipids, proteins

and glucids, experimentally obtained as explained in the previous sections (see Table 1).

In PCA analysis, the data are decomposed into separate sets of scores and loadings for each of the two modes of interest (samples and variables) and the whole variability of the data is explained in order to provide a clear and more interpretable visualisation of data structure in a reduced dimension. Giving the scale differences of the measured variables, the PCA was performed on the autoscaled data matrix allowing the variance of each variable to be initially identical.

PCA analysis has been carried out by using the PLS-Toolbox 3.5.4 for MATLAB© (distributed by Eigenvector Research, WA, USA).

Three principal components, explaining the 85.10% of the total data variance, have been chosen on the basis of their eigenvalues (greater than one). In order to obtain visualization of the entire data-set, a biplot, a combined scores and loadings plot, for the first two components is reported in Fig. 3, the first two principal components, accounting for 73.92% of the total data variance, shows that AHH horse-chestnuts categories (stars symbols) are well distinguished from AHP categories (triangles symbols). In particular, the first component clearly distinguishes the two categories with the samples of the first categories which get higher PC1 values and lies in opposite site of the AHH ones.

Moreover, the second component seems to discriminate the samples in their own categories. As far as loadings are concerned, the biplot shows the distribution of the investigated variables too and it is evident the presence of two main groups. The first one includes the C, N, H percentages, followed by lipids content, calculated by adding the whole lipids. The second group includes S percentage, TISS and CWS values and the percentages of moisture, humidity, proteins, ashes and glucidic contents.

Considering the first PC, which mainly separate the two horse-chestnuts categories, it can be possible to observe that the first and second variables groups, being located on the opposite side of the loading plot, seem to be the major responsible of the discrimination of the two horse-chestnuts samples.

Along PC1, the second group gets the highest loading values, highlighting their probably major content in the AHP samples with respect to AHH ones; on the other side, the opposite holds for the first group of variables.

As a final remark, we wish to underline that this work seems to be the first literature approach focusing on the characterization of these kind of horse-chestnuts categories, in relation to the different chemical-variables and compositions; hence, these statistical considerations, evicted from PCA Analysis, could be helpful to furnish some valid explanations on the different characteristics and properties of the two seeds categories.

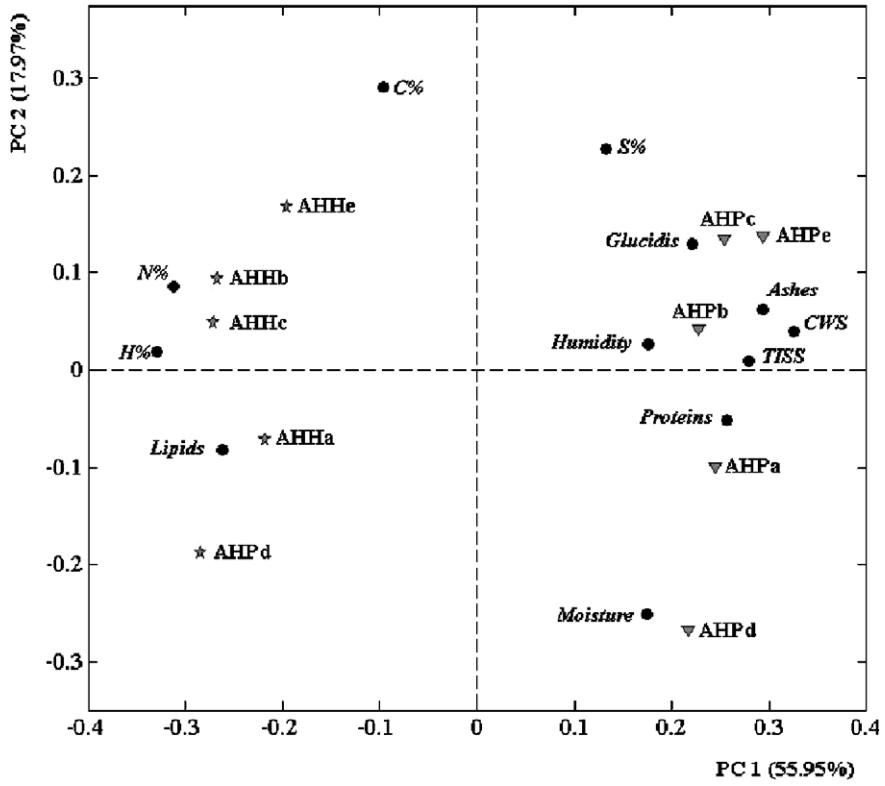

Fig. 3. PCA biplot for the first two principal components, PC1 vs. PC2, related to the horse-chestnuts samples and to the measured variables. The five samples belonging to the same species (AHH and AHP, respectively) have been labelled with letters from 'a' to 'e'. Each variable has been reported in Italic letters.

5. Conclusions

This study presents some results about morphological properties, physico-chemical behaviour and chemical composition by some accurate analysis of horse-chestnuts samples coming from two most common varieties of *Aesculus hippocastanum* L. largely diffused in Modena territory (Italy) and in Mediterranean areas.

These findings could be of some interest in research about the nutritional value of these foodstuffs, being horse-chestnuts largely utilised as starting material for nutritional supplements and bio-pharmacological targeting productions.

In particular, by comparing the relevant analytical patterns for AHP and AHH samples, it is possible to highlight that some constituents, pertaining to the investigated panel characters and the lipid fractions, can strongly discriminate the two seeds classes (confirmed by PCAnalysis), even if their macroscopic composition is rather similar.

In conclusion, our results suggests that in horse-chestnut, where natural variation is large and vegetative propagation through natural cross-hybridisation and grafting is very easy, the traditional method of selection variety may give good results in terms of selected fruits and seeds productions, also in relation to the optimisation procedures for technological transferability, and to obtain the best performances for bio-available supplements and specific salutary targets.

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