Determination of invertase in honey by immunoassay

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Abstract

Invertase (β-fructofuranosidase; EC 3.2.1.26) is an enzyme that catalyzes the hydrolysis of sucrose into glucose and fructose. Since invertase is produced from bees to make honey from nectar, certain levels of invertase can be found in honey. Invertase derived from yeast is used to make inverted sugar syrup from sugar (sucrose). It has been suspected that high levels of invertase in honey may be caused by the addition of invertase with sucrose to make adulterated honey products. A competitive immunoassay was developed for the quantitative determination of invertase in honey. Limit of detection was 0.1ppm with the detection range of 0.1 to 10ppm, and total assay time was 2 hours. Invertase (1ppm) was spiked in honey and water. Since honey products are heat treated by manufacturer before export, samples were treated by boiling for 1, 2 and 3 hours. Control was non-heat treated. The recoveries from heat treated honey samples for 1, 2 and 3 hours were 76, 69 and 68%, respectively. The recoveries from heat treated water samples for 2 and 3 hours were 109 and 104%, respectively. Non-heat treated honey and water samples had recoveries of 70 and 125%, respectively. Heating does not decrease the amount of invertase from honey and spiked water samples after 3 hours boiling. This indicates that the antibody used for the immunoassay can bind to both active and deactivated invertase. Twenty one honey samples were tested (11 domestic and 10 imported). The average levels of invertase from domestic and imported honeys were 18.7 and 48.5ppm, respectively.

Introduction

The amount of honey consumed in the U.S. is 400 million lbs. a year. Only 48% of the demand is supplied by U.S. beekeepers. The rest is imported from other countries in Latin America and Asia (Phipps, 2012 and Time Magazine, 2011). Adulteration of honey has been a problem for many years. A simple way to make an adulterated honey is adding a cheap sweetener such as sucrose, corn syrup, or inverted sugar (Time Magazine, 2011). Inverted sugar is a mixture of glucose and fructose obtained by hydrolysis of sucrose by acidic and enzymatic treatment. Since the composition and properties of honey are nearly the same as inverted sugar, adding inverted sugar to honey could be an attractive way to make an adulterated honey. However, inverted sugar usually contains invertase that was added to catalyze the hydrolysis of sucrose into glucose and fructose. Therefore, an adulterated honey produced by inverted sugar may contain more invertase than an authentic honey. Enzymes in honey have been studied to find if they can be used to distinguish between natural and adulterated honeys (White, 1975). However, more studies have been conducted to measure the activities of diastase and invertase in honey to determine its freshness as their activities are decreased during storage or by heat treatment (Sanchez et al., 2001). Honey imported from China is regularly treated with heat to reduce the water content to prevent spoilage. Therefore, it is necessary for an immunoassay to detect the concentration of invertase instead of determining its activity. A competitive immunoassay was developed for the quantitative determination of invertase in honey. The study shows that this immunoassay can detect both active and deactivated invertase.

Figure 1. Domestic and imported honey products.

Methodology

Polyclonal antibody production:
In the development of this assay, invertase was coupled to a modified protein carrier, which served as an immunogen. The antibody was produced through the immunization of New Zealand white rabbits. These rabbits were shaved and injections were administered intradermally on the back. The serum was collected monthly.

Sample preparation:
Honey products (1 g) were measured in a 50 mL centrifuge tube and 25 mL of PBS buffer was added to dissolve honey completely. Further dilution with PBS will be needed if the OD value is below the last calibrator (10 ppm).

Assay protocol:
Antibody solution (100 µL) and calibrator (or sample extract; 100 µL) were added in a mixing well. They were incubated for 30 minutes at room temperature. A hundred micro liter (100 µL) of the incubated solution was transferred to an invertase coated plate for a second incubation of 15 minutes. The plate was washed with deionized water 4 times. GAR-HRP (Goat Anti Rabbit- Horse Radish Peroxidase) conjugate was added and incubated for 30 minutes. It was washed with deionized water 4 times. Substrate (100 µL) was added to the wells and incubated for 30 minutes. The reaction was stopped with 100 µL of stop solution and the color intensity of the wells were read at 450nm-650nm.

4-parameter curve fit: Y = (A – D)/(1 + (X/C)^B) + D

A	B	C	D	R^2
1.95	0.945	0.537	0.416	0.999

IC 50: 1 ppm

Figure. 2. Standard curve.

Table 1. Invertase calibrators and inhibition ratios.

Calibrator (ppm)	%B0
0	
0.1	87
0.5	61
1	51
5	30
10	26

Results

Table 2. Recoveries of invertase from honey and water

Heating (hr)	Honey	Water
Zero	70%	125%
1	76%	-*
2	69%	109%
3	68%	104%

*Sample was spilt before test.

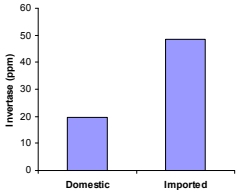

Figure. 3.
Average invertase contents in domestic and imported honeys.

Table 3. Invertase contents.

Domestic (ppm)	Imported (ppm)
9	12
11	18
12	23
12	33
12	41
12	44
17	49
18	57
21	94
26	114
56	-
Average = 18.7	Average = 48.5

*Values are in ascending order.

Discussion

There are several commercial assays for invertase in honey or soil samples. However, they determine the activity of invertase by addition of sucrose during the assay. Therefore, it is impossible to determine the concentration of inactivated invertase. For example, if a honey product contained added invertase and was heat treated, the presence of invertase would not be detected as there is no invertase activity. Due to this reason, those assays can not be used to determine if a honey product was adulterated by addition of invertase and sucrose to make a cheap honey. A competitive immunoassay was developed for the detection of invertase in honey. Antigen (invertase) was coated on the microtiter plate which competes with invertase from the sample or calibrator to bind to anti-invertase polyclonal antibody. The detection limit of the immunoassay is 0.1 ppm in PBS buffer and the linear range of the calibration curve is between 0.1 and 10 ppm. The detection range of invertase in honey samples is from 1 to 100 ppm. Due to the viscosity of honey, it should be diluted with PBS of at least 10 times the volume. However, there are no further steps required for sample preparation such as filtration or centrifugation. This immunoassay was successfully tested and evaluated to see if it could determine both active and inactive invertases. Invertase was spiked in honey and water samples, and boiled for 1, 2, or 3 hours. A significant decrease of invertase due to boiling was not observed. Invertase levels were not significantly changed from both honey and water matrices (Table 2). These results indicate that both active and deactivated invertase can be recognized by the antibody. There was some matrix effect from honey samples that decreased the recovery when spiked with 1 ppm of invertase. High levels of sugar and/or other enzymes in honey may cause this interference. Eleven domestic and 10 imported honeys were tested to determine invertase levels. The highest levels of invertase from both products were 56 and 114 ppm, respectively. Average invertase levels from both domestic and imported were 18.7 and 48.5 ppm (Table 3 and Figure 3). The high levels of invertase within imported honeys had almost 10 times more invertase than some domestic honeys. This may indicate possible adulteration of imported honeys by addition of invertase and sucrose. A competitive immunoassay developed by Beacon Analytical Systems could be used to determine the amount of both active and deactivated invertase in honey, and distinguish between authentic honey and adulterated honey by addition of invertase.

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