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Determination of Maltose in Honey

Research Article

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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. We quantified glucose, fructose, maltose and sucrose contained in honey products in the marketplace, and showed that some had been adulterated.

Keywords: Honey, Sugars, High-Performance Liquid Chromatography

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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%.[1] 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 (2.94 kcal) per gram.[2] 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.[3,4,5] The author 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.[6,7]

Methods and Reagents

Reagents

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

Machine

Shimadzu LC20A-PAD and RF

Sample

Commercially distributed honey products made in Japan were purchased as samples.

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 minutes. 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 3µm
Mobile phase: Acetonitrile/methanol (1:1) : 0.5% acetic acid = 3:7
Flow rate: 0.2 ml/min
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 minutes, cooled to room temperature, and applied to HPLC.

Chromatographic conditions of fructose and sucrose

Column: Intersil Ph-3 4.6×150 mm 5μm
Mobile phase: Acetonitrile/methanol (1:1) : water = 35:65
Flow rate: 1.0 ml/min
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. 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 1 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[3,4,5] . 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. Our study confirmed that maltose is added to some honey products marketed in Japan. The calibration curve of five points (UV and fluorescence) was the first regression line. As for r, 0.9999 was obtained (1, 10, 100, 500, and 1000,mg/L). This time, the method to determine the developed sugar was the fixed limit of the quantification value of 0.1 mg/dl. The results of addition-recovery (honey) experiments (1, 10 and 100 mg) of

Table 1: Quantification of sugars in honey

	Glucose (g/dl)	Fructose (g/dl)	Maltose (g/dl)	Sucrose (g/dl)
Honey a	33.104±0.003	35.721±0.005	0.000±0.000	0.000±0.000
Honey b	27.610±0.002	23.881±0.006	17.153±0.007	0.000±0.0 00
Honey c	35.104±0.003	33.721±0.005	0.000±0.000	0.000±0.000
Honey d	31.243±0.004	35.412±0.001	0.000±0.000	0.000±0.000
Honey e	35.104±0.003	33.721±0.005	0.000±0.000	0.000±0.000

Table 2: Recoveries of glucose fructose, maltose, and sucrose

Substance	Trials	Added	Recovery (%)
Glucose	5	100 mg	98.5
		10 mg	98.2
		1 mg	99.1
Fructose	5	100 mg	98.3
		10 mg	97.4
		1 mg	99.2
Maltose	5	100 mg	97.9
		10 mg	99.6
		1 mg	98.9
Sucrose	5	100 mg	99.1
		10 mg	98.4
		1 mg	97.3

glucose, maltose, fructose, and sucrose are shown in Table 2. The recovery rate was as high as 90%. The precision of quantification was marked.

By using cheap (machinery cheaper than MS) and simple(no complicated than post-column) method[6,7] for the determination we have developed, we can prove that honey that has been sweetened of maltose is present.

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