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A Study of Sugars in Honey

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Abstract: Honey is an excellent nutritious food and is widely produced and marketed. However, there have been complaints that some marketed honey is adulterated with maltose. Researcher quantified glucose, fructose, maltose and sucrose contained in honey products in the marketplace and showed that some had been adulterated.

Key words: Honey, sugars, high-performance liquid chromatography, nutritious, food, Japan

INTRODUCTION

Honey bees collect honeydew from flowers and store it in their nest but when they collect honeydew, they add invertase which decomposes the honeydew in their bodies. For this reason, the composition of honey, initially consisting primarily of sucrose, gradually changes in the nest. The nest of honeybees is always maintained at about 35°C due to the metabolic heat of bees and is continuously ventilated by the fanning behavior of worker drones. This promotes the evaporation of water and condenses honey to a sugar content of nearly 80% (Hayashi *et al*., 1966). It is highly nutritious as it contains isomaltooligosaccharide, gluconolactone, various vitamins, minerals and amino acids as well as glucose and fructose, with a heat quantity of 12.307 kJ g^{-1} (2.94 kcal) (Aso *et al*., 1958). Some honey products marketed in Japan are suspected to be adulterated with maltose to increase the sweetness and organizations such as the Consumer Affairs Bureau are requested to analyze them (Mishima *et al*., 2003; Okazaki, 1970).

The researchers investigated whether honey samples had been adulterated with maltose by quantifying their glucose, fructose, maltose and sucrose contents using the pre-column derivatization method which was reported previously (Fujita and Tobino, 2011).

MATERIALS AND METHODS

Reagents: Ethyl ester of aminobenzoic acid, phosphoric acid, acetic acid, phenylhydrazine (Wako Pure Chemical Industries) and sodium cyanoborohydrate (Nacalai Tesque).

Machine: Shimadzu LC20A system-PDA and RF.

Sample: Commercially distributed honey products made in Japan were purchased as samples (pure honey).

Preparation of samples: Exactly 0.1 g of honey was weighed and dissolved with 100 mL of pure water.

Derivatization of glucose and maltose: To 5 mL of the water solution of honey, 400 μL of 1.4 M sodium cyanoborohydrate solution in water, 400 μL of acetic acid and 2 mL of 0.6 M ethyl ester aminobenzoic acid (Methanol) were added and the mixture was heated at 80°C for 10 min. After it had been cooled to room temperature, 2 mL of distilled water was added. The water phase was washed with 4 mL of chloroform to remove ethyl ester of aminobenzoic acid from the water phase and the water phase was applied to HPLC.

Conditions of chromatography of glucose and maltose:

- Column: Cosmosil 3×100 mm; 3 μm (Nacalic tesques)
- Mobile phase: Acetonitrile/methanol (1:1) 0.5% acetic acid = 3:7
- Flow rate: 0.2 mL min^{-1}
- Column temperature: 45°C
- UV: 307 nm

Derivatization of fructose and sucrose: To 1 mL of the water solution of honey, 1 mL of hydrazine solution (Phosphoric acid, acetic acid, phenylhydrazine = 110:90:3) was added. The mixture was allowed to react at 150°C for 10 min, cooled to room temperature and applied to HPLC.

Chromatographic conditions of fructose and sucrose:

- Column: Intersil pH-3; 4.6×150 mm; 5 μm (GL sciences)
- Mobile phase: Acetonitrile/methanol (1:1) water = 35:65
- Flow rate: 1.0 mL min^{-1}
- Column temperature: 45°C
- Fluorescence: Excitation at 330 nm, emission at 470 nm

RESULTS AND DISCUSSION

The purchase of 100 samples (honey), the sample was quantified sugars arbitrarily choose the 5 samples. Table 1 shows the results of analysis of the 5 samples. Commercially distributed honey products made in Japan were purchased as samples (pure honey). In 4 samples, glucose and fructose together accounted for nearly 80% of the sugar content and no adulteration with maltose to increase the sweetness was observed.

In the remaining one sample, maltose was detected at about 17 g/100 g. If sucrose had been contained, there is the possibility that its degradation into glucose and fructose had not been sufficiently advanced but maltose was clearly an additive (Mishima *et al*., 2003; Okazaki, 1970).

Researcher reported to the Consumer Affairs Bureau. Sugar composition honey a, b, d and e is similar to the results of Okazaki (1970) was measured by AOAC method.

As a result of post-column and RI detector is the correlation was high. Analytical method that can be accurately quantified in the laboratory there is only a simple machine such as the Consumer Affairs Bureau as evidenced by the previous report. This study confirmed that maltose is added to some honey products marketed in Japan.

The calibration curve of 5 points (UV and fluorescence) was the first regression line. As for r = 0.9999 was obtained (1, 10, 100, 500 and 1000 mg L^{-1}). This time, the method to determine the developed sugar was the fixed limit of the quantification value of 0.1 mg/100 g.

Table 1: Quantification of sugars in honey

Honey composition	Glucose (g/100 g)	Fructose (g/100 g)	Maltose (g/100 g)	Sucrose (g/100 g)
a	33.104±0.003	35.721±0.005	3.213±0.000	0.313±0.0
b	35.104±0.003	33.721±0.005	3.253±0.007	0.335±0.0
c	27.610±0.002	23.881±0.006	17.277±0.000	0.327±0.0
d	31.243±0.004	35.412±0.001	3.219±0.000	0.319±0.0
e	35.104±0.003	33.721±0.005	3.237±0.000	0.317±0.0

Table 2: Recoveries of glucose fructose, maltose and sucrose

Substances	Trials	Added (mg)	Recovery (%)
Glucose	5	100	98.7
		10	98.3
		1	99.2
Fructose	5	100	98.4
		10	97.5
		1	99.5
Maltose	5	100	97.7
		10	99.8
		1	98.8
Sucrose	5	100	99.4
		10	98.6
		1	97.7

The results of addition-recovery (Honey) experiments (1, 10 and 100 mg) of glucose, maltose, fructose and sucrose are shown in Table 2. The recovery rate was as high as 90%. The precision of quantification was marked.

CONCLUSION

It is observed that by using cheap (machinery cheaper than MS) and simple (no complicated than post-column) method (Fujita and Tobino, 2011) for the determination we have developed, it can be proved that the honey has been sweetened due to maltose is present.

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