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Sugar composition of French royal jelly for comparison with commercial and artificial sugar samples

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ABSTRACT

A gas chromatographic method was developed to quantify the major and minor sugars of 400 Royal Jellies (RJs). Their contents were compared in relation to the geographical origins and different production methods.

A reliable database was established from the analysis of 290 RJs harvested in different French areas that took into account the diversity of geographical origin, harvesting season, forage sources available in the environment corresponding to natural food of the bees: pollen and nectar.

Around 30 RJ samples produced by Italian beekeepers, about sixty-ones from French market, and around thirty-ones derived from feeding experiments were analysed and compared with our database.

Fructose and glucose contents are in the range 2.3–7.8% and 3.4–7.7%, respectively, whatever the RJ's origin. On the contrary, differences in minor sugar composition are observed. Indeed sucrose and erlose contents in French RJs are lesser than 1.7% and 0.3%, respectively, whereas they reach 3.9% and 2.0% in some commercial samples and 5.1% and 1.7% in RJs produced from feeding experiments.

This study could be used to discriminate different production methods and provide an additional tool for identifying unknown commercial RJs.

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1. Introduction

Royal jelly (RJ) is the secretion of the cephalic glandular system (hypopharyngeal and mandibular glands) of young worker bees between the 5th and the 14th days of their life. After this time, the secretion stops by natural atrophy of the glands. RJ is used to feed all larvae for the first 3 days of their lives and the queen bee during all her life.

It is a yellowish and creamy product, currently consumed pure as a dietary supplement for its stimulatory effect, predominantly for elderly and convalescent people. It has today numerous applications in cosmetics, pharmaceutical and food industries.

The chemical composition of RJ has been studied by several authors (Boselli, Caboni, Sabatini, Marcazzan, & Lercker, 2003; Ferioli, Marcazzan, & Caboni, 2007; Garcia-Amoedo & De Almeida-Muradian, 2007; Howe, Dimick, & Benton, 1985; Lercker, Caboni, Vecchi, Sabatini, & Nanetti, 1992; Low & Sporns, 1988; Palma, 1992; Takenaka & Echigo, 1980; Zhou, Xue, Li, Zhang, & Zhao, 2007) but data available in the literature are highly variable due to the intrinsic variability of the product and the use of different analytical methods since no reference methods have been established for RJ.

Some international discussions (ISO committee TC34/WG13) are implemented in order to establish an international standard for definition, physicochemical parameters and quality of RJ but is still at its beginnings. This standard is particularly important for market exchanges and consumer protection.

French production is about 1–2 tonnes a year. GPGR's (Groupement des Producteurs de Gelée Royale) production is representative of the French production with more than 80% of the French production.

Because of an important domestic demand and in order to satisfy the national market, France needs to import large amounts of RJ, mainly from China. Indeed China is undoubtedly the largest RJ producer with some thousands of tons a year. This production has a highly competitive price on the worldwide market and the determination of geographical origin of RJ is an important issue for RJ quality control.

Sugar composition, moisture, protein and 10-HDA contents are the most common criteria used to characterise RJ quality. Protein and water contents are too large to be good indicators of the origin of RJ. Ten-HDA considered as the main marker of RJ freshness, quality and authenticity, is generally present in an appropriate content. Sugar studies usually relate contents of the major sugars namely fructose, glucose and sometimes sucrose (Bogdanov et al., 2004; Sesta, 2006; Sesta, Persano Oddo, Nisi, & Ricci, 2006). Indeed fructose and glucose account for 80% of the sugars present in RJ. In this

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study we have quantified the three main RJ sugars and 12 minor ones (galactose, mannitol, maltose, maltulose, turanose, trehalose, palatinose, isomaltose, gentiobiose, melezitose, erlose and maltotriose) and gluconic acid. Actually previous studies in our lab on honey (Cotte, Casabianca, Chardon, Lheritier, & Grenier-Loustalot, 2003; Cotte, Casabianca, Chardon, Lheritier, & Grenier-Loustalot, 2004) or by others authors (Lercker et al., 1986; Rybak-Chmielewska, 2007; Sabatini, Marcazzan, Caboni, Bogdanov, & Almeida-Muradian, 2009; Serra Bonvehi, 1991) have shown that some sugars, present in minor quantities, are better markers for adulteration. So the necessity to study the maximum of sugars will be demonstrated in order to determine adulteration process.

2. Material and methods

2.1. Samples

French RJ samples (290) have all been purchased from GPGR producers. They are around seventy producers in this group, spread throughout France in 34 areas. Belonging to this group implies the respect of a quality convention concerning the production, harvest, conservation and sell of the RJ. Feeding is not allowed, except with honey and pollens in the case of insufficient honeydew. Samples are harvested during all the 2007, 2008 and 2009 seasons.

Commercial samples (58) have been purchased in French health food stores, in import companies, on internet. It is representative of the different places where French people can buy RJ.

Italian RJs (26 samples) harvested between May and October 2009 have been provided by Italian producers located in 8 different areas. Among them 11 are produced without feeding the bees and 3 with a honey feeding (2 with chestnut and 1 with acacia honey). The others ones are produced either by feeding bees with pure sugar cane (4 samples) or with different syrups (8 samples).

French RJs (27 samples) derived from feeding experiments have been provided from GPGR producers that have dedicated some hives to these experiments. The feeding has been realised with either honey or different kind of sugars (sugar cane, starch hydrolysates, beet inverted sucrose) diluted at 50%. Frames of honey have been removed and replaced by feeders except in the honey feeding hive where honey frames were kept. These experiments have been carried out during a scarcity period (no pollen and nectar in the environment) in order to limit the effect of foraging activity. Bees have been fed continuously with sugar, the beekeeper controlling every day that the feeders are full and that no looting occurs.

The feeding experiments have been done with ten different C4 sugars (cane sugar: F1–F4; F15–F17 and F21–F23), nine C3 sugars (beet: F12–F14 and starch hydrolysate F6–F11) and four with chesnut honey F5, F18–F20. Four samples from C4 syrup Agenabon: F24–F27 correspond to one harvest every 3 days during 9 days and start value (F24), feeding starting after the introduction of the larvae, in an attempt to evaluate the feeding influence versus time. We have thus collected 14 samples from C4 feeding, 9 from C3 one and 4 from honey one, respectively.

2.2. Reagents

2.2.1. Sugars

The sugars were purchased from Fluka [Fructose (Ref. 47739, puriss \geqslant 99%), Galactose (Ref. 58928, puriss \geqslant 99%), Maltose (Ref. 63418, puriss \geqslant 99%), Isomaltose (Ref. 58928, puriss \geqslant 96%), Sorbitol (Ref. 85530, puriss \geqslant 99%)], from Sigma [Glucose (Ref. G5500, puriss \geqslant 99%), Maltulose (Ref. 50796, puriss \geqslant 98%), Turanose (Ref. 287377, puriss \geqslant 98%), Trehalose (Ref. T9531, puriss \geqslant 99%), Melezitose (Ref. M5375, puriss \geqslant 99%), Erlose (Ref. E1895, puriss \geqslant 97%), Maltotriose (Ref. M8378, puriss \geqslant 95%)], from Acros [Gentiobiose

(Ref. 22591–0010, puriss \geqslant 97%), Palatinose (Ref. 22595–0050)], from Merck: Mannitol (Ref. M687487,puriss \geqslant 99%) and from Bio Chemika: Sucrose (Ref. 84097, puriss \geqslant 99.5%).

2.2.2. Derivatisation reagents

The reagents were purchased from Fluka: Hexamethyldisilazane (Ref. 52619, puriss \geqslant 99%), trimethylchlorosilane (Ref. 33014, puriss \geqslant 99%), Pyridine (Ref. 82702, puriss \geqslant 99.8%). Anhydrous pyridine is obtained by distillation over calcium hydride.

2.3. Method

2.3.1. Sample preparation

For GC analysis a derivatisation step is necessary in order to make sugars volatiles. Thus a mixture of hexamethyldisilazane and trimethylchlorosilane (HMDS/TMCS) has been used in a pyridine solvent (Low & Sporns, 1988; Pourtallier, 1967). Note that elimination of water from the sample is essential because this reaction is sensitive to water.

About forty mg of lyophilised RJ and one mg of sorbitol as internal standard are accurately weighted and introduced in the glass reactor which is tightly closed. Then 1 mL of anhydrous pyridine is added. The mixture is stirred 5 min with the reactor sealed. Then 200 μ L of hexamethyldisilazane is added in the reactor and the mixture is again stirred 5 min before adding 100 μ L of trimethylchlorosilane. After 30 min of stirring, the mixture is left 20 h at room temperature with the reactor sealed. The derivatised sample is then analysed by GC.

2.3.2. GC analysis

The quantitative determination and separation of RJ sugars are performed with an Agilent Technologies 6890 series chromatograph equipped with an HP5-MS column (30 m \times 0.25 mm; 0.25 µm), a split–splitless injector, an autosampler, and a flame ionisation detector (FID). Helium is used as carrier gas (grade 5.0). Injection volume is fixed at 1 µL in a split mode with a ratio 1:20. The oven temperature is programmed as follows: the initial temperature (150 °C) is maintained 5 min, then increased to 325 °C at a rate of 3 °C/min. The final temperature is maintained for 10 min. The injector and detector temperatures are set at 280 °C. In the detector the hydrogen flow is of 40 mL/min and the air one 450 mL/min. Helium pressure is maintained constant at 22.04 psi.

2.3.3. Sugar identification

Instead of retention times, Kovats indices are used to identify the different sugars. The retention indices of each sugar are determined by injecting the standard with the same analytical and chromatographic conditions.

A mixture of paraffins from C15 to C40 is injected prior to each batch of samples for retention indices calculation.

2.3.4. Sugar quantification

Sugar quantification is achieved by internal calibration with sorbitol as internal standard. A response factor, or mass correction factor, is calculated for each sugar as follows in Eq. (1):

$$k_i = \frac{A_{SI}}{A_i} \times \frac{M_i}{M_{SI}} \tag{1}$$

with k_i = response factor of sugar i; A_{SI} = area of the internal standard; A_i = area of sugar i; M_{SI} = mass of the internal standard; M_i = - mass of the sugar i.

For compounds that are present within two anomeric forms, the area is calculated by adding the area of both anomers.

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