Amino acid analysis in royal jelly by HPLC

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Introduction

The "Service Central d'Analyse" (SCA) is a CNRS department. There were two principal activities at the food product laboratory : research and provision of services.
Royal jelly is the secretion of hypopharynged or brood food glands in the worker honey bee heads. Nowadays, there is a lot of foreign production of royal jelly, whose composition is unknown. The "Groupement des producteurs de gelée royale" (royal jelly producers) asked the SCA to analyze royal jelly, in order to determine the composition of the French royal jelly production. This article only deals with the identification and quantification of amino acids.
This analysis has never been done before. Our work was based on honey amino acid analysis. We used HPLC with a fluometric detector and a derivatization method. After the establishment of a method using standard amino acids, we further validated it and quantified royal jelly amino acids.

Experimental Conditions

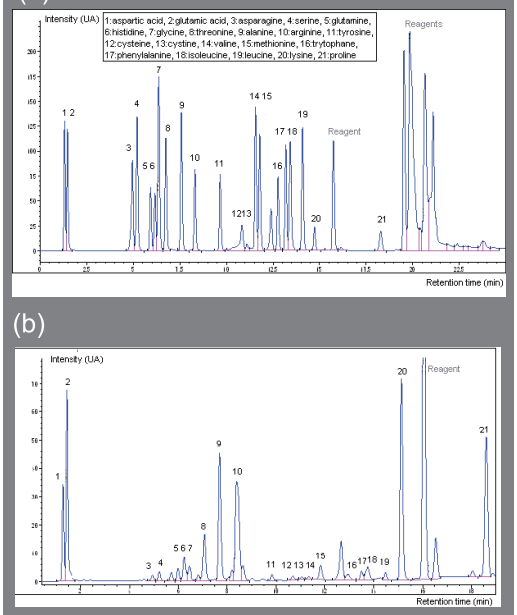
In order to quantify royal jelly amino acids, we used an HPLC Agilent 1200 series with fluometric detector. Amino acids were made fluorescent using a derivatization method with OPA, FMOC and borate buffer. The detector operated at two wavelengths :

	Excitation wavelength (nm)	Emission wavelength (nm)
Primary amino acid derivate with OPA :	340	450
Secondary amino acid derivate with FMOC :	266	305

We used a Hypersil ODS 200 x 2.1 mm; 5 µm column.
The oven was maintained at 40 °C.
There was a mobile phase gradient.Two phases were used: an aqueous phase (A) and a phase composed of acetonitrile and methanol (B).

Time (min)	A (%)	B (%)	Flow rate(ml/min)
0	100	0	0.45
17	40	60	0.45
18	0	100	0.70
25	0	100	0.70
25.1	100	0	0.45

We made a solution with 21 amino acids. Once derivatized, it was passed through a column.
To validate this method we studied: linearity, quantification and detection limit, specificity, repeatability and accuracy.
Once validated this method was used to analyse royal jelly samples. When the laboratory received royal jelly, a part of it was lyophilised. The lyophilised royal jelly (5mg) was dissolved by ultrasonication in ultra pure water for 5 minutes, then centrifugated 5 minutes. The supernatant was filtered on a PTFE 0.2 µm filter before analysation. A blank analysis with ethanol injection was used to clean the system before each series ran.



(a) Chromatogram of standard amino acids
(b) Chromatogram of royal jelly

Results and conclusion

The diagram (a) shows the chromatogram of the standard solution of 21 amino acids at 5ppm. The peaks were well separated and used to make an external calibration. The peaks at the end represent FMOC and OPA.
The chromatogram (b) is a royal jelly analysis. We can see small peaks representing amino acids in weak concentration. In royal jelly the major amino acids were: proline, lysine, arginine and glutamic acid.

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