Food Chemistry **19** (1986) 255–264

Carbohydrate Determination of Royal Jelly by High Resolution Gas Chromatography (HRGC)*

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(Received: 16 July, 1985)

ABSTRACT

Gas chromatography, using a short capillary column (qualitative and quantitative) can be carried out on the sugar components (such as trimethylsilyl derivatives, acetyl derivatives) of Royal Jelly up to a maximum of 24 carbon atoms.

The technique has made possible the identification of simple sugars contained in Royal Jelly (glucose, fructose and saccharose) and has, at the same time, given information about the other neutral constituents. These constituents, even if present only in small quantities, are characteristic and thus play a useful rôle in checking the authenticity of the product. In addition, the possibility of checking for fragments containing up to a maximum of four sugars linked together (tetrasaccharides) makes

* This paper was presented, in part, at the 'International Congress of Apiculture (Apimondia)', Budapest, Hungary, August 25–31, 1983.

it possible to identify products obtained from sugars originating from hydrolysed starch, or isomerized syrup containing variable amounts of fructose. Such syrups, readily available at prices low enough to encourage their use as adulterants, can themselves be identified by the presence of these characteristic di- and trisaccharides.

INTRODUCTION

Following our initial experiments on the lipid fraction (Lercker *et al.*, 1981*a*, *b*, 1982), we carried out further experiments in order to identify and determine the composition of the carbohydrate fraction in female bee larvae food.

The existing literature available to us generally reports the global composition of the glycid fraction of Royal Jelly as ranging from 7·5% to 15%. (Aeppler, 1922; Elser, 1929; Melampy & Jones, 1939; Haydak, 1943; Gontarski, 1954; Rembols, 1965; Rembols & Lackner, 1978).

Sugars present in more noteworthy quantities are glucose, fructose and saccharose (Townsend & Lucas, 1940; Shuel & Dixon, 1959; Patel *et al.*, 1961; Christensen, 1962; Simo & Christensen, 1962; Takenaka & Takashi, 1980; Tourn *et al.*, 1980; Takenaka, 1982).

Other sugars, such as maltose, trehalose, erlose, melibiose and ribose (Ammon & Zoch, 1957; Pourtallier *et al.*, 1970; Siddiqui & Furgala, 1965) have also been noted.

The chemical composition of larval food, in relation to its rôle in cast determination, has been dealt with in other reports, some of which are recent (Rembold, 1967, 1980; Haydak, 1968; Jung-Hoffman, 1968; Asencot & Lensky, 1975; Weiss, 1980; Brouwers, 1984).

In some of these reports earlier literature is also considered and discussed. Extraction, by an aqueous-alcoholic solution, of the lyophilized jelly from which the lipid fraction had already been removed, had, in previous research (Lercker *et al.* 1981*b*) led to an overall evaluation of total sugars.

According to the literature data, the use of chemical methods to determine the sugars—both before and after inversion of di-tri- and tetrasaccharides—produces a smaller quantity of total sugars.

In this work the identification and determination of a number of Royal Jelly sugars were carried out using HRGC.

MATERIALS AND METHODS

Royal Jelly samples used for the analytical reproducibility test were taken from a pool of artificially bred cells. The larval age ranged from 1 to 5 days after grafting.

The Royal Jelly samples collected to evaluate sugar composition variability were obtained, at the height of the active season (June–July), from artificially bred cells and were removed every 24 h from the first day after grafting until capping.

Each age group (five groups) was made up of seven or eight cells.

The age of larvae was determined at sight according to their size and position.

The neutral components were obtained (Capella & Losi, 1968) by treating 5 ml of a Royal Jelly suspension at 10 % concentration with 2·5 g of deionizing resin (Merck V).

After at least 1 h, 2 or 3 ml of the centrifugate solution were azeotropically distilled with 65 or 100 ml of absolute ethanol using a rotary evaporator ($T \leq 55\,°C$). The dry residue was dissolved in 5 ml of anhydrous pyridine; after at least 12 h 0·5 ml of pyridine–hexamethyldisilazane–trimethylchlorosilane (5:2:1 v/v/v) was added to 0·5 ml of solution.

After 15 min the solution was evaporated under a nitrogen stream ($T \simeq 100\,°C$), the residue dissolved in a small amount of benzene and then about 1–2 mg of the internal standard (methyl eicosenoate, *n*-C20: from 10 mg/ml benzene solution) added.

The hydrolysis of the neutral compounds mixture was carried out on 50 ml of the Royal Jelly suspension at 10 % concentration using 2 ml of hydrochloric acid, heating at 70 °C for 15 min (Metodi uff. di analisi per mosti, vini e aceti, Ministero Agr. e For., Roma, 1958).

A known amount of the 'inverted' solution was neutralized, then treated with deionizing resin following the procedure described above.

A C. Erba model 4160 instrument, equipped with a 10 m × 0·32 mm inside diameter glass capillary column, coated with 0·10–0·15 μ film thickness of SE 52 stationary phase was used for gas liquid chromatography (GLC). The sample was injected using the 'on-column system' (cold injector: Grob); the detector temperature was 350 °C and the oven temperature was programmed from 30 °C with a gradient of 11 °C/min. The carrier gas was helium with a flow rate of 2·5 ml/min. Quantitative

evaluation of the peak areas and the percentages were obtained using a Spectra Physics SP4100 integrator.

RESULT AND DISCUSSION

The method selected was originally used in the field of musts and wines (Capella & Losi, 1968) and, with a few modifications, could be perfectly well adapted to the substrate under examination.

The principle was based on contact (in an aqueous or weakly alcoholic solution containing about 1% sugars) with a mixed ion exchange (acid base) resin, for an appropriate amount of time. A known aliquot of solution was then dried in the 'rotavapor', using the azeotrope with absolute ethanol.

The residue was treated with pyridine and left long enough to be totally dissolved and subsequently silanized, and analyzed by GLC.

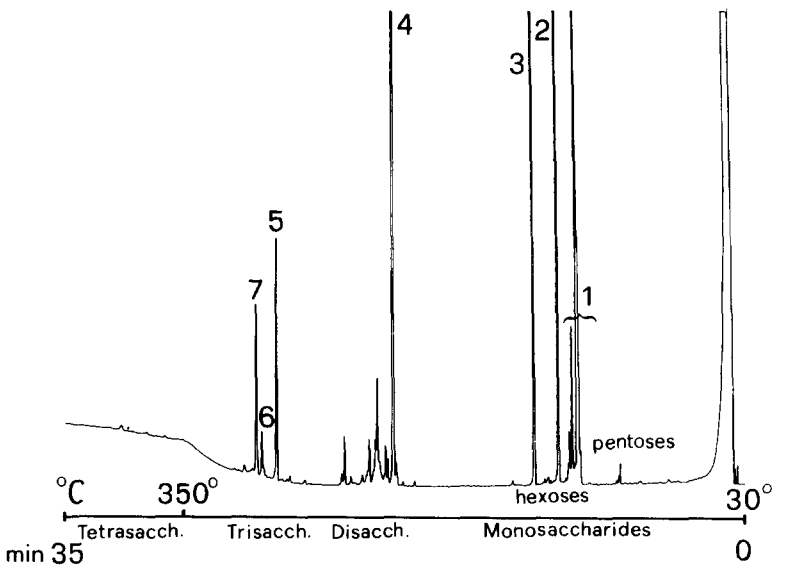

Fig. 1. Gas chromatographic (GLC) trace of the neutral components of Royal Jelly obtained after trimethylsilyl derivatisation, by apolar capillary column. Peaks: 1, fructose; 2, α-glucose; 3, β-glucose; 4, sucrose; 5, 6 and 7, unidentified components with hydrolyzable component. GLC conditions are reported in the text (see 'Materials and Methods' section).

The availability of capillary columns with thermostable stationary phases (and high resolution power) makes it possible to rapidly (using short columns) determine any sugars present up to a maximum of 24 carbon atoms. Under these analytical conditions, a practical example of which is given in Fig. 1, it is possible to check the nature of the substances eluted by comparing them to the corresponding standards.

In fact, with the method adopted, not only the sugars, but also all the neutral substances present, can be analyzed. In the case of Royal Jelly and also of honey, sugars are quantitatively predominant and neutral non-sugar substances represent only a small fraction of the total.

The high sensitivity of the analytical method allows the analysis of sugars with only a very little sample, e.g. worker bee food.

The peaks eluted after sucrose, in the area characteristic of disaccharides, belong to neutral non-sugars, except in the case of isomaltose, which was found to be present only in traces.

If hydrolysis (inversion) is carried out before the analysis, these gas chromatographic peaks are not eliminated: they are not sugar components. It emerges, however, that during the checking phase these substances act as a 'fingerprint' for the Royal Jelly.

All the substances eluted at the tri-saccharide level are hydrolyzable and are therefore potential sugars, but they have not yet been positively identified.

Measuring before and after hydrolysis and comparison with available standards did not reveal detectable amounts of maltose, melibiose, melezitose or trehalose.

TABLE 1

Reproducibility of the Proposed Method Applied to One sample of Royal Jelly. Values are the Means of Ten Entire Determinations: σ are the Standard Deviations.

Sugar	*Percent of sugars*		*Percent in the Royal Jelly (on a fresh matter basis)*	
	$\bar{X}$	σ	$\bar{X}$	σ
Fructose	36·0	4·5	1·45	0·18
Glucose	29·8	2·2	1·20	0·09
Sucrose	34·2	3·2	1·38	0·13
Total	100·0		4·03	

TABLE 2

Sugar Composition of the Royal Jelly Samples as a Function of the Larval Age (Identified Sugars and Hydrolyzable Neutral Components 5, 6 and 7)[a]

Larval age (*days after grafting*)	*1*		*2*		*3*		*4*		*5*	
	$\bar{X}$	δ	$\bar{X}$	δ	$\bar{X}$	δ	$\bar{X}$	δ	$\bar{X}$	δ
	%		%		%		%		%	
Total sugars	1·8	0·7	3·1	1·5	3·4	0·7	11·0	2·6	8·4	1·9
(*on a fresh matter basis*)	(0·7–2·8)		(2·0–5·2)		(2·5–4·7)		(6·4–15·1)		(6·2–10·8)	
Percent of total sugars	$\bar{X}$	δ	$\bar{X}$	δ	$\bar{X}$	δ	$\bar{X}$	δ	$\bar{X}$	δ
1 Fructose	21·8	6·6	23·1	7·3	40·8	2·8	39·9	3·3	41·5	4·5
	(10·4–32·0)		(14·2–33·9)		(38·4–46·4)		(36·2–45·6)		(33·7–46·2)	
2 + 3 Glucose	47·4	8·0	57·3	7·3	41·9	3·7	40·4	3·8	45·0	1·9
	(40·3–64·8)		(52·7–64·5)		(33·9–46·6)		(34·9–47·3)		(43·1–48·7)	
4 Sucrose	25·9	9·0	13·9	4·0	14·8	1·1	16·1	2·0	9·9	3·8
	(13·5–42·4)		(8·6–21·4)		(12·7–16·3)		(12·7–18·9)		(6·0–16·7)	
5[b]	2·6	1·0	3·0	0·5	1·2	0·4	1·5	0·7	1·2	0·5
	(0·9–3·6)		(2·3–3·9)		(0·7–1·6)		(0·8–1·8)		(0·6–1·8)	
6[b]	0·7	0·4	0·8	0·2	0·6	0·1	1·6	0·7	1·2	0·5
	(0·0–1·1)		(0·5–1·1)		(0·4–0·7)		(0·9–1·9)		(0·8–2·1)	
7[b]	1·7	1·2	2·0	1·0	1·0	0·5	0·9	0·7	1·4	1·0
	(0·0–3·2)		(0·4–3·4)		(0·3–1·6)		(0·1–2·0)		(0·0–3·2)	

[a] Average of 7–8 samples analyzed. $\bar{X}$ is the mean value (min–max), δ is the standard deviation.

[b] Constituent with retention time characteristic of trisaccharides, having a hydrolyzable component.

The method was tested on a Royal Jelly sample, which was particularly rich in sucrose, taking into consideration only the three main sugars, in order to estimate their respective reproducibility of determination (Table 1).

Since the results show that the compositions obtained have margins of difference greater than that of standard deviations and that it is therefore possible to quantify them, the method can clearly be used as planned.

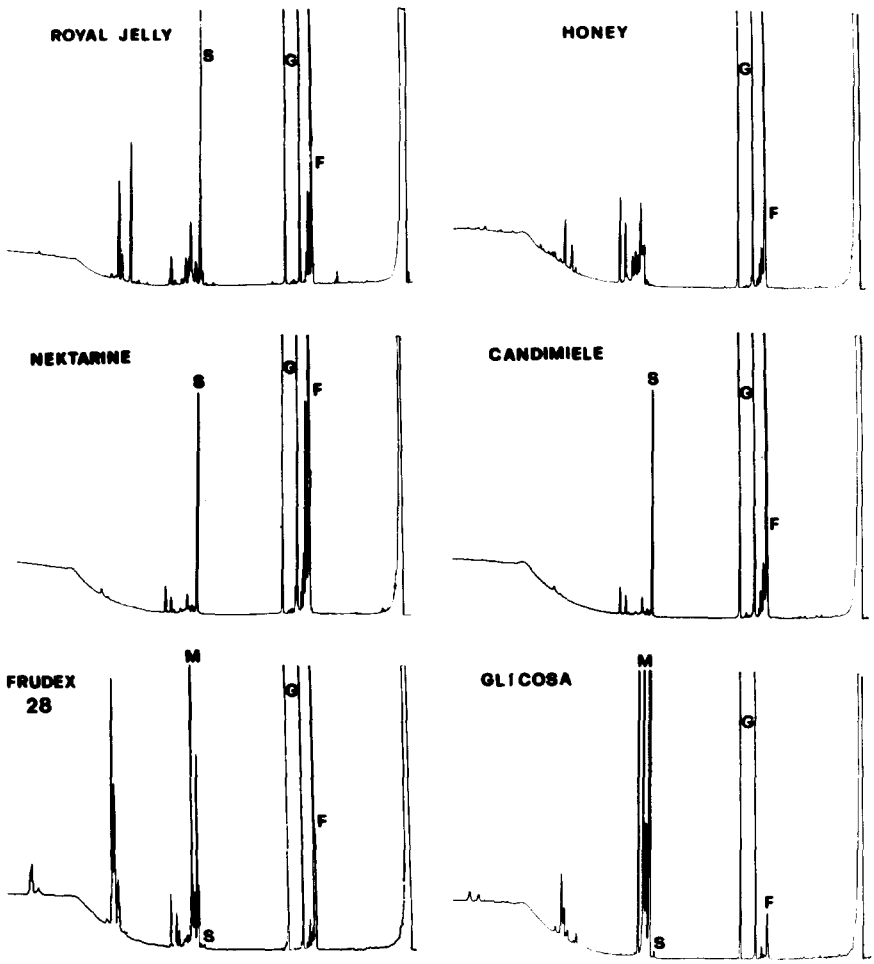

Fig. 2. GLC traces of the neutral components of a Royal Jelly, and chestnut honey samples and of some Italian commercial bee foods. Peaks: F, fructose; G, glucose (α and β); S, sucrose and M, maltose.

Analysis to determine the sugar composition of the Royal Jelly samples of cells containing larvae of known age (from 1 to 5 days after grafting) gave the results set out in Table 2.

It has been well known for a long time that the weight of Royal Jelly varies considerably from cell to cell and, under normal beekeeping conditions, this is probably related to the age differences of the larvae up to 24 h of age.

Since the amount and composition of Royal Jelly were found to be correlated to larval age (Table 2), it is to be hoped that the method described here could be used in testing commercial products.

Figure 2 shows the GLC graphs of the neutral components of some Italian commercial bee foods: it is possible to distinguish the examined samples by means of the profile of neutral compounds from both Royal Jelly and honey.

The characteristic and reproducible patterns of some gas chromatographic peaks of the commercial starch syrups are not present in the Royal Jelly, and vice versa, and so the detection of any adulteration is easy.

CONCLUSIONS

The method proposed for the analysis of Royal Jelly sugars by gas–liquid chromatography of neutral components appears satisfactory with regard to reproducibility of the data obtained.

The presence of non-sugar components in the GLC elution mixture is characteristic and is a useful 'fingerprint' for evaluating commercial foods.

The constancy of the sugar composition of Royal Jelly collected from cells from 3-day old larvae makes it possible to use the GLC sugar analysis for testing commercial Royal Jellies.

REFERENCES

Aeppler, C. W. (1922). Tremendous growth force. *Gleanings in Bee Culture*, **50**, 151–3.

Ammon, R. & Zoch, E. (1957). Zur Biochemie des Futtersaftes der Bienenkönigin, *Arzneimitt. Forsch.*, **7**, 699–702.

Asencot, M. & Lensky, Y. (1975). Des larves femelles d'abeille mellifère, nourries avec de la gelée d'ouvrières supplementée, naissent des reines adultes, *XXV Congrès International d'Apiculture*, Grenoble, Apimondia Bukarest.

Brouwers, E. V. M. (1984). Glucose/fructose ratio in the food of honeybee larvae during caste differentiation, *J. apic. Res.*, **23**(2), 94–101.

Capella, P. & Losi, G. (1968). Sulla determinazione degli zuccheri nei vini mediante gascromatografia. Nota 1, *Industrie Agrarie*, **6**, 7–13.

Christensen, G. M. (1962). Identification of sugars in royal jelly, *Nature*, **195**, 74.

Elser, E. (1929). Die chemische Zusammensetzung der Nahrungstoffe der Biene, *Markische Bienen Ztg.*, **19**, 210–15; 232–5; 248–52.

Gontarski, H. (1954). Untersuchungen über die Verwertung von pollen und Hefe zur Brutpflege der honigbiene, *Z. Bienenforsch.*, **2**(6), 161–80.

Hardak, M. H. (1943). Larval food and development of castes in the honeybee, *J. Econ. Entomol.*, **36**, 778–92.

Haydak, M. H. (1968). Nutrition des larves d'abeilles. In: *Traité de biologie de l'abeille*, Masson et Cie, Paris, 302–33.

Jung-Hoffman, I. (1968). Developpement des larves. In: *Traité de biologie de l'abeille*, Masson et Cie, Paris, 69–99.

Lercker, G., Capella, P., Conte, L. S., Ruini, F. & Giordani, G. (1981*a*). The components of royal jelly. Note I. Identification of the organic acids, *Lipids*, **16**, 912–19.

Lercker, G., Vecchi, M. A., Piana, L., Nanetti, A. & Sabatini, A. G. (1981*b*). Composition de la fraction lipidique de la gelée des larves d'abeilles reines et ouvrières (Apis mellifera ligustica Spinola) en fonction de l'âge des larves, *XXVIII International Beekeeping Congress*, Acapulco, Apimondia Bukarest.

Lercker, G., Capella, P., Conte, L. S., Ruini, F. & Giordani, G. (1982). Components of royal jelly. II. The lipid fraction, hydrocarbon and sterols, *J. apic. Res.*, **21**(3), 178–84.

Melampy, R. M. & Jones, D. B. (1939). Chemical composition and vitamins content of royal jelly, *Proc. Soc. Expt. Biol. and Med.*, **41**, 382–88.

Patel, N. G., Haydak, M. H. & Lovell, R. (1961). Chromatographic investigation of the methyl alcohol/chloroform soluble constituents of honey-bee larval food, *Nature*, **191**, 362–3.

Pourtallier, J., Taliercio, Y. and Mussot, J. M. (1970). Contrôle de la qualité de la gelée royale. Revue bibliographique des travaux sur la composition, les propriétés et l'utilisations de la gelée royale. *Bull. Apicole*, **2**, 145–60.

Rembold, H. (1965). Biologically active substances in Royal Jelly, *Vitamins and Hormones*, **23**, 359–82.

Rembold, H. (1967). Zur Biochemie der Königinnentstehung bei der Honigbiene, *XXI Int. Bienenz. Kongr.*, Maryland, Apimondia Bukarest.

Rembold, H. (1980). Der weiselfuttersaft. In *Königinnenzucht-biologische Grundlagen und technische Anleitungen*, cap 2, Apimondia Bukarest, 19–24.

Rembold, H. & Lackner, B. (1978). Vergleichende Analyse von Weiselfuttersaften, *Mitteilungen der Deutschen Geselleschaft für Allgemeine und Angewandte Entomologie*, **1**(2–4), 299–301.

Shuel, R. W. & Dixon, S. E. (1959). Studies in mode of action of royal jelly in honey-bee development. II. Respiration of newly emerged larvae on various substrates, *Canad. J. Zool.*, **37**, 803–13.

Siddiqui, I. R. & Furgala, B. (1965). Isolation and characterisation of disaccharides from royal jelly, *J. apic. Res.*, **4**(2), 89–94.

Simo, K. & Christensen, G. M. (1962). Quantitative analysis of sugars in royal jelly, *Nature*, **196**, 1208–9.

Takenaka, T. (1982). Chemical composition of royal jelly, *Honeybee Science*, **3**(2), 69–74.

Takenaka, T. & Takashi, E. (1980). General chemical composition of the royal jelly, *Bull. Fac. Agric., Tamagawa University*, **20**, 71–9.

Tourn, M. L., Lombard, A., Belliardo, F. & Buffa, M. (1980). Quantitative analysis of carbohydrates and organic acids in honeydew, honey and royal jelly by enzymic methods, *J. apic., Res.*, **19**(2), 144–6.

Townsend, G. F. & Lucas, C. C. (1940). The chemical nature of royal jelly, *Biochem. J.*, **34**, 1155–62.

Weiss, K. (1980). Uber die Entstehung der weiblichen kasten im Bienevolk, cap. 3, pp. 135–68; Der Einfluss von Aufzuchtfaktoren auf die Entwicklung der Königinnen, cap. 5, pp. 65–134; Der Umgang mit dem Zuchtstoff, cap. 6, pp. 135–68. In: *Koniginnenzucht-biologische Grundlagen und technische Anleitungen*, Apimondia Bukarest.