Vitamins B_1, B_2, B_6 and PP contents in royal jelly

Vitaminas B_1, B_2, B_6 e PP em geleia real

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ABSTRACT

Hydrosoluble vitamins B_1, B_2, B_6 and PP are essential organic substances for human organism, functioning as coenzymes on several metabolic cycles. In the present investigation four vitamins of B complex and its vitamers contents were determined in royal jelly samples marketed in São Paulo, Brazil. A single extraction process was employed, and each vitamin was determined by HPLC using C18 column and detected by fluorescence. Four samples from different suppliers were analyzed, and the results varied from 0.08 to 0.41 mg/100g (vitamin B_1 or thiamine); from 0.01 to 0.05 mg/100g (vitamin B_2 or riboflavin); from 0.13 to 0.38 mg/100g (piridoxal - vitamin B_6); from 0.26 to 1.38 mg/100g (piridoxamine - vitamin B_6); from 0.21 to 0.57 mg/100g (niacin - vitamin PP); and from 1.56 to 2.00 mg/100g (niacinamide - vitamin PP). These data show that royal jelly is not an important source of the analyzed vitamins, though the results indicate that the employed technique is suitable for determining these four vitamins and its vitamers.

Key words. royal jelly, vitamins, HPLC.

RESUMO

As vitaminas hidrossolúveis B_1, B_2, B_6 e PP são importantes substâncias para o organismo humano, que atuam como coenzimas em diversos ciclos metabólicos. No presente trabalho, foram determinados os teores de quatro vitaminas do complexo B e de seus vitâmeros em amostras de geleia real comercializadas no Estado de São Paulo. Foi utilizado um único processo de extração e a determinação de cada vitamina foi realizada por CLAE utilizando-se coluna C18 e a detecção foi feita por fluorescência. Quatro amostras de diferentes fornecedores foram analisadas e os resultados variaram de 0,08 a 0,41 mg/100g (vitamina B_1 ou tiamina); de 0,01 a 0,05 mg/100g (vitamina B_2 ou riboflavina); de 0,13 a 0,38 mg/100g (piridoxal – vitamina B_6); de 0,26 a 1,38 mg/100g (piridoxamina - vitamina B_6); de 0,21 a 0,57 mg/100g (niacina – vitamina PP) e de 1,56 a 2,00 mg/100g (niacinamida - vitamina PP). Conclui-se que a geleia real não é fonte importante dessas vitaminas, mas verifica-se que a técnica utilizada é adequada para efetuar as determinações das quatro vitaminas analisadas e de seus vitâmeros.

Palavras-chave. geleia real, vitaminas, tiamina, riboflavina, piridoxol, niacina.

INTRODUÇÃO

Vitamins are essential organic compounds for human metabolism. They are in most of the foods in low concentrations. Several compounds have vitamin activity and can be divided in two groups according to their solubility: liposoluble vitamins (A, D, E and K) and water-soluble vitamins (C, B_1, B_2, B_5 or panthotenic acid, B_6, B_{12}, PP ou niacin or B_3, folic acid, biotin, inositol and choline). The biological activity of each vitamin can be due to one or more compounds called vitamers[1, 2].

Vitamin B_1 or thiamine was the first compound to be called vitamin at the beginning of 20th century and it was related to beriberi disease. Riboflavin or vitamin B_2 was firstly identified as a resistant factor to high temperature present in yeast extract. Three different compounds are called vitamin B_2: riboflavin, flavin mononucleotide or riboflavin 5-phosohate and flavin adenine dinucleotídeo or riboflavin 5'-adenosilphosphate[2,3,4].

Vitamin B_6 is a group of six compounds (pyridoxol and pyridoxol phosphate, pyridoxal and pyridoxal phosphate, pyridoxamine and pyridoxamine phosphate) that acts as coenzymes in fatty acids and lipids metabolism. Vitamin PP has two active forms, niacin and niacinamide, that are part of the coenzymes nicotinamide adenin dinucleotide (NAD) and nicotinamide adenine dinucleotídeo phosphate (NADP)[5,6,7,8,9]. These vitamins are wide spread throughout the food, as flour, milk and dairy products. Some bee products are known to have high vitamin content, mainly royal jelly (RJ).

RJ is a honeybee's secretion from hypopharyngeal and madibular glands used for nutrition of honeybee workers, drones and queens, although only the queen receives RJ throughout its life[10].

For the importance of vitamins in nutrition and the lack of knowledge about vitamin content in many foods, it is necessary to have reliable data obtained through validated methods.

The official methods for water soluble vitamins are microbiological or spectrophotometric ones, both time consuming and difficult to perform. Nowadays, chromatographic methods have been developed for vitamins B_1, B_2, B_6 and PP determination. The procedure described by Moreschi[11] has a common extraction procedure followed by specific chromatographic determination to identify and quantify the different vitamers of these 4 vitamins (riboflavin, thiamine, pyridoxol, pyridoxal, pyridoxamine, niacin and niacinamide) and it was applied to determine these vitamins in RJ.

The objective of this work is to have preliminary data regarding B vitamins (B_1, B_2, B_6 and PP) in commercial samples of RJ from São Paulo and also to contribute to food composition databases.

MATERIAL AND METHODS

▪ Material

Analytical standards of thiamine mononitrate, riboflavin, pyridoxol hydrochloride, pyridoxal hydrochloride, pyridoxamine dihidrochloride, niacin and niacinamide from Merck were used.

The stock solution of vitamin B_1 (thiamine mononitrate) was prepared by dissolving 25 mg of the component in a volumetric flask of 500 mL of hydrochloric acid 0,1M.

Riboflavin (vitamin B_2) was weighed (25 mg) in an erlenmeyer and 1.2 mL of acetic acid and about 50 mL of deminarilized water was added; this solution was heated to boiling, cooled to room temperature and transferred to 500 mL volumetric flask and completed to mark with deionized water.

Niacin and niacinamide stock solutions were prepared by dissolving 25 mg of each vitamin in a 250 mL volumetric flask with deionized water.

Vitamin B_6 (pyridoxal, pyridoxol and pyridoxamine) standard solutions were prepared by dissolving 10 mg of each component in a 1000 mL volumetric flask with deionized water. These solutions were kept in dark glass volumetric flasks at 4°C in refrigerator for 1 month. Working solutions were prepared by dilution daily.

Mobile phase for vitamins B_1 and B_2 was prepared by dissolving 0.95g de hexanesulfonic (PIC-6) in 900 mL of deionized water, 95 mL of acetonitrile (HPLC grade) and 0.5 mL of ammonium hydroxide. The pH of this solution was adjusted to 3.6 with phosphoric acid and then 70 mL of deionized water and 22 mL of acetonitrile was added. The post-column reagent for vitamin B_1 determination is obtained by mixing 60 mL of 1% potassium ferricyanide solution and 440 mL of 15% sodium hydroxide solution.

Vitamin B_6 mobile phase was obtained by dissolving 6.80 g of potassium dihidrogenophosphate and 0.14g of heptanosulfonic sodium salt (PIC-7) in 500 mL of deionized water. The pH of this solution was adjusted

with phosphoric acid to 2.5. After the pH adjusted, the solution was transferred to 1000mL volumetric flask and made up to the mark with deionized water.

The mobile phase for vitamin PP determination was prepared by dissolving 9.54g of potassium dihidrogenophosphate in 500 mL of deionized water and adding 7.6 mL of peroxide of hydrogenium and 1.0 mL of copper sulfate solution 5mM. The volume was adjusted to 1000 mL with deionized water.

The reagents used to prepare the mobile phases were analytical grade and the solvents, HPLC grades. All the mobile phases, standards and sample solutions were filtered through 0.45 µm filter prior to injection in the chromatographic system.

The reagents used during the extraction procedures were hydrochloric acid 0.1 M and sodium acetate 2.5M prepared using analytical grades reagents.

Few trademarks of RJ are found in the market, most of them are from China. It was chosen two frozen samples of the same trade market, one sample (lyophilized) from other trademark and one sample acquired direct from local producer. All samples were kept frozen until the analyses were performed.

METHODS

▪ Instrumentation

Chromatographic systems (Waters®) were composed by isocratic pump (model 510), automatic injector (model WISP 717+) and fluorescence detector (model 474). For vitamin B_1 determination it was used a post column reagent pump (Water®) and for vitamin PP the post column reaction was performed under a UV radiation from a black light as described in Lahély et al.[12].

The fluorescence detector was adjusted at excitation wavelength (λ_{exc}) 360 nm and emission wavelength (λ_{em}) 435 nm for vitamin B_1. For vitamin B_2 determination it was used the λ_{exc} = 450 nm and λ_{em} = 530 nm, vitamin B_6 used λ_{exc} = 296nm and λ_{em} = 390nm and for vitamin PP the wavelength adjustment was λ_{exc} = 322 nm and λ_{em} = 380 nm.

Vitamin B_6 mobile phase flow was set up at 0.6 mL/min and the others vitamins were analyzed using a flow rate of 1.0 mL/min. The injection volume for all determinations was 20 µL.

Chromatographic columns were all C_{18} spherical, 5µm particle size and the length varying according to the vitamin. For vitamins B_1 and B_2 the column length was

Table 1. Analytical conditions for vitamins B1, B2, B6 and PP determination

	Vitamin B_1	Vitamin B_2	Vitamin B_6	Vitamin PP
Column	Lichrospher C18, 5µm, 125 mm		Lichrsospher C18 endcapped, 5µm, 250 mm	Superspher C18, 5µm, 250 mm
Mobile Phase	PIC-6; acetonitrile; ammonium hydroxide - pH 3.6		Potassium dihidrogenophosphate; PIC-7; acetonitrile - pH 2.5.	potassium dihidrogenophosphate; peroxide of hydrogenium; copper sulfate
Flow rate	1,0 mL/min		0,6 mL/min	1,0 mL/min
Injection volume	20 µL			
Wavelength	λ_{exc} - 360 nm λ_{em} - 435 nm	λ_{exc} - 450 nm λ_{em} - 530 nm	λ_{exc} - 296 nm λ_{em} - 390nm	λ_{exc} - 322 nm λ_{em} - 380 nm

Lichrospher C18, 5µm, 125 mm; for vitamin B_6 it was used a Lichrsospher C18 endcapped, 5µm, 250 mm and for vitamin PP a Superspher C18, 5µm, 250 mm. All the columns were acquired from Merck.

- **Procedure**

After the samples reach the room temperature, 5g were weighed in a dark glass 125 mL erlenmeyer. Add 50 mL of hydrochloric acid and take the erlenmeyer in a water bath with boiling water for 30 minutes. After this period, the samples are taken from the water bath, cooled to room temperature and adjusted the pH solution to 4.6 with sodium acetate 2.5 M. The solution is transferred to brown 100 mL volumetric flask and maked up to the mark with deionized water. The extract was filtered through analytical paper filter and then through 0.45 µm filter into chromatographic vials.

The standard curves were prepared for vitamins B_1, B_2, B_6 (hydroxol hydrochloride) and PP (niacin and niacinamide) with concentration from 0.1 to 2.0 µg/mL; vitamin B_6 (hydroxal hydrochloride and hidroxamine dihidrochloride) from 0.05 to 1.0 µg/mL. Dilution was performed with Milli-Q water.

The summary of the analytical conditions are presented in Table 1.

RESULTS AND DISCUSSION

The performance characteristics for this method (linearity, precision, trueness, limit of detection and quantitation, specificity and rudgeness) were determined previously[11]. To verify the method performance for royal jelly samples, it was evaluated precision under repeatability conditions and trueness by recovery of added standards in the sample just after the weighing. The amount added for each vitamin was equivalent to about 0.80 mg/100g for vitamin B_1, B_2 and pyridoxol, 0.40 mg/100g for pyridoxal and piridoxamine and about 4.0 mg/100g for both vitamers of vitamin PP.

The recoveries varied depending on the vitamers analyzed from 79 to 112% as showed in Table 2.

The average recoveries and the standard deviation obtained for the vitamins showed that the method performance is in line with the vitamins methods in food in the literature.

As the method performance showed adequate for the determination, analyses were done using the samples collected at local markets. Results obtained are showed in Table 3.

Detection and quantitation limit for the method was calculated based on signal/noise procedure as described by Moreschi[11].

The results showed that the vitamin content varies within the samples, probably due to different sources of the royal jelly and no vitamin B_2 was found. The vitamin B_6 was presented in the forms of pyridoxal and piridoxamine.

When comparing these values to those found in literature[4,8,9,10], vitamin B_1 is within the limits of the literature (0.14 – 0.67 mg/100g) as the vitamin B_6 (0.10 – 0.48 mg/100g). The values from the literature for vitamin PP (4.8 – 8.8 mg/100g) are higher than the obtained in this paper as well the vitamin B_2 content in literature reaches 2.5 mg/100g, much higher than the results presented here. It is possible to have results comparable to the literature ones if a bigger and more representative sampling is conducted latter.

Table 2. Recoveries of added standard for vitamers of vitamins B1, B2, B6 and PP

	Vitamin B_1 (*)	Vitamin B_2 (*)	Vitamin B_6			Vitamin PP (***)	
	Thiamin and mononitrate	Riboflavin	Hydroxol hydrochloride (*)	Hydroxal hydrochloride (**)	Hydroxamine dihidrochloride (**)	Niacin	Niacinamide
Average (%)	84	84	110	103	105	89	92
SD (%)	4	2	0.8	3	4	2	3

Average and standard deviation (SD) from 6 determinations for each vitamer.
(*) addition of about 0.80 mg/100g.
(**) addition of about 0.40 mg/100g.
(***) addition of about 4.0 mg/100g.

Table 3. Results of vitamins from different brands of royal jelly obtained from local market

Brand	Vitamin B_1	Vitamin B_2	Vitamin B_6			Vitamin PP	
	Thiamine	Riboflavin	Hydroxol	Hydroxal	Hydroxamine	Niacin	Niacinamide
A	< 0.15(**)	< 0.02 (**)	< 0.02 (**)	0.17 ± 0.02	0.29 ± 0.04	0.51 ± 0.04	1.91 ± 0.07
B	0.22 ± 0.01	< 0.02 (**)	< 0.02 (**)	0.32 ± 0.01	0.38 ± 0.01	0.39 ± 0.01	3.4 ± 0.1
C	0.41 ± 0.02	< 0.02 (**)	< 0.02 (**)	0.34 ± 0.03	1.40 ± 0.02	0.22 ± 0.01	1.6 ± 0.1
D (*)	0.26 ± 0.06	< 0.02 (**)	< 0.02 (**)	1.25 ± 0.05	0.89 ± 0.02	1.1± 0.1	7.8 ± 0.2

(*) Lyophilized royal jelly
(**) Quantitation limit of the method
Results expressed in mg/100g
Results are average and standard deviation of 3 determinations.

Vitamin B_1, B_6 and PP content is very low compared to Brazilian RDI for adults (1.3 mg/100g for vitamins B1 and B6 and 16 mg/100g for vitamin PP)[13].

CONCLUSION

Vitamin content in royal jelly was very low for most of vitamins analyzed and is below the limit of quantitation of the method for vitamin B_2.

Considering that royal jelly is a dietary supplement, the amount in the normal diet is very small so the vitamin intake from this supplement is not important.

Although the amount of vitamins found in the samples analyzed, the method showed to be adequate for the determination of vitamins B_1, B_2, B_6 and PP in royal jelly. Maybe more analyses should be carried out to determine the variation of the vitamins content in royal jelly coming from different bees and different regions.

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