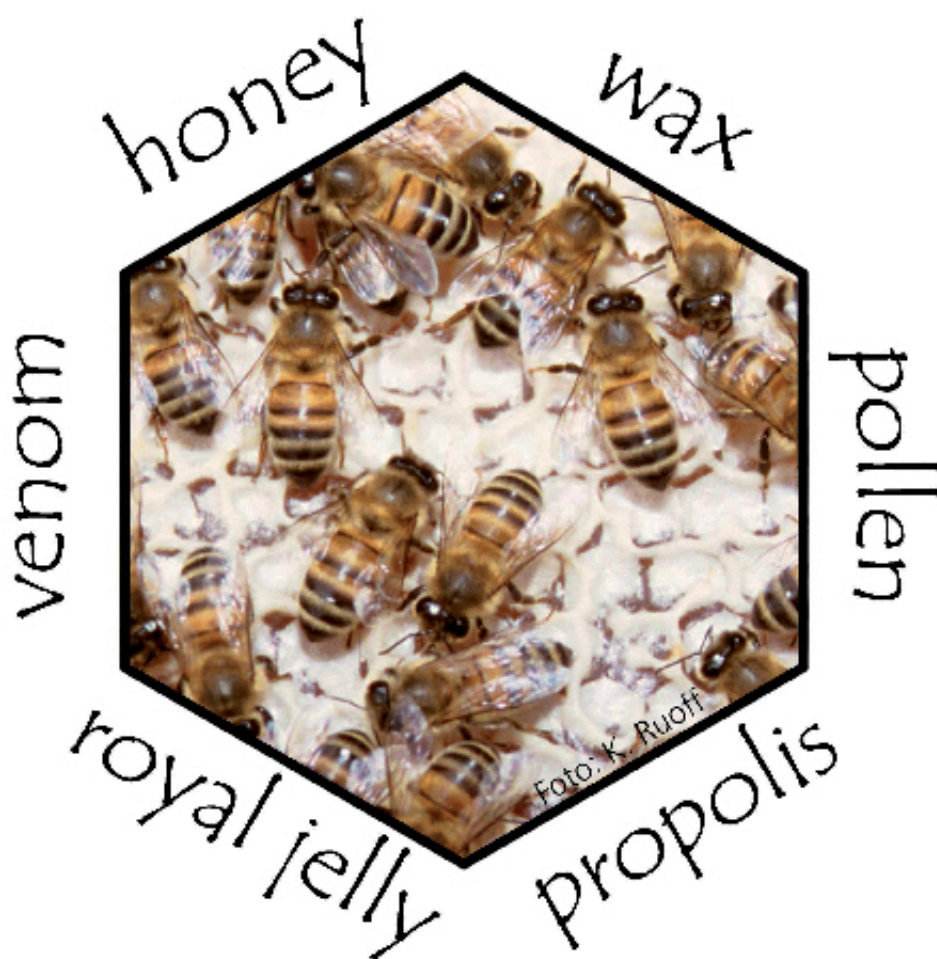


AUTHENTICITY OF HONEY AND OTHER BEE PRODUCTS STATE OF THE ART

Technical-scientific information



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Keywords:

Honey, beeswax, propolis, royal jelly, pollen, authenticity

1 Introduction

The authenticity issue is especially important for natural products such as bee products. Thus, existing standards for such products are a basis for authenticity testing. An important part of the work of the International Honey Commission (IHC) is to develop and improve these standards. The honey standard of Codex Alimentarius was set in 2001 essentially as proposed by the IHC. At present IHC groups are elaborating standards on all other bee products excepting venom. One of the authors of this paper (Stefan Bogdanov) is coordinating the IHC work on the standardisation of the bee products. This work was crowned by submitting publications proposing drafts for standards of poplar propolis, royal jelly and pollen (see below). The beeswax group, headed by Hansjoachim Roth from the Ceralyse laboratory in Bremen is working on a paper on the standardisation of beeswax. However, the example of honey shows that there is a lot of work remaining until eventually world standards for these bee products can be established within the frame of the Codex. A drawback for the proposed standard drafts (with the exception of the wax standard draft) is that they are based on existing national standards and on publications which were carried out with different methods and in different countries. This inevitably leads to a very large variation of the proposed limits. For bee products other than honey the next step is to set up standardised methods for the establishment of the standard.

Most of the recent work on authenticity issues has been dedicated to honey. Therefore honey occupies the major part of this review. The present work aims at surveying the state of the art in the field, in the hope that it will stimulate further work in this area.

ALP science

Title

Authenticity of honey and other bee products state of the art

First edition

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Publisher

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Layout

Marc Wassmer

Publication frequency

Several times yearly at irregular intervals

ISBN 978-3-905667-59-2

ISSN 1660-7856 (online)

2 Honey



The different colours of Swiss unifloral honeys: from left to right: acacia, dandelion, rape, linden and sweet chestnut

An extensive review on honey authenticity was published in 2002 (Bogdanov and Martin, 2002). This review covers more recent developments on the subject.

Authenticity of Production and Processing

The major concern about honey quality is to ensure that honey is authentic in respect to the legislative requirements. According to the definition of the Codex Alimentarius (Codex Alimentarius Commission 2001) and other international honey standards (2001/110/EC, EU Council 2002) honey shall not contain any food ingredient other than honey itself nor shall any particular constituent be removed from it. Honey shall not be tainted by any objectionable

matter, flavour, aroma or taint from foreign matter during harvesting, processing and storage. Honey shall not have started to ferment or effervesce. No pollen or constituent particular to honey may be removed except where this is unavoidable in the removal of foreign inorganic or organic matter. Honey shall not be heated or processed to such an extent that its essential composition is changed and/or its quality impaired.



Honey bee foraging pollen on willow

Adulteration by sweeteners

Adulteration by sweeteners is the most important authenticity issue. As a natural product of a relatively high price, honey has been a target for adulteration for a long time. Addition of sweeteners, feeding the bees during the nectar flow or extracting combs containing bee feed may adulterate honey. The following sweeteners have been detected in adulterated honeys: sugar syrups and molasses inverted by acids or enzymes from corn, sugar cane, sugar beet and syrups of natural origin such as maple.

Many methods have been tested for adulteration proof but most of them are not capable to detect unequivocally adulteration (Bogdanov and Martin, 2002). We discuss here only the most promising methods.

Adulteration by addition of cane- and corn sugar can be screened microscopically (Kerkvliet and Meijer, 2000) and verified by measuring the $^{13}\text{C}/^{12}\text{C}$ isotopic ratio (Brookes et al., 1991; Rossmann et al., 1992; White, 1992; White et al., 1998). Recently this method has been further developed to include Site-Specific Natural Isotopic Fractionation (SNIF) measured by Nuclear Magnetic Resonance (Cotte et al., 2007). A recent development is further the inclusion of sugar chromatography in this method (Elflein and Raezke, 2005; Cabanero et al., 2006), claiming, that the addition of beet sugar can also be detected. The addition of high fructose corn syrup may be detected by detection of oligosaccharides naturally not present in honey through capillary GC (Low and South, 1995). Recently infrared spectroscopic methods have been described for the detection of adulteration by adding beet and cane sugar to honey (Sivakesava and Irudayaraj, 2001; Kelly et al., 2006a; Kelly et al., 2006b). These results were obtained by adding the adulterants to honey and comparing to the products with the original product. In practice, this differentiation should be more difficult, due to the wide natural variation of honey. Also, when the adulterants were fed to bees both infrared spectroscopy and front phase fluorimetry were unable to detect 50 % honey adulteration (Ruoff, 2006).

Fermentation

Harvesting of honey with high moisture content, or subsequent addition of water can result in honey fermentation and spoilage. Honey spoilage can be first tested by a microscopic yeast count (Russmann, 1998b; Beckh and Lüllmann, 1999). This test on its own does not yield conclusive results, as counted yeast could be in an inactive status not taking part in the fermentation process. Determination of the fermentation products is more reliable (Beckh and Lüllmann 1999) i.e. by determining the glycerol or ethanol content (Russmann, 1998a; Beckh et al., 2005; Zucchi et al., 2006)

Heat defects

The use of excessive heat in honey processing for liquefaction or pasteurisation has adverse effects on honey quality, i.e. loss of volatile compounds, accumulation of HMF and reduction of invertase and diastase activities. Quantification of HMF content and enzyme activities are useful tools to detect heat induced defects in honey. However, it should be noted that improper storage of honey leads also to similar changes of HMF and enzyme activity.

Honey filtration

Honey should not be strained with a mesh size smaller than 0.2 mm in order to prevent pollen removal. On the other hand, the recently revised Codex Alimentarius Honey Standard (Codex Alimentarius Commission 2001) and EU Directive relating to honey (EU Council 2002) allow a removal of pollen if it is unavoidable for the removal of foreign matter. Such honey should be labelled as "filtered". Since microscopical pollen analysis is still the most important tool for the determination of botanical and geographical origin of honey, any removal of pollen by filtration will make authenticity routine testing much more difficult, if not impossible.

Organic honey, raw or unheated honey

The production of organic honey implies organic beekeeping which is defined in European regulation EEC No 2092/91, Annex I. The qualification of beekeeping products as being from organic origin is closely bound up both with the characteristic of the hives' treatments (e.g. application of veterinary drugs) and the quality of the environment. As all organic food honey is produced in our more or less polluted environment. And such contamination can never be excluded. On the other hand organic honey must not be contaminated by veterinary drugs introduced by bee keepers which are generally the most important contaminants (Bogdanov, 2006). A recent examination of 250 pesticides in organic Swiss honeys confirmed this, as only traces of one pesticide were found in 2 out of 33 samples (Edder et al., 2006). Thus, residues from synthetic veterinary drugs in honey, can prove mislabelling of organic honey.

The term 'natural' honey should be avoided. It is misleading, since honey is natural by definition. The terms raw and unheated honey are also misleading, as honey is not heated during the harvest. Pasteurisation is not mentioned in the Codex or European honey regulations. These regulations do not allow overheating of honey such as to significantly impair its quality. Quick pasteurisation does not significantly influence the honey quality and is often carried out in some countries. However, the labelling of honey pasteurisation is not compulsory as in milk. On the other hand the pasteurisation of organic honey is not allowed.

Use of non-registered products and drugs for honey production

According to the honey definition (Codex Alimentarius) honey should comply with the established Maximum Residue Limits (MRL). Contamination with non allowed products leads to an illegal honey product which can be banned from trading, as it is the case with honey, contaminated by antibiotics. This field is covered by a recent review (Bogdanov, 2006).

Authenticity of botanical and geographic origin

Misdescription of botanical source

The botanical source may be labelled if the honey originates totally or mainly from a particular source and has the organoleptic, physico-chemical and microscopic characteristics of that origin. As bees forage on different plants, absolutely pure unifloral honeys are extremely rare. The different unifloral honeys show typical sensory, melissopalynological and physico-chemical properties.

Pollen analysis is the classical method for the determination of the botanical origin of honey (Louveau et al., 1970; von der Ohe et al., 2004). However, due to the considerable variation of the pollen content it is now regarded as a side method, besides sensory and physico-chemical analysis. Recently the International Honey Commission has worked out standards for the main European unifloral honeys, comprising sensory, melissopalynological and physico-chemical characteristics (Persano Oddo and Piro, 2004).

In summary, the routine analysis of honey botanical origin includes organoleptic, physico-chemical and pollen analysis and a decision to whether a honey is unifloral or not is based on a global interpretation of all results (Persano Oddo and Bogdanov, 2004). Another approach is the chemometrical evaluation of physico-chemical parameters (sugars, electrical conductivity, optical rotation, nitrogen content etc.). The combination of these methods allows a good separation of some unifloral honeys (Bogdanov, 1997; Piro et al., 2002; Terrab et al., 2004). However, it should be noted that these methods may not allow discrimination between unifloral and polyfloral honeys. Of all honey measurands analysis of the volatile and aroma components is most promising (Bogdanov et al., 2004). Both quantitation and statistical evaluation of the volatile components can be used, but the quantization approach should be the more successful, as it is the more robust one. Recently promising in-situ spectroscopic techniques, combined with statistical analysis have been successfully used for the authentication of unifloral honey. Front phase fluorimetric spectroscopy (Ruoff et al., 2006b), near-infrared (Ruoff et al., 2006a) and mid-infrared spectroscopy (Ruoff et al., 2006c). Of all the mentioned techniques the mid-infrared technique is the most promising, as it allows also the measurement of the principal honey parameters (Ruoff et al., 2005).

Misdescription of geographical origin

Generally, in Western Europe and also in countries as the United States and Japan, honey imported from the Far East or South America has a lower price than the locally produced honey, and is therefore prone to mislabelling because of economic reasons.

Pollen analysis is at present mostly used to determine the geographical origin of honey. The possibilities of pollen analysis for the determination of the geographical origin of honey have been reviewed recently (Piana, 1997). Indeed, the differences of the pollen spectrum between honeys from quite different geographical and climatic zone are easy to detect. However, if the geographical zones are closer, differences are more difficult to distinguish. In such cases more sophisticated melissopalynological methods should be used. In recent years pollen analysis has been used for the determination of honeys originating from close geographical zones by the use of special statistical software (Ferrazzi and Medrzycki, 2002; Floris and Satta, 2002).

Recently the analysis of stable isotopes by IRMS has been developed for the authenticity proof of different foods (Piasentier et al., 2003; Kelly, 2003). These isotopes depend theoretically more on the climate and are added theoretically to honey rather through rain water than through the honey plants. This method should be tested first on unifloral honeys of different geographical origin in order to decide if it is useful for the determination of the geographical origin of honey.

Misdescription of the entomological source

The present EU Directive definition defines honey as derived from *Apis mellifera*, while according to the Codex standard honey is the product of all honey bees. *Apis mellifera*, originally indigenous to Africa and Europe, has been introduced into major exporting countries such as China, where honey is also produced from *Apis cerana*. This has created two problems which need to be solved. It is necessary to characterise the honey from species other than *Apis mellifera* so that the honey from these species can be accepted in international trade either as indistinguishable from *Apis mellifera* honey (*Apis cerana*, the Asian bee) or with compositional limits of its own (*Apis dorsata*, stingless bees). Indeed, it is planned that the honeys of bees others than *Apis mellifera* also are characterised and a Codex standard for these honey types are established.

3 Other bee products

The review will focus on the testing by physico-chemical methods. It is obvious, that sensory testing is also important, because all products have their specific, very typical sensory characteristics and an experienced person can quickly detect significant adulterations.

Beeswax



Candles and beeswax figures are popular objects and the customer expects that they are of pure genuine beeswax.

Beeswax is the most important bee product besides honey and apart from its use in beekeeping it is predominately used in cosmetic industry. An update on beeswax quality issues was published recently (Bogdanov, 2004).

As this product has a relatively high price, the authenticity issue is an important one. It can be adulterated with synthetic products (e.g. paraffin wax) or with plant waxes (e.g. carnauba wax). The current quality criteria for pure beeswax according to the pharmacopoeia i.e. acid value, ester value, saponification value, drop point, tests for paraffin and other waxes as well as for glycerol and other

polyols, summarised in table 1. are inadequate for its reliable determination (Ceralyse Laboratory, Bremen, personal communication) but are still used today because they are easy to carry out (Bernal et al., 2005). Today, adulteration can be detected very sensitively by gas chromatographic determination of wax components. Unambiguous detection of beeswax adulteration should be carried out by gas chromatography (Brüschweiler et al., 1989; Sabatini et al., 1989; Serra Bonvehi, 1990; Aichholz and Lorbeer, 2000; Boselli et al., 2002; Jimenez et al., 2003; Jimenez et al., 2004; Jimenez et al., 2006; Jimenez et al., 2007).

In the special case of adulteration by carnauba wax, a simple biological test can also be used (Aquino et al., 1999). Most of the wax produced world wide originates from *Apis mellifera* (about 60 %), while only a smaller portion (about 20%) is produced by the African bee *Apis mellifera scutellata* (Brand-Garnys and Sprenger, 1988). The wax produced by different races of *Apis mellifera*, and also of African *adansoni* wax, have the same composition but some components are in a different proportion (Brand-Garnys and Sprenger, 1988; Beverly et al., 1995). The waxes of Asian bees *Apis florea*, *Apis dorsata* and *Apis cerana*, are called Ghedda wax. The composition of wax from Asian honeybee species is less complex compared to the European and African species. It contains fewer compounds which are in different proportions (Tulloch, 1980).

Generally, the beeswax used in European beekeeping is home made, while the wax used in pharmacy and in other fields is mostly imported, the chief importer being China (Herrmann, 2005). Thus, the two types of waxes do not compete with each other. Geographical origin of beeswax has not been an issue in routine quality control so far. However, it is possible to analyse pollen in beeswax and potentially determine its geographical origin (Ricciardelli d'Albore and Acocella, 1999).

Table 1 Composition criteria for routine authenticity testing of beeswax according to the Ceralyse laboratory, Bremen, Germany (Cortesy of Hansjoachim Roth, Ceralyse)

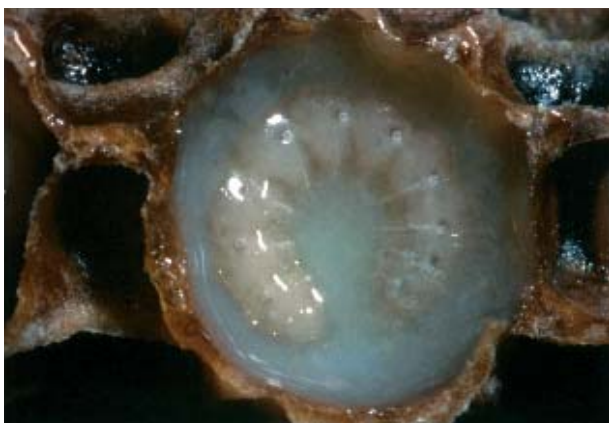
Quality Criteria	Value	Method
Water content	< 1%	DGF-M-V-2*
Refractive index, 75° C	0.4398-1.4451	EP**
Melting point	61-65 °C	EP
Acid Number	17-22	EP
Ester Number	70-90	EP
Ester/Acid ratio	3.3-4.3	EP
Saponification Number	87-102	EP
Mechanical impurities, additives	absent	DGF-M-V-3
Glycerols, polyols, fatty acids fats	absent	EP
Hydrocarbons	max. 14.5 %*	DGF-M-V-6

* - DGV, V2,3,6 – Methods of Deutsche Gesellschaft für Fettwissenschaft

** - European Pharmacopoeia, 4th Edition

Royal Jelly

Recently, a group of the International Honey commission proposed a first world composition standard for royal jelly (RJ, table 2). This is a first step towards the establishment of compositional criteria for testing of RJ.



Royal jelly is harvested from bee queen cells with 3-5 days old larvae.

The main quality factors of RJ have been described and studies have revealed the importance of the lipid fraction as a marker and hence a criterion by which to determine the product's genuineness. Presently, 10-Hydroxy-2-decenoic acid (10-HAD) is mostly used for routine testing of RJ authenticity. However, the concentration of this acid varies in vary wide limits. Further studies are necessary to

determine whether the determination of the stable isotopes of the elements C and N is a promising approach for the determination of the authenticity of production. (Stocker et al., 2006). Adulteration by honey results in a general diminution of proteins and lipids and a relative increase of sugars (Serra Bonvehi, 1991). Another promising parameter for the evaluation of RJ authenticity of production is the presence of apalbumine (Simuth et al., 2004).

Royal jelly is predominately produced in China and other far eastern countries and is marketed world-wide. The far eastern production has a highly competitive price on the world market and the determination of geographical origin of royal jelly is an important issue in RJ quality control. Microscopic analysis of RJ sediment according to the basic principles of melissopalynology enables the authentication of the geographical origin of the product (Ricciardelli d'Albore et al., 1978; Ricciardelli d'Albore and Quaranta, 1994; Ricciardelli d'Albore and Acoella, 1999). Pollen spectra from Chinese, Mexican, North-American, Russian, Polish, Romanian, Yugoslavian, Czech, Bulgarian and French royal jelly have been described in the above cited works. Pollen identification is made easier by the fact that only a few countries actually produce RJ and specialists are capable of formulating their respective characteristic pollen associations.

Table 2 Suggestion for composition criteria of royal jelly
(according to Sabatini et al., 2007, submitted for publication)

	Fresh (*)	lyophilized
Water g /100 g	60 – 70	< 5
Lipids g /100 g	3 – 8 (4-8)	8 – 19
10-Hydroxy-2-decenoic acid g /100 g	> 1,4 (1.4-6)	> 3,5
Protein	9 – 18	27 – 41
Fructose + glucose+ sucrose g /100 g	7 – 18 (11-23)	-
Fructose g /100 g	3 – 13	-
Glucose g /100 g	4 - 8	-
Sucrose g /100 g	0,5 – 2,0	-
Ash g /100 g	0,8 - 3,0	2 – 5
pH	3,4 - 4,5 (3.5-4.1)	3,4 – 4,5
Acidity (ml 0.1N NaOH/g)	3,0 - 6,0	-
Furosine (mg/100g protein)	< 50* (-)	-

* - values of the Swiss Food manual, where they differed from the proposed standard.

Propolis

The authentication of propolis is doubtlessly the most complex of all bee products. Botanical authenticity is more important than the geographical one, as there are different types of propolis, depending on the plant, from which propolis is produced. The different types of propolis have a specific composition (Bankova, 2005a; Bankova, 2005b), which can be determined. From commercial point of view the poplar and the Brazilian green propolis are most important. All other propolis types are of local importance only. A standard for poplar propolis, based on routine examination of the phenolic compounds has been proposed by the IHC propolis group headed by V. Bankova (Popova et al., 2007), see table 3.

Table 3 Proposed standards for raw poplar propolis, poplar propolis (Popova et al., 2007) and Brazilian green propolis (Marchini et al., 2005).

Component	Poplar Propolis min Values, g/100 g	Green Propolis Min Values g/100 g
1. Balsam	45	35
2. Total phenolics	21	5
3. Total flavones and flavonols	5	-
4. Total flavanones and dihydroflavonols	4	-
5. Total flavonoids	9*	0.5
6. Beeswax	Max. 40 **	
7. Insoluble matter	Max. 5**	

* - sum of 3 and 4

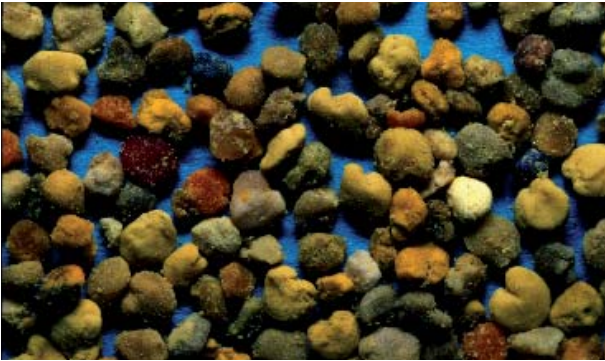
** - according to the Allwex trading company, Hamburg (personal communication)

The standard for Brazilian green propolis is cited from the Brazilian bee product legislation (Marchini et al., 2005). The Brazilian green propolis has less total phenolics and flavonoids. Rough adulteration of propolis can be detected by applying these recently published standards. Beeswax is a natural component of propolis, the average amount for poplar propolis ranging from 30 to 40 % (according to the propolis trading company Allwex, Hamburg, personal communication). The geographical authenticity of propolis is not an issue of quality control, but it can be determined by pollen analysis (Ricciardelli d'Albore, 1979).



Propolis originate from different plants and have different sensory colour, sensory and chemical properties.

Pollen



Bee pollen consists of the pollen loads carried by the bees.
The pollen types differ depending on the harvest time.

Pollen is produced in various countries as a feeding supplement for bees as well as food stuff or food supplement for human nutrition. Recently an IHC working group, headed by M. Campos has suggested a world standard of pollen (table 4). The limits for the different quality criteria vary within a broad range limits. This variation is due to the wide variety of pollen, gathered by the bees (Keller et al., 2005).

Table 4 Proposition for a standard of dried pollen according to Campos et al. (2007, submitted for publication)

Main Components	Minimum – Maximum (*) g/100g dry weight
Proteins	10-40
T	1-13 (1-10)
Total carbohydrates*	13-55
Dietary fibre, pectin	0,3-20
Ash	2-6
Undetermined	2-5

* - values of the Swiss Food Manual, where where they differed from the proposed standard.

Because of its unique appearance no adulterations with other products are known. At present mostly mixed pollen is sold on the market. Unifloral pollen is not very common and is of a local importance only. The authenticity testing of such a product can be easily done by microscopy.

In countries with higher prices there is a certain risk of selling lower priced pollen from abroad as a domestic product. Pollen analysis is at present the only way to detect eventual misdescriptions of the geographical origin.

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