In Search of Honey Authentication

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This book is dedicated to my daughter, Vassiliki, and my wife, Evangelia; my parents, Konstantinos and Adamantia; my brother, Vassilios, and my grandmother, Malamo “Malamaki”. It is also dedicated to those grandparents who have passed away, in their beloved memory: Ioannis, Stella, and George. Finally, the happiness I gain from my black labrador, Agapi, is a reason to include her in my dedications.

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PREFACE

The ability to determine the origins of honey — the product of *Apis mellifera* honeybees — has become very important for the protection of both consumers and beekeepers. The origins of honey can impact its pricing, which can lead to counterfeits being produced to increase the perceived value of lesser products.

In that sense, terms such as "protected designation of origin" (PDO), "protected geographical indication" (PGI) and "traditional specially guaranteed" (TSG) have emerged to highlight the quality of several products, including honey, made in different parts of the world. Some typical examples include the Spanish PDO ''Miel de la Alcarria'', the Greek fir-tree honey from Mainalon, and the pine-thyme honey (Pefkothymaromelo) from Crete. Such products have the potential to attract consumers, producers and exporters, and create a basis for different types of research, such as economic studies or studies related to authenticity by using a data set of several physicochemical parameters and chemometric analyses.

The official method to determine the botanical origin of honey is pollen recognition (melissopalynology). However, this approach has certain limitations: pollen-counting procedure and identification; interpretation of results which is difficult and requires trained analysts; the considerable time of analysis; and limited geographical-origin determination applications.

Therefore, specific physicochemical parameters such as sugar content, moisture content, electrical conductivity, acidity, colour attributes, etc., along with honey micro-constituents such as volatile compounds, polyphenols, minerals, organic acids, free amino acids, and isotopic data, may accurately determine honey's botanical and geographical origins, in combination with chemometrics.

This book, in being the ultimate research guide, may be used as a reference manual by both researchers/academics and practitioners in the honey industry to determine a honey's uniqueness or identity (authentication).

—Ioannis K. Karabagias, Ph.D.

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“*Without honey this world would be less sweet.*”
—Dr. Ioannis K. Karabagias

Chapter 1

Honey: History, Chemical Composition, Botanical Origins and Nutritional Value

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HISTORY

The bee appeared on our planet 65 million years ago, in the Third Period, much earlier than man. Assuming that honey appeared along with the bees, we can postulate that from then until just before the 16th century, honey was the only confectionary raw food in the "known" world.

The nutritional value of honey and its “divine” properties have been appreciated since ancient times. In Egyptian papyrus, 3,500 years ago, honey was referred to as a remedy. The Egyptians offered honey to their gods as a precious gift of loyalty and exile. In the book of Ancient India, life is prolonged when there is milk and honey in the daily diet; nectar was the food of the Olympian gods in Greece; Zeus was raised by the nymph

Melissa with honey; Hippocrates recommended honey for the treatment of many diseases; and Aristotle believed that honey extended the life of a human. Democritus and Dioscourides emphasised the benefits of honey for strengthening the body and mind.

Spain has the earliest depictions of honey collections in Mesolithic caves dating from the 6th millennium BC, showing people in gathering honey, with bees flying around them (Crane, 1999). Indeed, honey collection is an ancient activity. Humans began hunting for honey at least 8,000 years ago, as evidenced by the aforementioned cave painting in Valencia, Spain. The painting is a Mesolithic rock painting, showing two honey hunters collecting honey and honeycomb from a wild bee nest. The figures are depicted carrying baskets or gourds and using a ladder or series of ropes to reach the nest (Crane, 1983). The greater honey-guide bird directs humans to wild beehives (Isack and Reyer, 1989) and this behaviour may have evolved with early hominids (Short et al., 2003).

The oldest known honey residues were found by archaeologists in Georgia on the inner surface of clay vessels unearthed in an ancient tomb, dating back some 4,700–5,500 years (Kvavadze et al., 2006).

CHEMICAL COMPOSITION

Honey contains more than 180 different components, making it an excellent food. About 14-20% of it is water, while natural sugars such as fructose, glucose and sucrose make up the remaining 75-85% of the whole mass. Additionally, honey contains small amounts of proteins, trace elements, enzymes, and vitamins, showing a considerable nutritional value (Figure 1). What is impressive, is not so much the individual honey components, but the coexistence of all these substances in a product of well-defined optimal proportions.

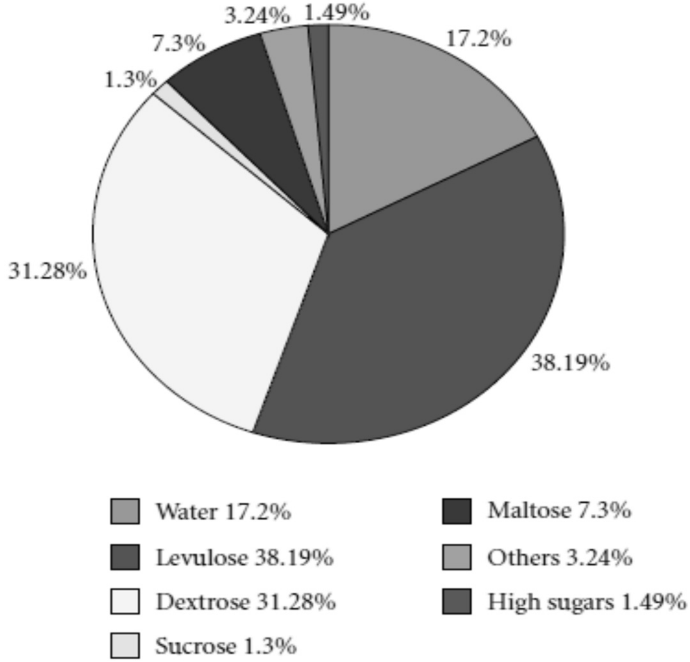


Figure 1. The chemical composition of honey (Jaganathan and Mandal, 2009).

Honey essentially consists of different sugars — predominantly fructose and glucose — as well as other substances such as organic acids, enzymes and solid particles derived from honey collection. The colour of honey varies from nearly colourless to dark brown. The consistency can be fluid, viscous or partly to entirely crystallized. The flavour and aroma vary but are both derived from the plant origins.

When placed on the market as honey or used in any product intended for human consumption, honey must not have any food ingredient added to it, including food additives or any other additions. Honey must, as far as possible, be free from organic or inorganic matters foreign to its composition. Furthermore, it must not have any foreign tastes or odours, have begun to ferment, have an artificially-changed acidity or have been heated in such a way that the natural enzymes have been either destroyed or significantly inactivated. No pollen or constituent particular to honey may be removed except where this is unavoidable in the removal of foreign inorganic or organic matter. In that sense, terms such as protected designation of origin (PDO), protected geographical indication (PGI) or traditional specially-guaranteed (TSG) [Regulation (EU) No. 1151/2012, 2012] have been set to highlight the quality of products (including honey) produced in different parts of the world. Some typical examples include the Spanish PDOs “Miel de la Alcarria” and “Miel de la Granada”, the Greek fir-tree honey from “Mainalon: Meli Elatis Vanilia” and pine-thyme honey (Pefkothymaromelo) from Crete (Ministerial Decision No. 313049, 1994; ES-PDO-0117-0079, 2010; De Alda-Garcilope et al., 2012; PDO-EL-02142, 2016). Such products have the potential to attract consumers, producers, exporters, and create a basis for different types of research, such as economic studies or studies related to authenticity using a data set of several physicochemical parameters and chemometric analyses (Thrasyvoulou & Manikis, 1995; Von der Ohe et al., 2004; Senyuva et al., 2009; Karabagias et al., 2014).

BOTANICAL ORIGINS

According to Council Directive 2001/110/EC (2002), honey is the natural, sweet substance produced by *Apis mellifera* bees from the nectar of plants, the secretions of living parts of plants, or the excretions of plant-sucking insects on the living parts of plants, which the bees collect and transform by combining them with specific substances of their own, before depositing, dehydrating and storing them in honeycombs to ripen and mature.

The main types of honey are classified as follows:

(a) according to origin:

(i) blossom honey or nectar honey

Honey obtained from the nectar of plants;

(ii) honeydew honey

Honey obtained mainly from the excretions of plant-sucking insects (*Hemiptera*) on the living parts of plants or the secretions of living parts of plants.

Another possibility is the classification of honey according to the mode of production and/or presentation:

(iii) *comb honey*

Honey stored by bees in the cells of freshly-built broodless combs or thin-comb foundation sheets made

solely of beeswax and sold in sealed whole combs or sections of such combs;

(iv) *chunk honey or cut comb in honey*

Honey which contains one or more pieces of comb honey;

(v) *drained honey*

Honey obtained by draining decapped broodless combs;

(vi) *extracted honey*

Honey obtained by centrifuging decapped broodless combs;

(vii) *pressed honey*

Honey obtained by pressing broodless combs with or without the application of moderate heat not exceeding 45°C;

(viii) *filtered honey*

Honey obtained by removing foreign inorganic or organic matter in such a way as to result in the significant removal of pollen.

Baker's honey

Honey which is (a) suitable for industrial uses or as an ingredient in other foodstuffs which are then processed and (b) may:

— have a foreign taste or odour, or

— have begun to ferment or have fermented, or

— have been overheated.

When placed on the market as honey or used in any product intended for human consumption, honey must meet the following composition criteria:

Table 1. Compositional criteria of honey intended for human consumption (Council Directive 2001/110/EC, 2002).

Compositional criteria for honey	Blossom honey	Honeydew honey, blends of honeydew honey with blossom honey	Heather honey (*Calluna*), and baker's honey	Baker's honey from heather (*Calluna*)	Pressed honey
Fructose and glucose (sum of both)	≥60 g/100 g	≥45 g/100 g	-	-	-
Sucrose content	≤5 g/100 g	≤5 g/100 g	-	-	-
Moisture content	≤20%	≤20%	≤23%	≤25%	
Water-insoluble content	0.1 g/100 g	0.1g/100 g	0.1 g/100 g	0.1 g/100 g	0.5 g/100g
Electrical conductivity	≤0.80 mS/cm	≤0.80 mS/cm	-	-	-
Free acid	≤50 meq/kg	≤ 50 meq/kg	≤80 meq/kg		
Diastase activity (Schade scale)	≥8	≥8	-	-	-
HMF	≤40 mg/kg	≤40 mg/kg	-	-	-

meq:milli-equivalents. 1 kg: 1000 grammes. HMF: hydroxymethylfurfural.

However, honey types such as false acacia *(Robinia pseudoacacia)*, alfalfa *(Medicago sativa)*, Menzies

Banksia *(Banksia menziesii)*, French honeysuckle *(Hedysarum)*, red gum *(Eucalyptus camadulensis)*, leatherwood *(Eucryphia lucida, Eucryphia milliganii),* and *Citrus* spp. could contain a sucrose content ≤10 g/100 g, whereas the sucrose content in lavender *(Lavandula* spp.), and borage *(Borago officinalis)* honeys should be ≤15 g/100 g.

Honeydew and chestnut honeys and blends of these types should have electrical conductivity values < 0.80 mS/cm. Exceptions to this are the strawberry tree (*Arbutus unedo*), bell heather *(Erica*), eucalyptus, lime (*Tilia* spp.), ling heather (*Calluna vulgaris*), manuka or jelly bush (*leptospermum*), and tea tree (*Melaleuca* spp.).

In honeys with low natural enzyme content (e.g., citrus honeys) and an HMF (hydroxymethylfurfural) content of not more than 15 mg/kg, the diastase activity should be ≥3.

For honeys of a declared origin from regions with a tropical climate (and blends of these honeys) the HMF content should be ≤ 80 mg/kg. The diastase activity and HMF content are determined after processing and blending.

Table 2. Typical compositional criteria for Spanish and Greek PDO honeys.

Compositional criteria	MIEL DE LA ALCARRIA[i]	MELI ELATIS VANILIA-VYTINA[ii]	PEFKOTHYMAROMELO (PINE-THYME) KRITIS[iii]
Moisture content	≤17.5%	15%(14.0-15.5%)	≤17.0%
HMF	≤15.0 mg/kg		≤25 mg/kg
Free acidity	≤35.0 mg/kg		20-50 meq/kg
Electrical conductivity	≤0.62 mS/cm		≥0.60 mS/cm
Fructose+ Glucose			≥50 g/ 100g (50%)
Sucrose content	-	≥10 g/100g (8.0-18.0 g /100g	
Diastase			≥8 DN
Colorimetry	L*≥55.0;-2.0≤a*≤+22.0;hab≥74.0	-	-
Pfund (mm)	-	-	70-130
Non-soluble substances	-	-	0.1 g/100g

i:Official Journal of the European Union: Council Regulation (EC) No 510/2006, amendment application in accordance with article 9 "MIEL DE LA ALCARRIA" EC No: ES-PDO-0117-0079-22.09.2010. C302/24-30 (06.10.2012), ii:Ministerial Decision No. 313049, Recognition of Protected Designation of Origin (P.D.O.) for Honey 'ELATIS MAINALOU VANILIA', 1994. (O.J.no. 16/14-01-94), iii: Official Journal of the European Union: PEFKOTHYMAROMELO KRITIS, PDO-EL-02142-17.05.2016. C 108/20-22 (06.04.2017).

The usual method to determine the botanical origin of honey is pollen recognition (melissopalynology). The usual process for qualitative melissopalynological analysis involves a 10g sample of each honey being diluted in 20ml of distilled water and centrifuged at 3000rpm for 10 minutes. The sediment of the solution should then be dried at 40°C and mounted on Entellan Rapid resin (Merck, 1.07961.0500). The honeydew elements and pollen grains are counted and identified in 20 optical areas at 200x magnification by using a light microscope (i.e., Olympus BX 40). The determination of a honey's botanical origin is based on the relative frequencies of nectariferous species (Von der Ohe et al., 2004). Pollen types from nectarless species are recorded and counted separately. In particular, only pollen-grain types with frequencies higher than 1% are considered (Figures 2a and 2b).

However, this approach has certain limitations such as the pollen-counting procedure and identification, the interpretation of results (which is difficult and requires trained analysts), the considerable amount of time it takes to perform analysis, and the limited geographical-origin determination applications (Von der Ohe et al., 2004). Hence, the combination of pollen analysis with physicochemical and sensory characteristics can overcome the limitations of pollen analysis.

The high sucrose content of the PDO Greek fir-honey from Mainalon is due to its melezitose content which has an average value of 9g/100g. In terms of organoleptic characteristics, the fir honey from Mainalon-Vitina is characterized by a particularly good taste, a distinct appearance, a light colour and the sensation of shades, presenting a milky-pearl opacity (Ministerial Decision No. 313049, 1994).

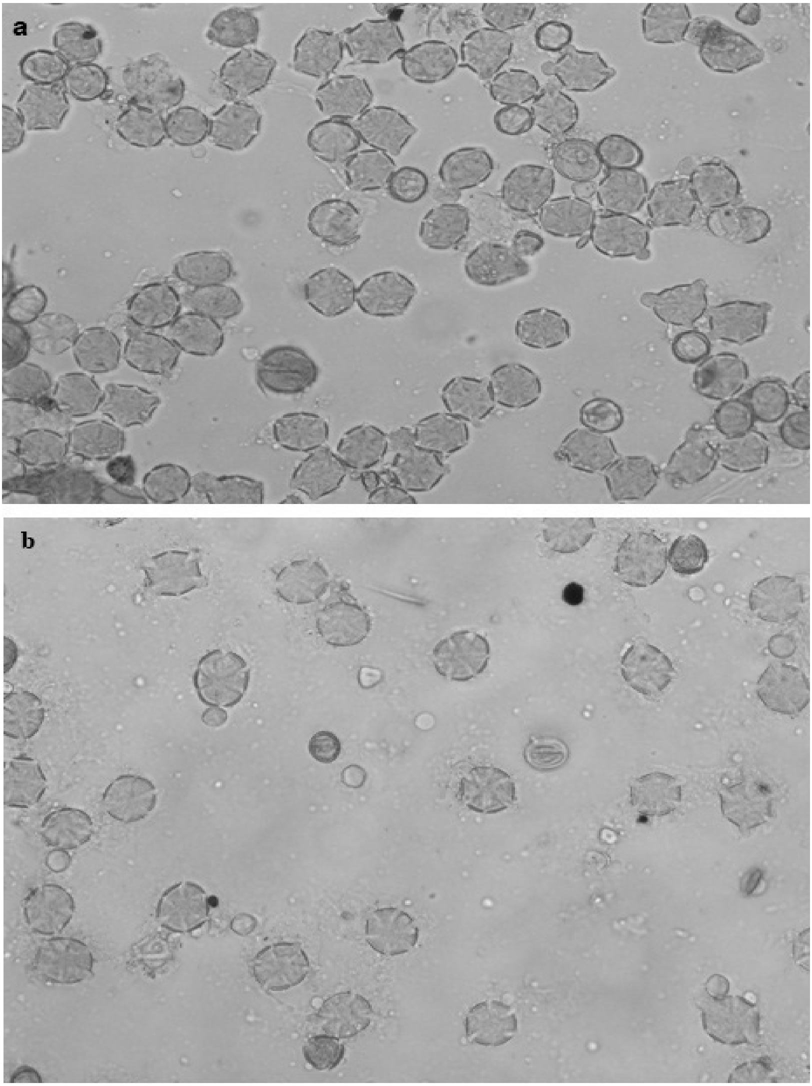


Figure 2 (a and b). Pollen loads of *Thymus capitatus* L. honeys from Greece.

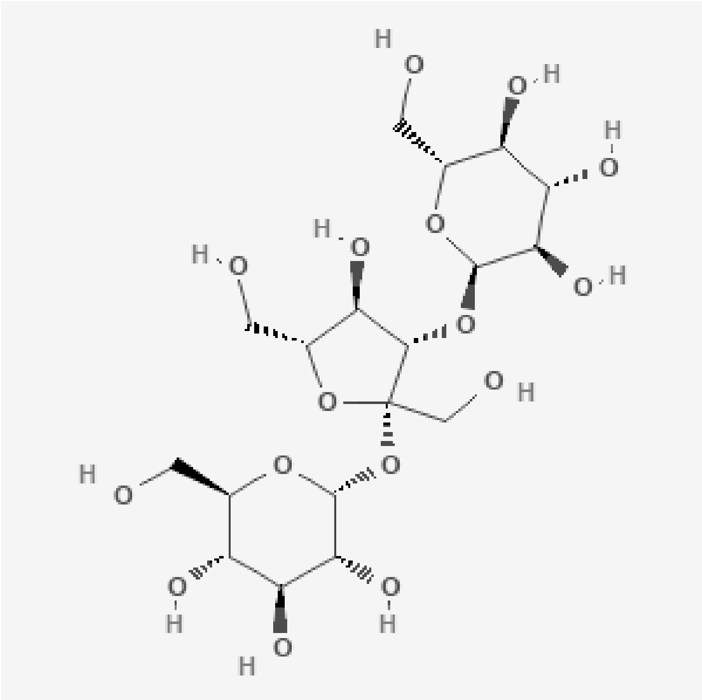


Figure 3. Structure of melezitose ($C_{18}H_{32}O_{16}$), a marker trisaccharide of the Greek PDO fir honey (Source: PubChem, 2019).

Pine-thyme honey from Crete, according to its pollenic characteristics, is classified under honeydew honeys (forest honeys-dasomela) but has the specificity to possess in its sediment, pollen grains from a considerable number of nectarous plants which can vary and can reach up to 20 different species in each sample of honey. The main species is *Coridothymus capitatus* (L.) or *Thymus capitatus* L., which is present in all honey samples at a percentage of $\geq$ 10% of the total pollen-like nectarous species. The ratio of honeydew elements/pollen grains (HDE/P) ranges from 0.5 to 6.5. The honeydew elements (HDEs) consist of spores of *Cladosporium* and *Fumago* and more rarely of *Altenaria* and *Stemphylium*. In honeydew, elements do not show the characteristic spores with the sharp edges of the *Coleosporium* species that appear in other honey that has been mixed with pine-tree honey.

The pollen characteristics of Miel de la Alcarria are related to the honey's type. In particular, this involves monofloral lavender-honey (lavender pollen >10%), monofloral rosemary-honey (rosemary pollen ≥15%), and multifloral honey, in which the total grains of thyme (*Thymus* t.), savory (*Satureja* spp.), rosemary and lavender pollen constitute ≥ 5%. Further attention should also be given to additional requirements: the percentage of grains of pollen from plants of the heath family (Ericaceae), except for common bearberry (*Arctostaphylos uva-ursi* L. *Sprengel*), is ≤ 1%. The percentage of grains of pollen from rockrose (*Cistus ladanifer* L.) is ≤ 3%. The percentage of grains of pollen from French lavender (*Lavandula stoechas* L.) is ≤ 3%. Finally, the total percentage of pollens from non-ornamental plants (excluding aromatic plants) cultivated in the production area is ≤ 15%.

The organoleptic characteristics of Mel de la Alcarria Spanish honey are listed in Table 3.

Table 3. Organoleptic characteristics of the Spanish PDO honeys, Miel de la Alcarria.

Organoleptic characteristics	Monofloral rosemary honey	Monofloral lavender honey	Multifloral honey
Colour	From extra-white to light amber	From extra-light amber to amber	From extra-light amber to amber
Aroma	Subtle floral aroma. Slight to average intensity and persistence	Aromatic, with balsamic notes. Average to high intensity and persistence	Very varied. Fruity, aromatic, warm, subtle, animal, from a fairly intense flowery aroma to a fresh green plant aroma. Varying intensity and persistence.
Taste	Sweet with acid notes. Slight to average intensity and persistence. Slight aftertaste	-	-
Flavour	-	Sweet with varying levels of acidity. Average to high intensity and persistence. Intense aftertaste.	Sweet with varying levels of acidity. Varying intensity and persistence. Generally fresh aftertaste.

The Greek National Decision 127/2004 sets qualitative criteria for domestic monofloral honeys that are about to be marketed (Table 4).

Table 4 . Qualitative characteristics of Greek monofloral honeys according to National Decision 127/2004.

Physicochemical and pollenic parameters	Pine	Fir	Chestnut	Erica	Thyme	Orange blossom	Cotton	Helianthus
Moisture (%)	-	≤18.5	-	-	-	-	-	-
Electrical conductivity (mS/cm)	≥0.9	≥1.0	≥1.1	-	≤0.6	≤0.45	-	
Dominant pollen grains of nectarous plants (%)	-	-	≥87	≥45	≥18*	≥3	≥3	≥20

HDE/P	Varying	Varying	-	-	-	-	-	-
PK/10 g	Varying, important presence of honeydew elements (fungi+smoky items)	Simple presence of honeydew elements (fungi)	≥100000	-			<90000	<55000

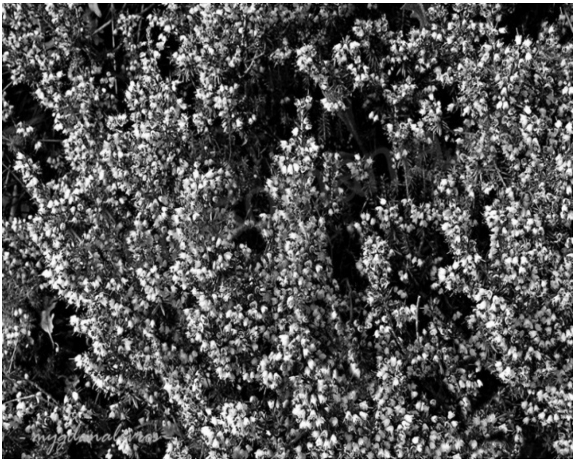
*. The percentage of accompanying pollen grains of plant species (rather than *Thymus* spp.) should not exceed 45%. **. Honeydew elements/Pollen. ***.Total number of pollen grains per 10g of honey sample.

Rhododendron spp.(Ericaceae)

Arbutus unedo(Ericaceae)

Borago officinalis (Boraginaceae)

Thymus capitatus (L.) (Lamiaceae)



Erica spp. (Ericaceae)

Resin substances on a pine tree

Figure 4. Characteristic plant types and honeydew secretions used for honey production by honeybees (Source: Wikipedia, 2019).

NUTRITIONAL VALUE

In a 100g serving, honey provides 304 kilocalories with no essential nutrients in significant content (USDA, 2018). It is mainly composed of 17% water and 82% carbohydrates and has low amounts of fat, dietary fibre, and protein. Honey is mainly fructose (about 38%) and glucose (about 32%), with remaining sugars including maltose, sucrose, and other complex carbohydrates. Its glycemic index ranges from 31 to 78, depending on the variety (Arcot and Brand-Miller, 2015). The specific composition, colour, aroma, and flavour of any batch of honey depends on the flowers foraged by the bees that produced the honey.

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