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Chemical characterization of a traditional honey-based Sardinian product: *Abbamele*

Nadia Spano [a], Marco Ciulu [a], Ignazio Floris [b], Angelo Panzanelli [a], Maria I. Pilo [a], Paola C. Piu [a], Roberta Scanu [a], Gavino Sanna [a,*]

[a] *Università degli Studi di Sassari, Dipartimento di Chimica, via Vienna 2, 07100 Sassari, Italy*
[b] *Università degli Studi di Sassari, Dipartimento di Protezione delle Piante, Via De Nicola 1, 07100 Sassari, Italy*

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Abstract

The first chemical characterization of *abbamele*, a traditional honey decoction from Sardinia (Italy) is hereby reported. Water content (from 17.7% to 27.7%), electrical conductivity (from 0.19 to 0.81 mS cm^{-1}), pH (from 3.21 to 3.92), free acidity (from 26.1 to 87.6 meq kg^{-1}), invertase activity (from 0 to 1.02 U kg^{-1}), 5-(hydroxymethyl)-2-furaldehyde, HMF (from 881 to 4776 mg kg^{-1}), total polyphenols (from 188 to 984 mg kg^{-1}) and free amino acid contents of thirteen *abbamele* samples, from industrial and traditional producers, were obtained in an attempt to compare this traditional product with honey and to study the relationship between its main features and the production procedures. The long thermal treatment involved in the production of *abbamele* has been identified as the main cause of very low (or absent) invertase activity and free amino acid content as well as the very high content of HMF.

Keywords: *Abbamele*; Honey; 5-(hydroxymethyl)-2-furaldehyde

1. Introduction

Abbamele is one of the most ancient gastronomic products of the rural culture of Sardinia (Italy) where it is mainly used to flavour and sweeten the most famous traditional Sardinian sweets. It has swiftly achieved renown abroad over recent decades. It can be defined as a decoction of honeycomb, honey and pollen in water, and traditional production involves the following simple steps. After the honey is extracted from the honeycombs, the latter are crumbled and dipped into warm water (40 °C). Then, the emerging wax separates and the remaining liquid (water, some honey and pollen) is heat-treated (up to 100 °C) until a brown, honey-like product, sometimes flavoured with orange or lemon rinds, is obtained. As a result of this careful production process, the value of *abbamele* is usually much higher (up to 10 times) than that of honey. In spite of its ancient origins (it is at least several centuries old), to our knowledge no detailed studies on the chemical composition of *abbamele* so far have been reported. In addition, information about closely related food products is scarce. In this context the very recent study by Edris, Murkovic, and Siegmund (2007) is worthy of note, as it focusses on the analysis of the aroma and the HMF determination of Egyptian treacle. The principal aim of this study was to characterize the chemical composition of *abbamele* by defining its main features. As a consequence, we present data on water content, electrical conductivity, pH, free acidity, invertase activity, 5-(hydroxymethyl)-2-furaldehyde (HMF) amounts, total content of polyphenols and the chromatographic profile of amino acids from thirteen *abbamele* samples collected from industrial and traditional producers in Sardinia.

* Corresponding author. Tel.: +39 079 229500; fax: +39 079 229559.
E-mail address: sanna@uniss.it (G. Sanna).

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2. Materials and methods

2.1. Samples

Thirteen *abbamele* samples, nine from local markets (1–9) and four from traditional producers (10–13), were collected in 2006 from different geographical areas in Sardinia. All samples were collected within their shelf life period, and stored at 4 °C before analysis. Prior to any analytical determination, the *abbamele* samples were homogenized for 15 min with an Ultra-turrax mixer mod. T18 (IKA, Staufen, Germany).

2.2. Water content

The water content of the samples was determined by measuring the refractive index at 20 °C according to the harmonized method for honey developed by the International Honey Commission, IHC, (Bogdanov, 2002). This determination, performed in duplicate, was conducted using an Abbe Refractometer MD002WAJ (Carlo Erba, Milan, Italy).

2.3. Electrical conductivity

Electrical conductivity was measured in duplicate, after checking the cell constant, at 20.0 °C in 20% (w/w) aqueous solutions of *abbamele*, according to the IHC method for honey (Bogdanov, 2002) and using a Hi 8633 (Hanna Instruments, Padova, Italy) conductivity meter equipped with a Hi 76301W (Hanna Instruments, Padova, Italy) conductivity probe. The sample solution was prepared using ultra pure water (Merck, Milan, Italy). The cell constant value was checked with 0.1 M aqueous solution of KCl (puriss. p.a., ACS reagent, $\geqslant$99.0%) from Fluka, Milan, Italy.

2.4. pH and free acidity

The measurement of pH and determination of free acidity were performed at 20 ± 0.1 °C on stirred solutions (obtained after dissolving 10 g of sample in 75 ml of carbon dioxide-free water) by potentiometric titration with a 0.1 M NaOH solution from Merck, Milan, Italy, until pH reached 8.3 according to IHC harmonized methods (Bogdanov, 2002). Potentiometric measurements were performed with an Orion model 720 A (Thermo Fischer Scientific, Milan, Italy) ion selective meter equipped with a mod. 411/CGG/12 combined glass electrode (Amel, Milan, Italy). Three titrations were performed for each sample.

2.5. Invertase activity

Invertase activity was determined, following the method outlined by Persano Oddo, Piazza, and Pulcini (1999): KH_2PO_4 (RPE ACS ISO p.a.) was from Carlo Erba, Milan, Italy; $Na_2HPO_4 \cdot 2H_2O$ (puriss. p.a., $\geqslant$99.5%) and HCl (puriss. p.a., min 37%) were from Riedel de Haen, Milan, Italy; the 4-nitrophenyl-α-D-glucopyranoside (Biochemika, $\geqslant$98.0%) was from Fluka, Milan, Italy; and the tris(hydroxymethyl)aminomethane (ultrapure grade, 99.9+%) was from Aldrich, Milan, Italy. UV/VIS measurements were taken using a Model U-2010 double beam spectrophotometer (Hitachi instruments, Milan, Italy), using 1 cm quartz cells. Data are expressed in units per kilogramme of *abbamele* ($U\,kg^{-1}$), where 1 U is the enzyme activity that can transform 1 μmole of substrate in 1 min under optimal conditions. Each determination was performed in duplicate.

2.6. HMF

The HMF concentration was determined using the same RP-HPLC method as previously developed and validated for honey by our research group (Spano et al., 2006). This method was chosen because of its high linearity interval, also towards high HMF concentrations. The chromatographic mobile phase consisted of ultra pure water, methanol (HPLC grade) from Riedel de Haen, Milan, Italy, and a properly diluted H_2SO_4 solution from Merck, Milan, Italy. All solvents used were previously filtered through a 0.45 μm membrane, from Millipore, Bedford, MA, to remove any impurities. The HPLC equipment was comprised of a Series 200 binary pump, a sampling valve, a 20 μl sample loop and a Series 200 UV–VIS variable wavelength detector, all from Perkin–Elmer, Milan, Italy. Separation was performed on an Alltima C18 column 250 mm × 4.6 mm, 5 μm particle size (Alltech, Sedriano, Italy) fitted with a guard cartridge packed with the same stationary phase. Data were elaborated using Turbochrom Workstation Software (Perkin–Elmer, Milan, Italy). Each sample was analyzed in triplicate.

2.7. Total content of polyphenols

The total content of polyphenols was spectrophotometrically determined according to Floris et al. (1994) using a method based on Folin Ciocalteu's reagent, that was conveniently modified to prevent interference by reducing sugars. Petroleum ether (puriss. p.a., ACS reagent, reag. ISO, min.95%), methanol (HPLC grade) and ethyl acetate (puriss. p.a., ACS reagent, reagent ISO, 99.5%) were from Riedel-de Haën, Milan, Italy; metaphosphoric acid (puriss., p.a., ACS reagent >33.5%), $(NH_4)_2SO_4$ (puriss. p.a., ACS reagent, $\geqslant$99.5%), Na_2CO_3 (purum p.a., $\geqslant$99%), caffeic acid (purum p.a., anhydrous, $\geqslant$99%) and Folin Ciocalteu's reagent (Biochemika) were from Fluka, Milan, Italy. Each sample was analyzed in duplicate, and analytical data were expressed in mg of caffeic acid per kilogramme of *abbamele*.

Table 1
Chromatographic conditions for amino acid determination

Elution program		
Time (min)	Solution $A = 10{:}90$ (v/v) mixture of (i) acetonitrile and (ii) an aqueous solution 0.23 M CH_3COONa, 6.5 mM in triethylamine and CH_3COOH until pH 6.4.(%)	Solution $B = 40{:}60$ (v/v) water–acetonitrile mixture (%)
0–13	84	16
13–18	54	46
18–20	0	100
20–22.5	100	0
22.5–23.5	84	16

Operative wavelength: 254 nm; flow rate: 1.2 ml min^{-1}.

2.8. Amino acids

2.8.1. General

The qualitative–quantitative determination of free amino acids in *abbamele* was performed, following a recent RP–HPLC method based on the extraction and derivatization of analytes with phenyl isothiocyanate, developed and validated for honey by our research group (Spano et al., submitted for publication).

2.8.2. Amino acid extraction phase

Abbamele samples (20 g) were dissolved in 50 ml of 2 M HCl. The solution, after filtration through paper, was placed in an Amberlite column (IR-120, H-form, previously activated with 2 M HCl, diameter 10 mm, length 170 mm) from Lancaster Synthesis, Milan. Different washes with HCl solution, pH 2.1, were performed until Fehling's test proved negative. Free amino acids were then eluted with a 2 M ammonia solution.

2.8.3. Amino acid derivatization phase

The extracts (300 μl) were dried under reduced pressure, at $t \leqslant 38$ °C, and allowed to react for 10 min with 18 μl of a 1:1:7 (v/v/v) solution of water, triethylamine (BioChemika, for amino acid analysis, $\geqslant 99.5\%$) and ethanol (absolute, $\geqslant 99.8\%$), and 2 μl of phenyl isothiocyanate, PITC, (puriss., $\geqslant 99.0\%$). All reagents were from Fluka, Milan, Italy.

2.8.4. RP–HPLC analysis

The solution was evaporated to dryness and the residue was dissolved in 200 μl of solution A, the composition of which is shown in Table 1. CH_3COONa and CH_3COOH were both RPE ACS ISO p.a. and were supplied by Carlo Erba, Milan, Italy. This solution was injected and analysed by RP–HPLC, using the same equipment as previously described for HMF analysis. The chromatographic conditions are listed in Table 1.

3. Results and discussion

The analytical data are shown in Table 2.

The water content of *abbamele* is heavily influenced by many factors, the most critical being: (i) the water content of the honey, (ii) the residual moisture in the honeycombs, (iii) the amount of water initially added when treating honeycombs and (iv) the temperature and the length of the heating process. Bearing in mind that almost all unifloral European honeys (except heather (*Calluna*) and strawberry tree (*Arbutus*) (Persano Oddo, Piazza, Sabatini, & Accorti, 1995) have an average moisture content of under 20% (Persano Oddo & Piro, 2004), it is evident that the average water content of *abbamele* (22.32%) is much higher. Indeed, more than 30% of the samples analyzed (1, 3, 5, 12) exceeded the maximum moisture limit of 25% established by European legislation regarding the water content of any kind of honey. Moreover, the long heating process at high temperatures inactivates the osmotolerant yeasts (e.g. *Saccharomyces spp*), preventing the risk of any fermentation processes in *abbamele*.

Without doubt, the thermal inactivation processes are also the cause of the absence of invertase activity in all samples. Like the HMF concentration, this parameter is an important indicator of the freshness of honey: invertase hydrolyses saccharose to glucose and fructose and is strictly heat-labile (Dustmann, Van Praagh, & Bote, 1985; Sancho, Muniategui, Huidobro, & Simal, 1992). Hence our results are not surprising, considering that heating for a long time at high temperatures (close to 100 °C) is supposed to cause the almost total denaturation of this enzyme.

By contrast, the "thermostable" analytes in *abbamele* do not show appreciable variation when compared to common honey levels. In particular, the electrical conductivity and the acidity parameters (pH and free acidity) were found to be well within the ranges expected for pure and fresh honey (between 0.1 and 2.0 mS cm^{-1} for the electrical conductivity, between 3.5 and 5.5 for pH and between 10 and 60 meq kg^{-1} for free acidity).

Also, total polyphenols seem to be scarcely affected by thermal effects. Table 2 also confirms that there is a marked variability of this parameter among different samples, probably due to the floral origin of the honey. Moreover, the polyphenol concentration in the *abbamele* samples might be similar to that of the original honey. Our findings are supported also by Larrauri, Rupèrez, and Saura-Calixto (1997) who observed only an 18.6% decrease in the total concentration of polyphenols after heat treatment applied to red grape pomace peels at 100 °C.

Table 2
Results of analyses conducted on *abbamele* samples

Sample	pH ± SD	Free acidity (meq kg^{-1} ± SD)	Water content (% ± SD)	Electrical conductivity (mS cm^{-1} ± SD)	Invertase activity (U kg^{-1} ± SD)	Polyphenols[b] (mg kg^{-1} ± SD)	HMF (mg kg^{-1} ± SD)
1	3.73 ± 0.02	26.1 ± 1.8	25.4[a] ± 0.2	0.60 ± 0.02	0.05 ± 0.01	512 ± 33	1269 ± 3
2	3.92 ± 0.04	26.4 ± 2.3	17.8 ± 0.2	0.41 ± 0.01	1.02 ± 0.06	293 ± 20	1228 ± 3
3	3.50 ± 0.03	56.5 ± 3.6	25.8[a] ± 0.3	0.56 ± 0.02	0.00	188 ± 9	4776 ± 6
4	3.36 ± 0.01	75.7 ± 5.4	23.4 ± 0.2	0.81 ± 0.03	0.00	395 ± 8	4033 ± 4
5	3.92 ± 0.02	30.9 ± 3.2	27.7[a] ± 0.3	0.42 ± 0.01	0.19 ± 0.03	207 ± 37	1969 ± 43
6	3.67 ± 0.03	34.0 ± 2.4	18.2 ± 0.2	0.45 ± 0.02	0.00	984 ± 14	1089 ± 16
7	3.61 ± 0.03	37.3 ± 3.9	21.4 ± 0.2	0.52 ± 0.02	0.05 ± 0.01	334 ± 39	2403 ± 5
8	3.67 ± 0.05	45.4 ± 5.2	17.4 ± 0.2	0.53 ± 0.02	0.00	299 ± 19	1055 ± 1
9	3.64 ± 0.01	36.4 ± 2.6	22.2 ± 0.2	0.46 ± 0.02	0.05 ± 0.02	502 ± 29	3871 ± 7
10	3.84 ± 0.00	30.2 ± 2.1	19.4 ± 0.2	0.32 ± 0.01	0.08 ± 0.02	288 ± 37	881 ± 25
11	3.21 ± 0.02	26.2 ± 1.7	21.4 ± 0.2	0.19 ± 0.01	0.08 ± 0.03	282 ± 33	1151 ± 1
12	3.76 ± 0.03	87.6 ± 4.0	25.4[a] ± 0.2	0.60 ± 0.02	0.05 ± 0.03	518 ± 6	1274 ± 13
13	3.60 ± 0.04	48.0 ± 3.6	24.6 ± 0.2	0.45 ± 0.02	0.02 ± 0.02	407 ± 10	1767 ± 19

SD: standard deviation.
[a] Water content greater than 25% was estimated by a linear extrapolation.
[b] Expressed as caffeic acid.

Another interesting result is the absence of free amino acids in *abbamele*. Even proline, which is normally the most abundant free amino acid in honey, was found to be at levels below the detection limit (0.05 mg kg^{-1}) in all samples. Other studies (Pawloska & Armstrong, 1994; Pätzold & Brückner, 2006; Von der Ohe, Dustmann, & Von der Ohe, 1991) have revealed that even moderate heating of the honey causes a sharp decrease in the free amino acid content, probably due to an increased rate of the Maillard reaction.

HMF is one of the chief products of carbohydrate degradation in food, known as non-enzymatic browning. It has been demonstrated that the HMF level in a saccharidic foodstuff increases greatly during thermal treatment and its concentration follows a kinetic model of pseudo first order (Tosi, Ciappini, Re, & Lucero, 2002). Nevertheless, the extremely high HMF values found in all the samples represent the most striking result of this characterization. According to EU honey legislation (Council Directive 2001/110/EC of 20 December 2001 relating to honey, 2002), the maximum content of HMF permitted is 40 mg kg^{-1} (80 mg kg^{-1} for honeys produced in tropical countries). However, in sample 10, that with the lowest HMF concentration, this value was found to be ten times higher than the highest value allowed by EU guidelines. For most of the samples we found values between 1000 and 2000 mg kg^{-1}, whereas the highest value, sample 3, was just below 5000 mg kg^{-1}. Therefore, so as to avoid the confusion that arises when comparing *abbamele* with honey, the former is to be regarded as a different, distinct saccharidic foodstuff. Furthermore, the HMF values detected in *abbamele* ought to be compared with those of other heated food products, such as those listed in Table 3.

Like other heated food products, the formation of significant amounts of HMF in *abbamele* is unavoidable. Even so, HMF content might be reduced during industrial production by the optimization of the amount of water initially added and careful temperature control, as well as a reduction in heating time, by carrying out the thermal process under reduced pressure.

Table 3
HMF amounts measured in different thermally treated saccharidic foodstuffs

Foodstuff	Highest observed HMF concentration (mg kg^{-1} ± SD)	References
Aceto Balsamico Tradizionale	3100 ± 17	Cocchi, Ferrari, Manzini, Marchetti, & Sighinolfi, 2007
(Italian traditional balsamic vinegar)	3420	Masino, Chinnici, Franchini, Ulrici, & Antonelli, 2005
Coffee	605	Kanjahn & Maier, 1997
Instant coffee powder	4200	Kanjahn & Maier, 1997
	6200	Schultheiss, Jensen, & Galensa, 1999
Coffee substitutes	13500	Schultheiss et al., 1999
Liquid caramel	2528 ± 34	de la Iglesia, Lazaro, Puchades, & Maquieira, 1997
	9500	Bachmann, Meier, & Känzig, 1997
Boiled juices	4500	Kus, Gogus, & Eren, 2005
Concentrated apple juice	963	Burdurlu & Karadeniz, 2003
Treacle	179	Edris et al., 2007
Dried pears	3500	Bachmann et al., 1997
Abbamele	4776 ± 6	This work

SD: standard deviation.

In conclusion, this paper reports, for the first time, the chemical characterization of abbamele, one of the most typical transformation products of traditional apiculture in Sardinia. Thirteen samples of this foodstuff were analysed in terms of moisture, electrical conductivity, pH, free acidity, invertase activity, HMF, total content of polyphenols and free amino acids. The analytical results were compared to those typical of its principal ingredient (i.e. honey). Some attempts have also been made to understand how heating, during the production process, may affect the values of selected parameters. In particular, attention has focussed on the moisture level, invertase activity, the concentration of free amino acids and (mainly), the HMF concentration, that ranged from 881 to 4776 mg kg^{-1}. On the whole, we believe that *abbamele* does not represent a potential food threat (at least no more than other products, such as instant coffee, coffee substitutes and caramel) because its "bare" and continuative consumption is quite negligible and, in "normal" use, *abbamele* is only a minor ingredient, often mixed with other ingredients and hence substantially diluted in the final product.

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