The Enzymes of Honey

Introduction

Enzymes are complex proteins formed in living cells that bring about the many processes and reactions in living materials. Honey naturally contains small amounts of enzymes. The predominant enzymes in honey are diastase (amylase), invertase (α-glucosidase) and glucose oxidase. Others, including catalase and acid phosphatase, can also be present. Honey's enzyme content can vary widely by floral source and region.[1]

Table 1: Honey Enzymes

Common names	Chemical reactions catalyzed
• Diastase, Amylase	• transforms starch to other carbohydrates (dextrins, oligo-, di- and monosaccharides)
• Invertase , Sucrase, Sucrose Hydrolase, Saccharase	• converts sucrose to glucose and fructose (invert sugar)
• Glucose Oxidase	• converts glucose to gluconolactone, which in turn yields gluconic acid and hydrogen peroxide.
• Catalase	• converts peroxide to water and oxygen
• Acid Phosphatase	• removes phosphate from organic phosphates
• Protease	• hydrolyzes proteins and polypeptides to yield peptides of lower molecular weight
• Esterase	• breaks down ester bonds
• β-glucosidase[1]	• converts β-glucans to oligosaccharides and glucose (β bonds)

Enzymes play an important role in honey and contribute to its functional properties. Honey's enzymes make it a unique ingredient, far more complex than other sweeteners. Table 1 presents an overview of honey enzymes and their functions.

Enzymes Not Effective as a Quality Indicator[2]

In the past, the diastase content (more accurately identified as amylase) was used as an indicator of honey quality. The rationale was that honey, when heated, would show a decrease in the diastase number (DN) which would indicate that the honey had been subjected to excessive heat during processing.

Likewise, heating honey causes an increase in hydroxymethylfurfuraldehyde (HMF) content. In the early 1900's, HMF was used as an indicator of honey adulteration with invert syrups. Cane sugar (sucrose) is "inverted" by heating with a food acid, and this process creates HMF. However it was quickly realized that heated honey also had slightly higher levels of HMF and therefore was not an accurate measure of adulteration when found in moderate quanties (less than 100mg/kg).

Numerous studies also indicate that honey shows significant variations in amylase content based on composition, pH value and floral source. In fact, research reveals that heating is not the only factor influencing amylase content in honey. Amylase can also be modified by varying the pH level of honey. Consequently, the amount of amylase is an Insufficient indicator of honey quality.[3, 4, 5, 6]

Honey's Enzymes in Food Manufacturing

The enzymes in honey do not generally influence the final food product because they are present in relatively low concentrations. However, enzymes play a vital role as nectar is ripened into honey.[4] The enzymatic reactions in nectar and ripening honey result in a wide array of compounds which contribute to the unique character and functionality of honey. The complicated carbohydrate profile of honey is partially the result of enzymatic action; research has shown that the minor sugars in honey arise from the transglucosylation activity of α- and β-glucosidases.[5]

In some food products, little or no amylase is desired in applications that use honey. In salad dressings, for instance, the presence of amylase could contribute to unwanted separation due to the degradation of starch. For that reason, some food manufacturers will either heat the honey they use or purchase honey that has been further processed to remove the amylase. In order to achieve the greatest reductions in honey amylase, a heat treatment of 85 ºC for about 5 minutes is recommended. [6, 7]

Honey can be blended with the enzyme pectinase to significantly improve clarification in fruit juices. Honey acts in unison with pectinase to produce a significant combined effect upon flocculation. Using honey at refrigeration temperatures to clarify juices and wine is an effective way to add sweetness to a natural beverage and capitalize on honey's natural and functional benefits.[8]

Enzymes and Honey Fermentation

Acid phosphatase is an enzyme of honey whose values have been related to honey fermentation. Acid phosphatase is mainly present in pollen, although it is also a component of nectar. Honeys that ferment more easily have shown higher acid phosphatase activities than unfermented honeys. Acid phosphatase activity is higher in honey from oceanic climates. The pH of honey has demonstrated to have a strong influence on the activity of acid phosphatase. The higher the pH, the greater the acid phosphatase activity. [9]

Enzymes and the Antibacterial Properties of Honey

Honey has been proven to have significant antibacterial properties and is a useful constituent in wound and burn care. The antibacterial activity was originally thought to be due the high osmotic properties of honey, [10] but some activity persisted after dilution.

Although first termed "inhibine" in the 1930's, by 1966 it was concluded that inhibine was actually hydrogen peroxide generated by the action of glucose oxidase.[11] The antibacterial properties arise from the presence of glucose oxidase which converts glucose to gluconolactone, which in turn yields gluconic acid and hydrogen peroxide.[12]

Diastase

Diastase is the common name for the enzyme α-amylase. It is found in nectar and is also added by the honey bee during the collection and ripening of nectar.[1] The diastase content of fresh unheated honey is known to vary over a wide range (Table 2).

Table 2

Floral Source	Diastase Content (DN)
Orange blossom	4.25
Clover	5.73
Pepper	13.2
Multifloral	22.0
Eucalyptus	24.0
Buckwheat	36.8

[1] Persano Oddo, L. et al. 1990. Diastatic Activity of Some Unifloral Honeys. Apidologie 21:17.

Diastase digests starch to simpler compounds but no starch is found in nectar. What its function is in honey is not clear. Diastase appears to be present in varying amounts in nearly all honey and it can be measured. It has probably had the greatest attention in the past, because it has been used as a measure of honey quality in several European countries.

The floral origin of honey also influences its diastase content. For example, citrus and clover honeys tend to contain less diastase. Other factors may affect diastase values: the natural difference in pH among honeys, nectar flow and foraging patterns of the bees. Long storage at moderate temperatures and exposure to high temperatures will inactivate diastase in honey. Diastase levels do not correlate with honey quality. Therefore, specifying the diastase level will not guarantee quality.

Controlling pH is also a method for modifying the amylase content in honey. The optimum pH range for the enzyme is 4.6 to 5. Complete control of amylase activity can be achieved at a pH of less than 3.9.[13] Amylase is stable at pH values from 7 to 8. In starch-containing foods, ingredients that acidify the final product would also prevent the reaction of amylase with starch.[14]

Invertase

Invertase is the enzyme that hydrolyzes sucrose to fructose and glucose. It is added to the nectar by the bee. The resulting chemical reaction is a key step in the ripening of nectar to honey. Invertase has been considered responsible for most of the chemical changes that take place during the conversion of nectar to honey. Invertase is generally present in small amounts and is inactivated by heating. [15]

Glucose Oxidase

Glucose oxidase is another enzyme in honey that originates from bees. Like invertase and diastase, it plays a part in the formation of honey in the hive: it oxidizes glucose in the un-ripened honey. It yields gluconolactone which equilibrates with gluconic acid, the principal acid of honey. It also yields hydrogen peroxide which contributes to the antibacterial properties of honey.[16,17] Glucose oxidase is an active enzyme in nectar but is virtually inactive in honey. The enzyme may become active again if the honey is diluted. This enzyme is sensitive to light and heat.

Enzymes as an Indicator of Quality

The levels of some enzymes such as diastase are relatively easy to measure and have been used for many years to estimate the extent of heating to which a honey has been exposed. Such information has been required by some countries where heating of honey is believed to reduce or destroy potentially health-promoting properties. In fact, because the enzyme content of fresh honeys can vary widely, enzyme levels in packed honey are a poor indicator of processing and storage conditions.

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