

Determination of foreign enzymes in honey to detect adulterations with sugar syrups

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1. General remarks

Honey is considered to be a pure and valuable natural product made by bees. The European Council Directive 2001/110/EC defines the product “honey” precisely and establishes minimum quality standards for honey. Honey bees collect nectar from flowers or secretions of living parts of plants (extrafloral nectar) or the secretions of plant-sucking insects on the living parts of plants (honeydew). The nectar and honeydew, respectively, are then transformed into honey by the bee enzymes diastase (α -amylase, EC 3.2.1.1) and invertase (α -Glucosidase, EC 3.2.1.20) and the storage and maturation in the beehive. During this process, diastase and invertase catalyse the conversion of the sugars of nectar and honeydew into fructose and glucose, the main constituents of honey.

2. Production of invert sugar syrups and analytical methods for the determination of honey adulteration

As the techniques of sugar syrup production and imitation of the honey sugar profile become more and more sophisticated, the consistent further development of analytical methods is necessary to accurately detect adulteration of honey with inferior sugar syrups and protect consumers from fraud. Besides the well-established stable carbon isotope ratio analysis ($^{13}\text{C}/^{12}\text{C}$ -EA/IRMS, $^{13}\text{C}/^{12}\text{C}$ -LC/IRMS) to determine the addition of sugar syrups to honey [1-4], additional analytical methods are necessary to cover as many types of sugar adulteration as possible, particularly the adulteration with sugar syrups produced from C3 plants (e.g. beet, rice, wheat). As the sugar syrups are produced almost exclusively in enzymatic procedures nowadays (enzymatically catalysed hydrolysis of starch or sucrose), it is only natural to use the enzymes used in these procedures as marker substances for the detection of adulteration (Fig. 1).

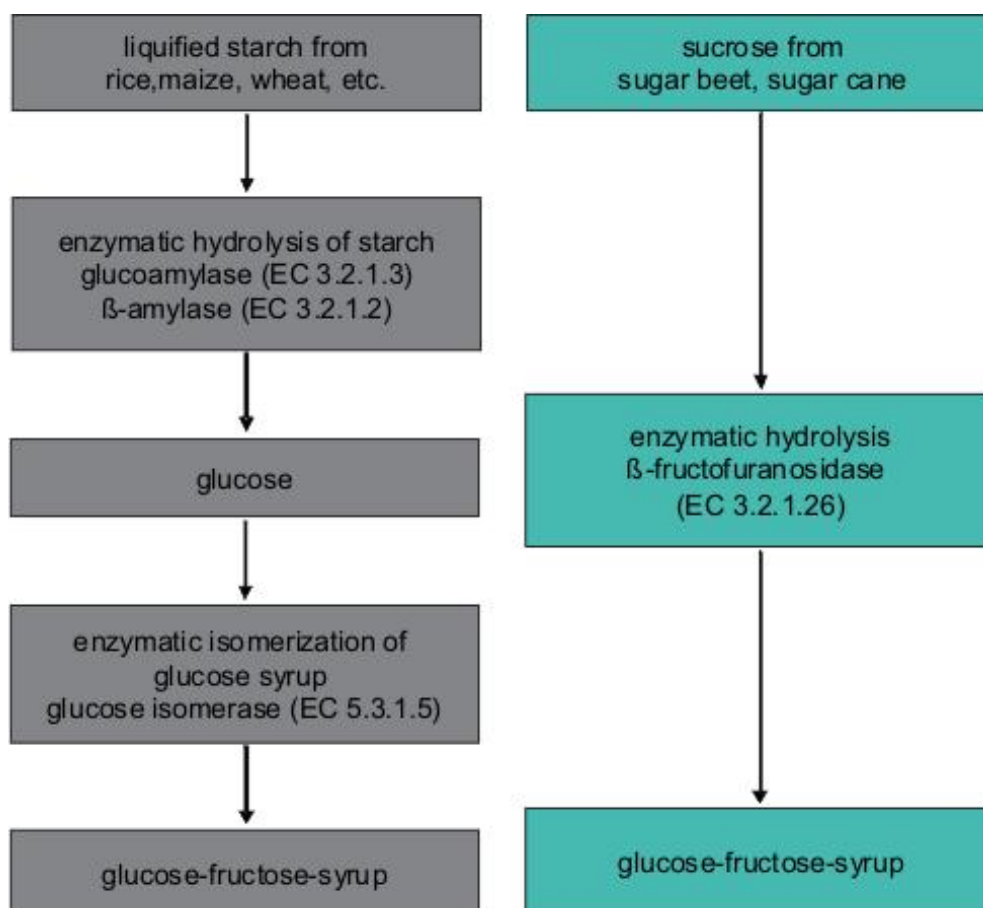


Fig. 1: Enzymatic production procedures for invert sugar syrups

3. Determination of foreign enzymes in adulterated honeys

It is possible to indirectly detect foreign enzymes in honey by comparing the diastase activity results obtained from two separate methods (Schade method vs. Phadebas method). The methods show different selectivities regarding the natural honey enzymes and the foreign enzymes, respectively. This fact leads to significant differences in the analytical results for diastase activity obtained from both methods indicating an adulteration with enzymatically produced invert sugar syrup or compensation of decreased natural enzyme activity (caused by addition of sugar syrup) with added foreign enzyme [5].

The enzymes used to produce invert sugar syrups, i.e. β -fructofuranosidase (EC 3.2.1.26), β -amylase (EC 3.2.1.2) and glucoamylase (γ -amylase, EC 3.2.1.3), do not occur naturally in honey (foreign enzymes). Therefore, adulteration with the foreign enzymes and the sugar syrup produced with them, respectively, can be directly detected by the specific analytical determination of these foreign enzymes serving as marker substances.

Suitable analytical methods (Fig. 2) were already developed and validated [6, 7] for β -fructofuranosidase (β FF).

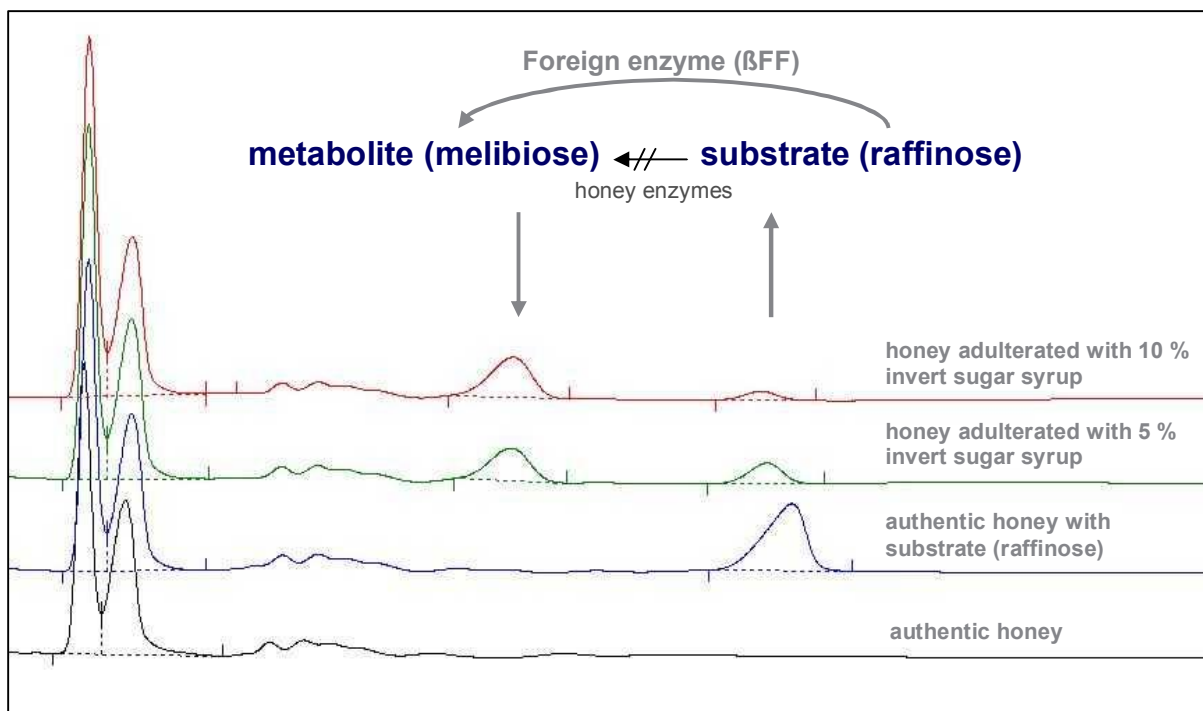


Fig. 2: Determination of the metabolization of the enzyme-specific substrate by β -fructofuranosidase activity in honey which is adulterated with C3 invert sugar syrup

Since November 2009, a validated analytical method for the determination of β/γ -amylase activity in honey has been available as well (Fig. 3) [8]. The natural honey enzymes do not interfere with the determination of the foreign enzymes due to the specificity of the methods. Therefore, both foreign enzyme methods represent a useful complementation of the authenticity analysis of honey by stable isotope ratio analysis.

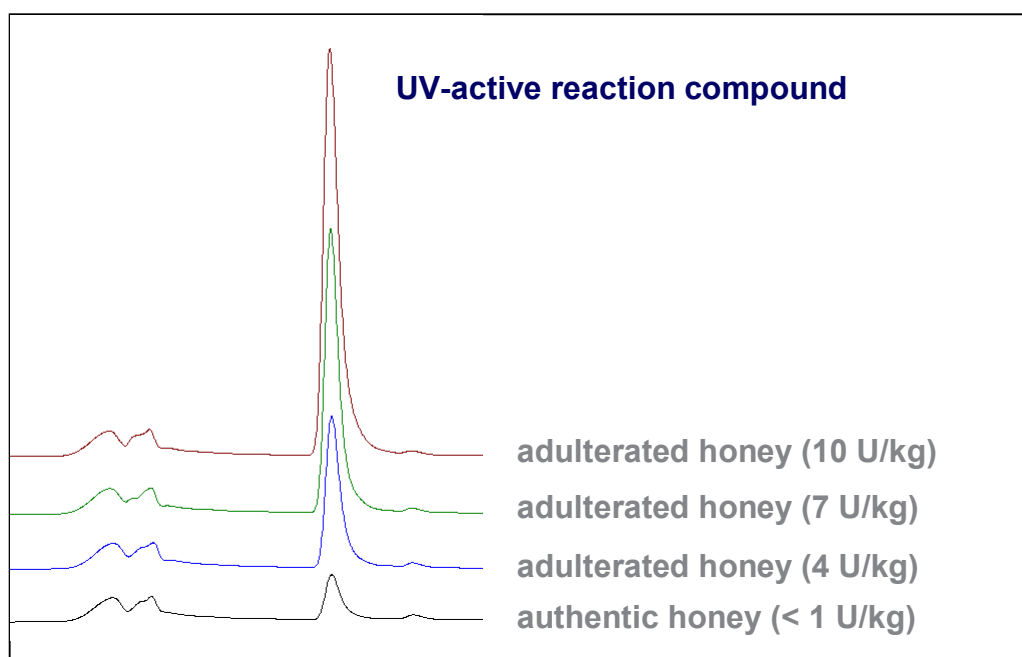


Fig. 3: Determination of the UV-active reaction compound produced by β/γ -amylase activity in honey which is adulterated with C3 invert sugar

4. Further literature references

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