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Assessment of physicochemical and antioxidant characteristics of *Quercus pyrenaica* honeydew honeys

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ABSTRACT

Consumers are exhibiting increasing interest in honeydew honey, principally due to its functional properties. Some plants can be sources of honeydew honey, but in north-western Spain, this honey type only comes from *Quercus pyrenaica*. In the present study, the melissopalynological and physicochemical characteristics and the antioxidant properties of 32 honeydew honey samples are described. *Q. pyrenaica* honeydew honey was defined by its colour, high pH, phenols and flavonoids. Multivariate statistical techniques were used to analyse the influence of the production year on the honey's physicochemical parameters and polyphenol content. Differences among the honey samples were found, showing that weather affected the physicochemical composition of the honey samples. Optimal conditions for oak growth favoured the production of honeydew honey.

1. Introduction

Honeydew honey is increasingly valued by consumers and the food industry, due to its valuable nutritional and medicinal qualities, which are different from floral honeys (Castro-Vázquez, Díaz-Maroto, & Pérez-Coello, 2006; Madejczyk & Baralkiewicz, 2008). Honeydew honey is a type of honey that honeybees produce from excretions of plant-sucking insects (Hemiptera) on the living parts of plants or secretions from the living parts of plants.

In the Iberian Peninsula, some trees can be sources of honeydew, but the main Spanish honeydew honey-producing plants are the holm oak (*Quercus ilex*) and oak (*Quercus* sp.) (Castro-Vázquez et al., 2006). In the northwest, the Pyrenean oak or *Quercus pyrenaica* Wild. (*Q. toza auct.*) is the principal honeydew source. This taxon grows frequently in a mixed forest of deciduous oaks (*Quercus robur* or *Q. pyrenaica*) and sweet chestnut (*Castanea sativa*). The plant is characterised by an Atlantic–Mediterranean distribution, which comprises south-western France, the Iberian Peninsula and northern Maroc. At the end of the summer, depending on the climatic conditions, the Pyrenean oak exudes a large amount of phloem sap in its acorns. This sweet sap contains natural sugars and minerals and is ingested by bees and deposited in hives as a dark, thick, fragrant honey (Jerković & Marijanović, 2010; Krakar, 2012). Some biotic elements such as plant pathogen fungi, anemophilous pollen, yeast or microalgae deposited on the green parts of the Pyrenean oak could be collected when honeybees suck the honeydew. These microscopic elements were used as indicative of the source of the honey (Escuredo, Fernández-González, & Seijo, 2012; Louveaux, Maurizio, & Vorwohl, 1978; Seijo, Escuredo, & Fernández-González, 2011).

The growing market for honeydew honey in many European countries requires its differentiation from other honey types in response to consumer demands (Simova, Atanassov, Shishiniova, & Bankova, 2012). Differentiation between honeydew and nectar honey is not easy. However, honeydew honey has a unique chemical composition. As a rule, honeydew honey has been found to contain higher di- and trisaccharide contents, as well as lower mean contents of glucose and fructose that nectar honey (Astwood, Lee, & Manley-Harris, 1998; Bentabol, Hernández-García, Rodríguez-Galdón, Rodríguez-Rodríguez, & Díaz-Romero, 2011; Escuredo, Míguez, Fernández-González, & Seijo, 2013; Sanz, González, De Lorenzo, Sanz, & Martínez-Castro, 2005). Additionally, honeydew honey also presents a high polyphenol content, antioxidant and antibacterial activity (Escuredo et al., 2013; Vela, De Lorenzo, & Pérez, 2007). Some physicochemical parameters such as pH, acidity, ash, diastase and electrical conductivity can be used to indicate the presence of honeydew in honey (Bentabol et al., 2011; Díez, Andrés, & Terrab, 2004; Escuredo et al., 2012; Mateo & Bosch-Reig, 1998; Sanz et al., 2005; Soria, González, De Lorenzo, Martínez-Castro, & Sanz, 2005; Vela et al., 2007). However, a wide dispersion of these parameters was associated with several factors such as geographical

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origin, botanical source and climatic conditions (Soria et al., 2005). This dependence often reduces the usefulness of these parameters in honey classification. The problem is made even more complicated by the fact that there are different types of honeydew honey (Simova et al., 2012), depending on the source plant-sucking insects and host plants (Persano-Oddo & Piro, 2004).

Considering the economic interest in honey as natural food, especially honeydew honey, the objective of this work was to typify *Q. pyrenaica* honeydew honey from northwest Spain. The characterisation was performed according to palynological and physicochemical parameters, polyphenol content and antioxidant activity. In addition, whether differences in the physicochemical and antioxidant data are correlated significantly with the production year was investigated.

2. Material and methods

2.1. Honey samples

Honey samples ($n = 32$) were provided directly from beekeepers in 2009, 2010 and 2011 (Table 1). They were refrigerated from the time of production until analysis in the laboratory.

2.2. Palynological analysis

The pollen analysis was based on the method established by Louveaux et al., (1978). 10 g of honey was dissolved in bi-distilled water and centrifuged at 3373 *xg* for 10 min. The obtained sediment was re-dissolved in water and centrifuged for 5 min. The qualitative analysis was performed with two aliquots of 100 μl of the sediment. A minimum of 800 pollen grains per sample were counted and identified using a Nikon Optiphot II microscope at 400× or 1000× when necessary. The results are expressed as the percentage of representation of each pollen type and divided into the following frequency classes: I, important pollen (between 3% and 15%); A, accompanying pollen (between 15% and 45%); and D, dominant pollen (equal to or upper than 45%).

For quantitative analysis, an aliquot of 10 μl of the obtained sediment (final volume of 2 ml) was used. The quantity of pollen grains in the aliquots was quantified using light microscopy at 400×. At the same time, different fungal elements (HDE) such as fungal spores, yeast and algae were counted. The results are expressed as the number of pollen grains per gram of honey, number of HDE per gram of honey and as the honeydew index, which relates the number of fungal elements to the number of pollen grains (HDE/P).

2.3. Physicochemical analysis

The physicochemical analysis of honey (HMF, diastase activity, electrical conductivity and moisture) was performed using the official method of analysis in Spain (Codex Alimentarius Commission, 2001). The HMF content in honey was determined using the White spectrophotometric method based on the determination of the difference between the absorbance at 284 nm of a honey solution and the same solution after addition of bisulphite. The HMF content was calculated after subtraction of the background absorbance at 336 nm. The results are expressed in mg/100 g. Diastase activity was based on the rate of starch hydrolysis by diastase present in a honey buffer solution at 40 °C. The endpoint for this reaction was established by measuring the absorbance at 660 nm with a UV–VIS spectrophotometer until it was less than 0.235. The results

Table 1
Palynological characteristics of honeydew honeys. D, dominant pollen (>45%); A, accompanying pollen (16–45%); I, important pollen (3–15%).

Samples	Year	D	A	I
1	2009	–	*Castanea sativa* (41.0%), *Rubus* (40.9%)	*Cytisus* type (10.5%), *Erica* (3.2%)
2	2009	*Castanea sativa* (58.5%)	*Rubus* (25.8%)	*Cytisus* type (4.1%), *Erica* (4.7%)
3	2009	*Rubus* (49.7%)	*Castanea sativa* (38.7%)	–
4	2009	–	*Rubus* (40.8%), *Castanea sativa* (35.5%)	*Cytisus* type (8.0%), *Crataegus monogyna* type (3.6%)
5	2009	*Cytisus* type (48.6%)	*Rubus* (24.8%)	*Castanea sativa* (13.9%), *Erica* (7.2%)
6	2009	*Castanea sativa* (53.0%)	*Rubus* (18.2%)	*Erica* (12.8%), *Cytisus* type (7.3%), *Eucalyptus* (3.9%)
7	2009	–	*Rubus* (37.6%), *Castanea sativa* (34.2%)	*Echium* (13.1%), *Cytisus* type (6.5%)
8	2009	*Castanea sativa* (55.4%)	*Rubus* (32.6%)	*Cytisus* type (3.2%)
9	2009	*Rubus* (51.1%)	*Castanea sativa* (34.1%)	*Erica* (6.2%)
10	2009	*Rubus* (51.0%)	*Castanea sativa* (20.6%)	*Cytisus* type (11.7%), *Echium* (7.1%)
11	2009	*Rubus* (53.8%)	*Castanea sativa* (24.5%)	*Cytisus* type (8.1%), *Frangula alnus* (5.2%)
12	2009	–	*Cytisus* type (43.0%), *Erica* (27.1%)	*Rubus* (12.1%), *Frangula alnus* (10.4%)
13	2009	*Castanea sativa* (56.2%)	*Rubus* (29.6%)	*Eucalyptus* (4.4%), *Cytisus* type (3.4%)
14	2009	–	*Castanea sativa* (44.4%), *Rubus* (42.5%)	*Cytisus* type (6.8%)
15	2009	*Castanea sativa* (55.7%)	*Rubus* (20.6%), *Sesamoides* (16.0%)	*Cytisus* type (3.8%)
16	2009	*Castanea sativa* (47.9%)	*Rubus* (22.2%)	*Erica* (14.2%), *Cytisus* type (8.2%)
17	2009	*Rubus* (60.0%)	*Castanea sativa* (30.0%)	*Erica* (3.0%)
18	2010	–	*Rubus* (39.5%), *Castanea sativa* (34.3%), *Erica* (15.8%)	*Cytisus* type (7.2%)
19	2010	*Rubus* (68.5%)	*Castanea sativa* (22.7%)	*Echium* (3.3%)
20	2010	*Rubus* (58.1%)	*Castanea sativa* (30.0%)	–
21	2010	*Rubus* (59.0%)	*Castanea sativa* (30.4%)	*Echium* (3.9%), *Cytisus* type (3.8%)
22	2010	*Rubus* (66.6%)	–	*Castanea sativa* (14.4%), *Cytisus* type (7.9%), *Erica* (7.7%)
23	2010	*Castanea sativa* (50.6%), *Rubus* (45.5%)	–	–
24	2010	*Castanea sativa* (55.0%)	*Rubus* (24.9%)	*Cytisus* type (11.8%), *Erica* (3.0%)
25	2010	*Rubus* (50.8%)	*Castanea sativa* (44.1%)	–
26	2011	*Castanea sativa* (54.3%)	*Rubus* (22.4%), *Cynoglossum* (19.7%)	–
27	2011	*Rubus* (51.8%)	–	*Castanea sativa* (12.7%), *Eucalyptus* (11.5%), *Cytisus* type (7.2%), *Crataegus monogyna* type (4.4%)
28	2011	*Castanea sativa* (60.7%)	*Rubus* (22.1%)	*Cytisus* type (5.4%), *Erica* (3.8%)
29	2011	*Rubus* (48.3%)	*Castanea sativa* (37.0%)	*Cytisus* type (3.4%), *Erica* (5.3%)
30	2011	*Castanea sativa* (54.9%)	*Rubus* (35.9%)	–
31	2011	–	*Rubus* (39.3%), *Castanea sativa* (28.2%), *Frangula alnus* (16.4%)	*Cytisus* type (7.9%)
32	2011	*Rubus* (47.2%)	*Castanea sativa* (41.2%)	*Cytisus* type (3.0%), *Erica* (5.0%)