PCR Detection of Rice DNA in Honey

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Summary

Adulteration of Asian honeys is often based on artificially added cheap rice syrup. By the help of Polymerase Chain Reaction (PCR), rice syrup added to honey can be detected indirectly by amplifying a specific rice DNA sequence coming from the added rice syrup. The PCR method described here might serve as support to mass spectrometry methods that are used for detection of honey adulterations with sugars from C_3 plants.

Keywords

Honey, rice, sugar from C_3 plants, DNA, Polymerase Chain Reaction, PCR, IR-MS

Introduction

For European consumers, honey as natural product is of significant value which is also legally underpinned by the European Community's Honey Directive: The Directive forbids any treatment, modification and adulteration of honey [1]. However, there are honey adulterations which often can be related to Asian honeys imported to the EC. These kind of honey adulterations are characterised by addition of cheap sugar syrups, e.g. derived from rice (*Oryza sativa*).

Up to now, Isotope Ratio Mass Spectrometry (IR-MS) – which is used for the routine detection of adulterations with sugars of C4 plants – cannot be used for the direct detection of rice syrup in adulterated honey. The IR-MS detection of sugars of C3 plants like e.g. rice, that have been artificially added to honey, is very problematic and not reliable [2]. This is due to the fact that sugars of C3 plants are also naturally occurring in honey. Moreover, the amount of sugar syrups of C3 plants added to honey cannot be quantified by IR-MS. Similar analysis methods are suboptimal, too [3, 4].

Hereby, we suggest a cheap and fast alternative to IR-MS methods: the detection of rice syrup DNA by Polymerase Chain Reaction (PCR).

Material and methods

For the detection of rice syrup DNA in honey, the primers Cyc1 and Cyc2 were used in order to amplify a 121 bp DNA sequence of the cyc fragment of the rice cytochrome C gene [5]. The primers were tested towards their applicability, sensitivity and specificity by using several matrices: DNA extracted from rice flour and rice syrup was used as matrix for the sensitivity test. DNA of rape, soybean and potato was used for the specificity test. Subsequently, different amounts of rice syrup (5%, 10%, 20%) were added to several unadulterated

polyflora honeys of different origin. By doing so, it was tested if rice syrup DNA can be isolated from honey. After mixing thoroughly, 10 g each of the honey and rice syrup mixtures were diluted with double distilled water and centrifuged for 10 min. at 4000 rpm. From the remaining sediments, DNA was extracted by the help of the Nucleospin Food Kit, Macherey Nagel (without using Proteinase K), and used for the rice specific PCR. The PCR products were separated in an agarose gel and visualised by help of UV rays. Positive results were verified by restriction fragment analysis and restriction enzyme Hpy188I, respectively. Additionally, raw honeys from of different geographical and botanical origin were screened in order to detect rice DNA potentially being present (see tab. 1).

Tab. 1: Honeys of different origin analysed by the help of rice specific PCR

Origin	**Honey**	**Result PCR**	**Result IR-MS (δ13C)**
Germany	Rape/clover	negative	nm*
	Summer honey flow	negative	nm*
	Heather	negative	nm*
Argentina	Polyflora	negative	nm*
Bulgaria	Acacia	negative	+0,6
	Acacia	negative	-0,1
Rumänien	Acacia	negative	+0,5
	Rosemary	negative	+0,8
India	Polyflora	**positive**	+0,9
	Polyflora	**positive**	+0,2
	Polyflora	**positive**	+0,5
	Polyflora	negative	-0,3
	Polyflora	**positive**	-0,2
Vietnam	Polyflora	negative	+0,2
	Polyflora	negative	-0,3
China	Polyflora	**positive**	+1
	Polyflora	negative	nm*
	Polyflora	negative	nm*
	Polyflora	negative	nm*
	Rape	**positive**	nm*
	Rape	negative	nm*

*nm = not measured

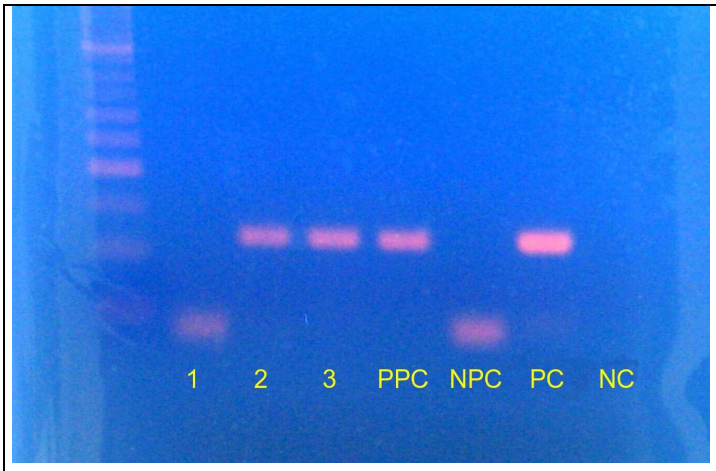

Fig. 1: Detection of a 121 bp sequence of the rice cyc fragment that was extracted from polyflora honeys artificially contaminated with different amounts of rice syrup (1: 5%, 2: 10%, 3: 20% of rice syrup in honey; PPC: Positive process control; NPC: Negative process control; PC: Positive PCR control; NC: Negative PCR control)

Results and discussion

Regarding sensitivity and specificity, the rice specific primer pair Cyc1/Cyc2 was applicable for the detection of the 121 bp cyc fragment of the rice cytochrome C gene. By the help of the rice specific PCR, it was possible to detect the fragment in honeys that were artificially adulterated with at least 10% of rice syrup (see fig. 1). In case the amount of rice syrup is below 10%, the detection of rice DNA only works sporadically and isn't always repeatable. The detection of rice DNA did also work for some Indian and Chinese import honeys that didn't show any adulteration with C3 plant sugars when analysed with IR-MS (i.e. $\delta^{13}C > 1,0$; see tab. 1).

The results of the PCR method presented here confirm that it is possible to isolate rice syrup DNA from honey as well as to detect it. Nonetheless, at this point it has to be critically examined if – for a corresponding honey – the detection of rice DNA is the real result or rather a false-positive result due to a natural "contamination" with rice DNA from e.g. rice pollen taken into the beehive by bees. If so, carrying out PCR couldn't be used because it would be impossible to differentiate between a natural phenomenon and artificial adulteration. However, it's a botanical fact that rice is a member of the sweet grass family (Gramineae) and of no interest for honey bees due to its lack in nectar and pollen [6]. The reason for this is that rice plants have blossoms that don't open until they had pollinated themselves inside the bud [7]. Hence, bees aren't vectors for rice DNA material to be transported into the bee hive. Moreover, an accidental honey contamination with rice pollen, e.g. transported by wind into the beehive or open honey drums, can be excluded because of self-pollination explained above. In case, pollen is coming out of the bud after all, it just can be transported for a few centimetres. Even if rice pollen ends up in honey by accident, detecting its DNA can be excluded due to the fact that they have a very thick outer cell layer (exine) that cannot be opened by the help of the extraction chemicals used for this method. Thus, no rice DNA can escape from the pollen which only is possible if the pollen had been cracked mechanically before, e.g. with glass beads.

When rice DNA is detected in honey by help of the method described, one can assume that rice DNA material like e.g. rice syrup had been used for adulteration of the corresponding honey. Hence, the described high-sensitive and specific PCR method is suitable for detection of rice DNA adulterations in honey.

Acknowledgement

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Literature

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