

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/304012435>

# Beeswax: Production, Properties, Composition, Control

Article · April 2016

---

CITATIONS  
10

READS  
11,607

**1 author:**



**Stefan Bogdanov**

Bee Product Science

124 PUBLICATIONS 5,874 CITATIONS

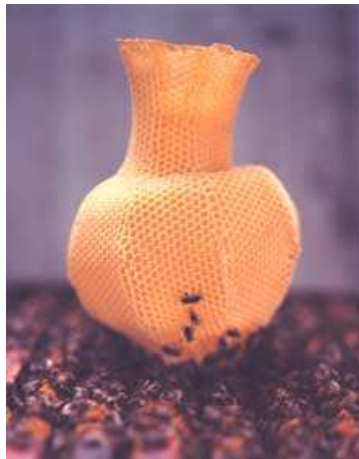
SEE PROFILE

# Beeswax



*The honeybags steal from the humble-bees  
And for wax tappers crop their waxen thighs  
And light them at the fiery glow-worms' eyes  
To have my love to bed, and to arise*

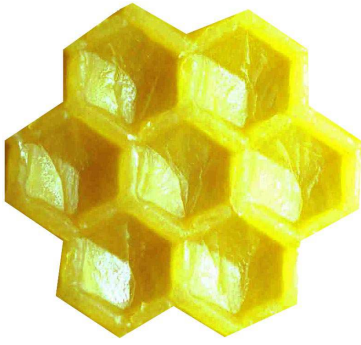
William Shakespeare, *Midsummer Nights Dream*, Act III



Be aware that this online book is only for private use and should not be copied and reprinted as some of the images are not copyrighted.

I would appreciate your feedback at [info@bee-hexagon.net](mailto:info@bee-hexagon.net)

Stefan Bogdanov, Muehlethurnen, Switzerland



# Beeswax: Production, Properties Composition and Control

Stefan Bogdanov

## BEES PRODUCE WAX

Bees need wax as construction material for their combs. They produce it in their wax glands, which are fully developed in 12 to 18 days old workers. In older bees the wax glands diminish their activity. However in emergency situations wax-synthesis can be reactivated. Greatest quantities of wax are produced during the growth phase of bee colonies, under moderate climate conditions during April to June. A bibliography on the synthesis of beeswax is given in the monograph of Hepburn <sup>15</sup>.

The main raw materials for wax formation are carbohydrates, i.e. the honey sugars fructose, glucose and sucrose <sup>55</sup>. The ratio of sugar to wax can vary from 3 to 30:1, a ratio of around 20:1 being typical for central Europe <sup>55</sup>. The stronger the colony, the smaller the ratio, the more economical the wax production for the colony. One Langstroth frame, containing only 100 g of wax can hold 2-4 kg of honey.

Wax production and comb construction activity in the bee colony are determined by following factors:

- **Nectar flow:** the greater the flow, the more combs are needed for storage.
- **Brood rearing (egg laying):** the more eggs are laid, the more comb cells are needed.
- **The presence of a queen:** only colonies with a queen build combs.
- **Temperature:** temperatures higher than 15° C favour comb building activity
- **The presence of pollen as a protein source**



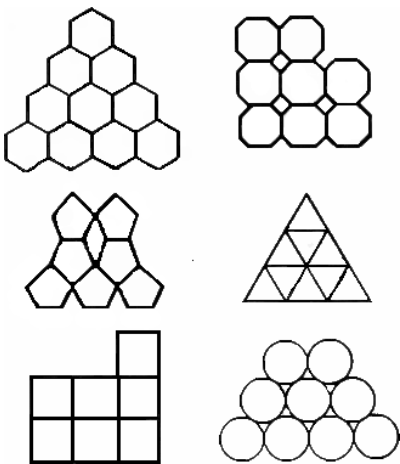
Building of swarms is a good way to make bees produce new wax

The wax economy of bees seems to function according to the supply-demand principle: there is no unnecessary wax production!



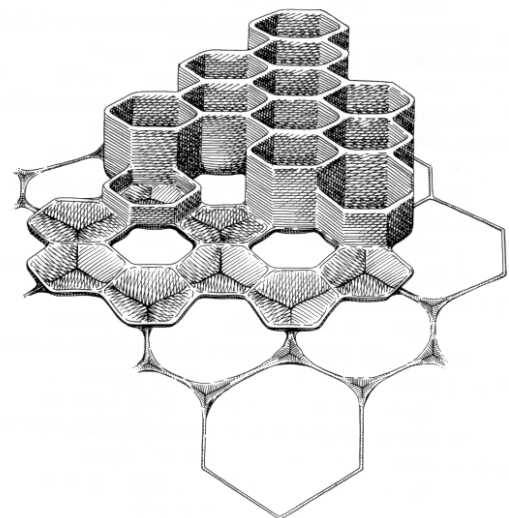
*Apis mellifera* bees produce the wax in their specialized wax glands on the ventral side of the abdomen(right photo). A bee has four pairs of glands. The liquid wax is delivered by these glands and cools down immediately to fine, white wax scales (left). These scales are taken by the hind legs and processed with the mouth tools. A wax scale weighs about 1 mg, so that 1 million of scales are needed to 1 comb. More details on the biology of beeswax production in the bees are given elsewhere <sup>15</sup>.

### The comb hexagon – an ideal form for the honey combs



A hexagonal shape of the combs cells are optimal regarding spent material ensuring a maximum strength. One gram of wax will serve for the construction of 20 cm<sup>2</sup>. Recently the mechanism of the building of combs has been elucidated <sup>34</sup>. From a mathematical point of view it seems that bees have also intuitively chosen the best possible form <sup>33</sup>. The comb is not only the place for storage of honey, pollen and the cradle and house of the larvae, but it serves also as a communication net for the honey bee colony <sup>45-47</sup>.

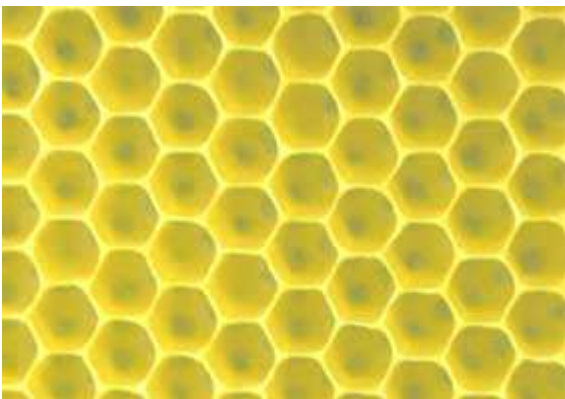
Honey combs are built with amazing precision. *Apis mellifera* worker cells are 5 to 6 millimeters in diameter and are about 0.25 mm in thick. A single cell of honey comb has a hexagonal shape. There are mathematical arguments, why the bees have chosen to build hexagon combs cells. The diameter of *Apis mellifera* cells varies between 5.1 to 5.5 mm. Drone cells have a diameter varying between 6.2 and 6.9 cells. All European races accept foundation wax with 750 to 950 cells/dm<sup>2</sup>. The diameter of the cells of the different bee races differs more. The nest of a honey bee colony with about 30000 workers comprises an area of about 2.5 m<sup>2</sup> (double sided), weighing about 1.4 kg and containing 100000 cells <sup>39</sup>. A standard Langstroth deep frame can hold 1.8-3.8 kg of honey, the wax necessary to produce these 7100 cells weighing only 100 g <sup>37</sup>. An individual beeswax scale weights only 1.1 mg so that 910,000 are necessary for 1 kg of wax. About 1 billion of scales are necessary for the



construction of the 2.5 m<sup>2</sup> combs surface present in the bee colony nest. The topic of cell building has been extensively reviewed in chapter 9 of Hepburn's book on beeswax<sup>15</sup>.

## MANAGEMENT OF COMBS

Successful comb management is an important part of the beekeeping practice. Combs used for brood rearing change in different respects. The comb colour turns from yellow to brown and black. The dark colour of old combs is caused by larvae excrements, pupae skins and from propolis rests. The properties of the combs change too: cells become smaller and thicker. These changes result in the production of smaller bees (see table..). Apart from these changes old combs are sources of infections. Honey, stored in dark combs will also get dark and dirt particles will contaminate it. Feed will also crystallise more readily in old combs, thus making hibernating more difficult<sup>27</sup> Old combs contain less wax and more protein and will be more readily attacked by the wax moth.



### Changes in combs with increasing number (n) of bee colony generations , after<sup>27</sup>

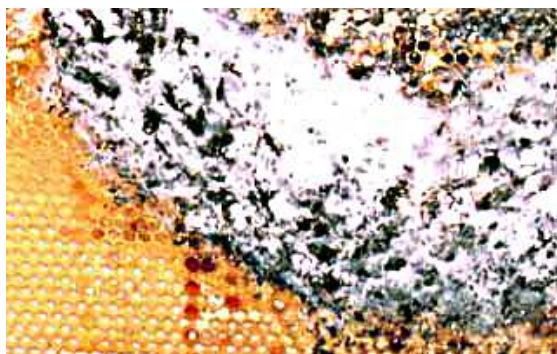
n	comb colour	cell volume cm <sup>3</sup>	comb thickness mm	cell diameter mm	bee mass mg	% wax
0-1	yellow	0.282	0.22	5.42	123	86-100
2-5	brown	0.269	0.40	5.26	120	60
6-10	dark-brown	0.255	0.73	5.24	118	49
13-15	black	0.249	1.08	5.21	106	46

Each year beekeepers should discard old combs out of the hive, thus stimulating bees to build new combs, by giving at least 2-3 foundations per colony. Brood combs should be exchanged at an interval of about 2-3 years.

The raw products for wax manufacture are old combs and capping. Thus, all old combs and pieces of wax should be saved for rendering into wax blocks. Old combs should be rendered separately from newer ones since the newer combs yield a higher quality wax. The price for old combs depends on the age of combs: the darker the comb, the lower the wax content and the price. Cappings, containing almost exclusively pure wax, achieve the highest prices. Dark combs contain propolis and cocoons which lower the quality of the wax. Honey should be preferably removed from the stored combs, this will prevent eventual fermentation and moulds. Old combs, free of sugar feed and honey should be packed in plastic bags and be given to wax manufactures for recycling into pure wax as soon as possible. Thus the beekeeper can avoid problems with the wax moth and with moulds, which arise often when storing combs. It is safer to recycle combs into raw wax by a sun wax melter (see figure).

**Control of wax moths in stored combs, after <sup>9</sup>.**

Method		Details, remarks
<b>Technical</b>	<b>Sort comb</b> <ul style="list-style-type: none"> <li>• Immediately melt old wax</li> <li>• Storage in a cool, light and airy place</li> </ul>	Simple, no residues
<b>Physical</b>	<b>Cool storage (&lt;15°C)</b> cellar or cool place, good air circulation in comb stack <ul style="list-style-type: none"> <li>• <b>Frost treatment</b></li> <li>• 2 hours at -15°C or</li> <li>• 3 hours at -12°C or</li> <li>• 4.5 hours at - 7°C</li> <li>• <b>Heat treatment</b></li> <li>• 80 minutes at 46°C</li> <li>• 40 minutes at 49°C</li> </ul>	Effective, needs infrastructure; a long term method.  Effective, kills all moth stages, needs expensive infrastructure  good air circulation and accurate temperature control necessary effective, kills all stages needs infrastructure (warm air blower);risk of wax melting
<b>Biological</b>	<b>Bacillus thuringiensis:</b> Melonex, B-401	<ul style="list-style-type: none"> <li>• Observe instructions and sell-by-date and storage conditions:</li> <li>• No residues, long-term effect (2-3 months), affects also the lesser wax</li> <li>• labour intensive</li> </ul>
<b>Chemical</b>	<b>Sulphur</b>	<b>Burning</b> 1 sulphur strip per 100 litres (about 3 DB supers); treat every four weeks from above (SO <sub>2</sub> heavier than air); do not breathe in vapours (respiratory and eye irritant). controls moulds at pollen conservation regular repeats necessary; ineffective against eggs;- fire danger
	<b>Acetic acid</b>	<b>spray SO<sub>2</sub> from spray can</b> <ul style="list-style-type: none"> <li>• 1 second (=2.5g SO<sub>2</sub>) per honey super or</li> <li>• 3-4 seconds per 100 litres hive volume</li> <li>• Treatment from above with 200ml acetic acid (60-80%) per 100 litres per hive volume (vapours heavier than air); in summer repeat treatment 2 times with an interval of 2 weeks;</li> <li>• effective, no problematic residues; kills all moth stages; kills Nosema spores; attacks metal parts, regular repeating necessary.</li> <li>• do not breathe in vapours, avoid contact with skin caution when handling.</li> </ul>
	<b>Formic acid</b>	<ul style="list-style-type: none"> <li>• Treatment from above with 80ml formic acid (85%) per 100 litres hive volume, in summer, treatment repeated 1-2 times with an interval of 2 weeks; regular repeats necessary</li> <li>• Effective; no problem residues, kills all moth stages , attacks metal parts, caution when handling, do not breathe in vapours, avoid contact with skin</li> </ul>



Storage of combs at warm temperatures results in damage by the wax moth (left)

## Control of the wax moth

Combs but not pure beeswax, are highly susceptible to damage by the Greater wax moth *Galleria mellonella* L. In order to control it effectively, different measures can be used. Pesticide control, e.g. with p-dichlorobenzene or naphthalene, should not be used because it leaves toxic residues in honey and wax<sup>5</sup>. The different control measures are summarised in the table above

## MANUFACTURE OF BEESWAX

Industrial wax production began in the 19<sup>th</sup> century. In 1857 Mehring from Germany started industrial productions of comb foundations<sup>11</sup>. The industrial production, is extensively described elsewhere<sup>11</sup>. Here we will show the principles of smaller scale productions units, as used in many European countries.

World-wide, beeswax is produced mainly by specialized beeswax manufacturers. Beekeepers provide either old combs or crude wax.

The good quality of beeswax depends greatly on the production methods. There are two wax extraction methods: melting and chemical extraction. Melting is the most frequently used procedure. Wax can be melted by boiling water, by steam, or by electrical or solar power. Chemical extraction by solvents is feasible only in a laboratory, where small scale wax production is needed. Good wax solvents are gasoline and xylene. The disadvantage of this method is that all organic wax contaminants and constituents of the pupae, propolis and pollen are dissolved. Thus the quality of wax can be impaired. This method is feasible only in a laboratory, where small scale wax production is needed.

The wax recovery depends on the combs and on the method used. Generally, recovery from old combs are around 50 %. If more cappings and new combs are used it could be higher. The comb debris or comb cake left after separation of pure wax contains still some wax (about 30 %). This rest can be removed by solvents, but this wax will not have the best quality. According to Temnov<sup>49</sup> beeswax in combs is in a free and bound state. When heating combs in sun melters and at temperatures below 100 °C only the free wax will be liberated. The bound wax can be liberated only by pressing or extracted by solvents.

During the manufacturing of wax formation water emulsions can be often built. There are two emulsion types: in the first one water particles are dispersed into wax, in the second one wax particles are dispersed into water<sup>43</sup>. These emulsions are built with the help of emulsifiers. Emulsifiers for the first type of emulsions are proteins and dextrans, contained in honey, pollen and salts of wax fatty acids with sodium and potassium. The second type of emulsion is caused by the salts of wax fatty acids with calcium, copper and iron cations. Cations are contained in hard water, or diffuse out of the vessels, used for wax production. That is why soft water should be used, together with vessels from stainless steel. If emulsions are formed, they can be destroyed by letting wax for a longer time remain in the water bath at a temperature of 75-80°C.

Wax, produced by the comb cappings has the best quality, as far as general quality criteria are concerned. However, this wax does not have less pesticide residues than normal beeswax<sup>4</sup>

Beekeepers can produce raw beeswax in a simple and cheap way. Combs are placed on the sun melter and are melted directly by the sun heat. The melter should be directly towards the sun 2-3 times a day. This method is efficient and the energy is “free of charge”. This method is preferable for the production of raw wax, as comb storage can be avoided.

### Manufacturing methods for beeswax

- **Hot water extraction using forced immersion**

The combs are placed in a tightly tied jute sack. Place sacks in a recipient with water and boil. As wax is lighter than water, it will filter through the jute and rise to the surface. After all combs have all melted, let the pot cool down. The wax solidifies as it cools, forming a block on the water surface. Throw out waste left in the sack.

- **Extraction with boiling water and a wax press**

The combs placed into a 120 litre container with 20 to 30 litres of boiling water and left to melt. When all the wax has melted, remove the wiring and tip the contents into a jute-lined press, then start pressing.

- **Combined steam and press extraction**

A metal basket of old combs is plunged into a tank of boiling water, closed with a watertight cover. A piston, capable of exerting up to 15 T of pressure presses the combs, then tank is kept simmering for about one hour. Wax runs to the top of the tank.

- **Steam extraction**

Combs with frames are placed into a container where vapour is introduced. The trester is sieved, wax flows into the lower part of the container and can be collected. There are different commercial devices

- **Centrifugal extraction**

Combs are melted in boiling water and boiling mixture is poured into baskets of a centrifugal wax extractor, spinning at more than 1500 rpm, kept at temperatures over 65°C to prevent the wax from setting. Pure wax runs out of through an opening from the extractor. Method used for bigger manufacturing units, due to expensive installation.

- **Heat extraction with electric elements**

Press combs or frames between two electrically heated metal plates. Plates are pushed together, the wax melting into a recipient.



Old combs are melted into wax blocks by a sun melter, an effective way of avoiding wax moth losses. The melted wax must be then further purified by specialized wax producers.



## Wax defects and how to prevent them

- **Wax darkening**
  - Do not heat at too high temperatures and for a too long time may damage the wax and darken its colour.
  - Wax should not be heated in containers made of iron, zinc brass or copper vessels because these metals make the wax turn dark. Do not use lead containers because of contamination. Stainless steel or aluminium, is suitable, but can be attacked by oxalic acid. Wooden containers can be suitable, if acid treatment is involved.
- **Wax off odour**

Do not melt combs containing fermented honey
- **Contamination by *Paenibacillus larvae* larvae**

heat-resistant spores of *Paenibacillus larvae* larvae are not killed by boiling of wax in water. Only heating under pressure (1400 hPa) at 120°C for 30 minutes kills all spores<sup>29</sup>.
- **Water-wax emulsions**
  1. the wax-water appears milky, due to the presence of calcium or iron in the water  
Use 2-3 g of oxalic acid per kg wax and 1 l of water to bind calcium, prevent emulsion and to brighten wax at the same time.
  2. wax absorbs a greater amount of water: heat wax at 105 °C to remove water.
- **Wax has a crummy structure**

This is due to saponification of wax. The process can be reverted by boiling wax with sulfuric or oxalic acid. Use soft water to prevent this, e.g. rain water. Water with a low mineral content should be used if such problems arise. However, in some cases, water/wax emulsions can occur, even with soft water. In such cases, raw molten wax in contact with water should be kept below 90°C.
- **Incorporation of water.**

Water is often incorporated in the process of wax manufacture. Surplus water can be removed by heating at about 105 °C. Prevent foam building by defoaming agents (e.g. silicon). When no more bubbles rise, the wax is free of water.
- **Impure wax**

After melting the wax is not pure enough. For additional cleaning heatable water tanks from high-grade steel are suitable. The wax should remain for longer time in the water bath at a temperature of 75-80°C (best over night). Since wax is lighter than water, it floats. The dirt sinking at the lower part of the wax must be scraped off after cooling. Under industrial conditions liquid wax can be cleaned by filtration with heated chamber filters. Wax can also be purified by hot filtration.

**ATTENTION: When using chemicals of the kind described above, use protecting gloves and goggles, as well as protective clothing.**



Dark wax (right) which was bleached by boiling the wax with diluted oxalic acid: boil 1 kg wax, 1 l water and 2-3 g of oxalic acid anhydride

## Wax brightening and bleaching

### Acids

Acids will bind a part of the iron which is responsible for wax darkening. Also they help breaking of emulsions and help the settling of impurities.

e.g. add 2-3 g concentrated citric acid or oxalic acid, or 1 ml concentrated sulfuric acid to 1 l of water per kg wax and (*add acid to water and not vice-versa!*).

### Hydrogen peroxide solution

Add concentrated hydrogen peroxide solution (about 35 % in basic milieu) to hot wax (100 °C). It is essential that the peroxide is used up in the bleaching process. Excess peroxide could cause problems in the manufacture of creams and ointments

### Sun bleaching

Bleaching in a solar extractor will lighten the colour of the wax. In order to achieve bleaching, the wax should be exposed to the sun for several days.

### Potassium permanganate

Heat wax at about 90 °C for 30 minutes in 0.01 % potassium permanganate in slightly acidic milieu. Exchange solution with water.

**Do not use complexing agents** because they are problematic from ecological point of view



**Small scale wax producing units:**

Combs with frames are placed into a container where vapour is introduced. The trester is sieved, wax flows into the lower part of the container and can be collected. In this device up to 36 combs with frames can be melted within 20 minutes. The generator already produces steam after 30 seconds steam.

## Industrial purification

For industrial purposes beeswax will be purified by filtration and centrifugation. A plate and frame press is suitable. Tightly woven cotton cloth, canvas or paper filters can be used. Paper filters can be disposed of after usage. Filtration is carried out under pressure. Filtration is extensively described elsewhere<sup>11</sup>.

## Wax purification in small scale production

After melting the wax is not pure enough. For additional cleaning heatable water tanks from high-grade steel are suitable. The wax should remain for longer time in the water bath at a temperature of 75-80°C (best over night). Since wax is lighter than water, it floats. The dirt sinking at the lower part of the wax must be scraped off after cooling and only the pure upper layer of wax should be kept. Let the wax cool down as slowly as possible and to avoid all movement of the container during cooling.

## Storage

Wax blocks are dried and stored in a dark and cool place. They can be stored in wrapping paper, placed on shelves or in containers made of stainless steel, glass or plastic, for best preservation of colour and aroma. This will keep of building of “dust”, which is supposed to be a salt of wax fatty acids<sup>49</sup>. This dust will be eliminated by liquefying the beeswax or storage in a warm room.

The hardness and the rigidity of beeswax increases upon storage. Within a storage time the coefficient of hardness increases by 61 to 74 %<sup>49</sup>.

Upon a longer storage beeswax is covered by a whitish layer soluble in organic solvents, probably a salt of unsaturated organic acids with a melting point of 35-36°C<sup>48</sup> or according to another Russian author Chudakov this product has hydrocarbon-like properties<sup>10</sup>.

## Production of comb foundations

Combs are produced basically by two methods: by sheeting and by casting (milling).



*Sheeting of beeswax* was the first method used in production of foundations. In a first stage wax sheets are produced and in a second the foundations are produced by calendering. The wax sheet is run through a foundation mill, which will print the foundations. Foundations produced by sheeting and milling is today the preferred method world-wide.



*Casting or Milling of wax* will produce foundations that are more brittle in the cold than milled sheets. Cast foundations are produced mainly by beekeepers, as this method is easy to perform in small beekeeping units.

*Photos courtesy G. Ratia*

## PROPERTIES AND COMPOSITION

### Beeswaxes from different honeybees

Publications on the physical constants for the comb waxes of Asian and European beeswaxes first appeared a century ago. It was soon shown that carbon chain length was, on average, shorter in the Asian beeswaxes than in *A. mellifera*, which explains the lower melting points of the former. The Asian waxes are more similar to one another than to *A. mellifera*. In Asian beeswaxes, the amounts of C31 and C33 in the pool of free fatty acids are reduced, but C25 hydrocarbons are increased compared to that of *A. mellifera*. The major compound families in beeswax are alkanes, alkenes, free fatty acids, monoesters, diesters and hydroxymonoesters, while fatty alcohols and hydroxydiesters are minor constituents. There are notable species-specific differences in the beeswaxes among honeybee species, but all share a complex mixture of homologous neutral lipids<sup>16</sup>

The wax produced by different species of *Apis mellifera*, and also of African *adansoni* wax, have the same composition but some components are in a different proportion<sup>3,7</sup>. The waxes of Asian bees *Apis flavae*, *Apis dorsata* and *Apis cerana*, are called Ghedda. The composition of wax from Asian honeybee species is much simpler and contains fewer compounds in different proportions<sup>26</sup> The different Ghedda waxes resemble each other much more, than any of them to the *Mellifera* waxes<sup>51</sup>. Thus, Ghedda waxes cannot be used as substitutes for *Apis mellifera* wax. Ghedda waxes from the Asian honeybee species are described as softer and more plastic, but do not have a significantly different melting point<sup>54</sup>.

The beeswax composition seems to vary also within the bee hive. There are different subtypes of beeswaxes in the bee colony serving as cues for bees to recognise bases, sexes and comb age<sup>13, 14</sup>

### Other waxes

Besides beeswax there are other ones, the most frequent are:

**Jojoba**, produced from the jojoba plant; **carnauba**: made from the leaves of the carnauba plant; **lanolin**, made of lamb wool. Beeswax has generally a melting point which is about 10-20 °C lower than other waxes. According to Tulloch this difference is due to the large number of different compounds found in beeswax<sup>51</sup>. This property permits the bees to use a softened material in the beehive and is also very useful in the uses in different crafts.

### Properties

The colour of the freshly produced beeswax is white, later it turns to yellow. The typical yellow colour originates from propolis and pollen colorants. However, depending on the relative amounts of different pollen and propolis pigments, wax colour can vary (see for review<sup>11</sup>. Beeswax has a typical odour, originating from bees, honey, propolis and pollen.

The colour of newly made beeswax is white and it changes with the length of use to yellow, dark yellow and brownish. The yellow colour is due to colourants originating from propolis and pollen, while the brown colour is due to the pigments of the larval excrements. The taste of beeswax is normally pleasant and is not specific – any unpleasant taste is a sign of quality deterioration due to foreign matter.

The structure of beeswax is crystalline. The crystallisation of beeswax depends on the the storage. The crystallisation process increases upon storage of wax until 3-4 months, while at the same time, its stiffness and elasticity increase. The mechanical properties of wax are important in connection with its use as “the house of the bees”. Fresh “scale wax has a greater strength and extend to greater extent upon strain than and is less stiff than comb wax, differences are due to the different physical structure and also of the chemical composition of these two types, see p. 84-88 of Hepburn’s Wax Book<sup>15</sup>. The hardness of beeswax is an important quality factor – the harder the wax, the better the wax quality.

Beeswax is an inert material with high plasticity at a relatively low temperature (around 32 °C). By contrast, at this temperature most plant waxes are much harder and of crystalline structure. Upon heating the physical properties of wax change. At 30-35 °C it becomes plastic, at 46-47°C the structure of a hard body is destroyed and between 60 to 70°C it begins to melt. Heating to 95-105 °C leads to formation of surface foam, while at 140°C the volatile fractions begin to evaporate. After cooling down beeswax shrinks by about 10 % Heating at 120°C for at least 30 minutes causes an increase of hardness due to the removal of the remaining water. The above information is taken from page 91 of a Bulgarian book on bee products<sup>42</sup>.

Beeswax is also insoluble in water and resistant to many acids. It is soluble in most organic solvents such as acetone, ether, benzene, xylol, toluene, benzene, chloroform, tetrachlormethane. However in at room temperature it does not fully dissolve in any of these solvents, but upon heating above the wax melting point it is readily soluble in all of them, and also in ethanol.

### Sensory properties of beeswax:

• <b>Colour</b>	yellow to yellow-brown
• <b>Odour</b>	heat wax , odour should be pleasant and honey-like.
• <b>Chewing test</b>	wax should not stick to teeth
• <b>Breakage test</b>	upon breaking should have a fine-granular, blunt, not crystalline structure
• <b>Cutting test</b>	wax should not stick to the knife
• <b>Splinters test</b>	scratch wax with nail or knife. Splinters should have a spiral form
• <b>Kneading test</b>	kneading for 10 minutes, wax should be plastic
• <b>Consistency</b>	should not stick upon cutting

Beeswax is an extremely complex material containing over 300 different substances<sup>51</sup>. It consists mainly of esters of higher fatty acids and alcohols. Apart from esters, beeswax contains small quantities of

hydrocarbons, acids and other substances. In addition, approx. 50 aroma components have been identified<sup>12</sup>.

The ratio of ester values to acids, a character used by the various pharmacopoeias to describe pure beeswax is changed significantly by prolonged or excessive heating. Heating at 100 °C for 24 hours changes the ratio of ester to acid beyond the limits set for pure beeswax. Longer heating or higher temperatures lead to greater degradation and loss of esters<sup>51</sup>. These changes also influence the physical characteristics of the wax. Thus, excessive heating during rendering or further processing changes the wax structurally and alters the beneficial characteristics of many of its minor compounds, not only the aromatic and volatile compounds. Besides the lipophylic substances of which wax is composed, there are also some proteins, which are added by the bees<sup>28</sup>. However, the ratio is not mentioned in the new 2008 European Pharmacopoeia.

### Composition of wax, after<sup>51</sup>

Component	Quantity %	Number of components in fraction	
		Major	Minor
Monoesters	35	10	10
Diesters	14	6	24
Triesters	3	5	20
Hydroxy monoesters	4	6	20
Hydroxy polyesters	8	5	20
Acid esters	1	7	20
Acid polyesters	2	5	20
Hydrocarbons	14	10	66
Free acids	12	8	10
Alcohols	1	5	?
others	6	7	?
<b>total</b>	<b>100</b>	<b>74</b>	<b>210</b>

## QUALITY CONTROL

With the collaboration of Hansjoachim Roth, [www.ceralyse.de](http://www.ceralyse.de)

The quality control of beeswax requires a great amount of specific knowledge and experience. The Ceralyse laboratory in Bremen, Germany, is the only laboratory in the world, specialized on the analysis and quality determination of beeswax.

Beeswax is specified in the Pharmacopoeia of different countries. Two types of wax are mentioned: white (cera alba) and yellow (cera flava), white beeswax - being defined as bleached yellow wax. Bleached wax has lost the colourants of normal beeswax and has not its pleasant odour. Beeswax is a natural product and no additives are permitted.

The quality control can be divided into 4 steps:

- Sensory Analysis
- Physico-chemical testing after the Pharmacopoeia
- Analysis of wax components by Gas Chromatography
- Analysis of residues

### Sensory Testing

The sensory properties, described on p. 10 are tested

### Physico-chemical testing after the Pharmacopoeia

There are different national Pharmacopoeia, which have only small differences. Official wax control is based mainly on the European and the American Pharmacopoeia. The International Honey Commission has proposed following criteria:

**Quality criteria for routine beeswax testing**

Quality Criteria	Value	Method
Water content	< 1%	DGF-M-V-2*
Refractive index, 75o C	1.4398-1.4451	EP**
Melting point	61-65° C	EP
Acid Number	17-22	EP
Ester Number	70-90	EP
Ester/Acid ratio	3.3-4.3	
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

EP - European Pharmacopoeia 7<sup>th</sup> - Edition, 2008 <sup>56</sup>

\*- wax from African and Africanized bees: max. 13.8%

**Physical properties of beeswax and artificial waxes, used as adulterants after** <sup>25, 32, 49</sup>

	Melting point °C	Density	Acidic number	Saponification number	Hardness ASTM D-5
Beeswax	61-65	0.950-0.965	17 – 24	87-100	15
<b>Artificial waxes</b>					
Ceresin	65-80	0.91-0.92	0	0	
Paraffin	45-70	0.88-0.91	0	0	
Stearin	52-55	0.89	205-209	-207-210	
<b>Natural waxes</b>					
Bayberry-myrtle	48-50	-0.875-0.980	4-30	205--217	7.5
Candelilla	65-69	0.97-0.99	-1-19	45-65	1.5
Caranday	82-85	0.99-1.00	3-10	62-80	1
Carnauba	.82-86	0.99-1.00	.2-11	-78-88	1
Castor bean wax	86	0.98-0.99	2	17	2
Esparto grass wax	78	0.99	24	70	1.5
Japan wax	50-56	0.97-0.99	6-20	217.237	
Montan crude wax	76-86	0.99-1.00	25-48	88-112	8
Ouricury	85	0.97-1.06	8-20	70-100	1
Retamo ceri nimbi	76-78	0.98-0.99	45-50	88	2
Shellac wax	72-86	0.97-0.98	2.25	45-85	2
Spermaceti	45-49	0.94-0.95	1	116-125	16
Sugar cane wax	75-79	0.98-0.99	6-10	25-35	3
Wool lanoline	31-42	0.92-0.96	1-40	80-140	

ASTM D-5 – standard penetration test, see [www.astm.org/Standards/D5.htm](http://www.astm.org/Standards/D5.htm)

Bee wax can be classified generally into European and Asian types. The ester/acid ratio is lower (3.3-4.3-) for European beeswax, and higher (8-9) in Asian beeswax. However, the quantity of Asian beeswax has decreased in recent years.

Determination of the sensory and physico-chemical characteristics according to the Pharmacopoeia is not a safe adulteration proof but in some cases can give hints on possible adulteration. If the values obtained are outside the limits, further analysis by gas and column-chromatography, should be carried out.

The ratio of ester values to acids, a parameter determined in the pharmacopoeia gives information whether pure natural beeswax is changed significantly by prolonged or excessive heating. When heating wax at 100°C for 24 hours the ratio of ester to acid is changed beyond the limits set for pure beeswax. Longer heating or higher temperatures lead to greater degradation and loss of esters <sup>51</sup>. These changes also influence the physical characteristics of the wax. Thus, excessive heating during rendering or further wax processing changes the structure of wax and alters the beneficial characteristics of many of its minor compounds, not only the aromatic and volatile compounds.

Determining the saponification cloud point is an easy, sensitive method for determining adulteration with hydrocarbons<sup>56</sup>. The method is limited to detecting quantities greater than 1 % of high melting (80-85 °C) paraffin waxes, or more than 4-5 % of low melting (50-55 °C) paraffins. If the solution becomes clear at or below 65<sup>0</sup> C, the wax is probably unadulterated with paraffin. If it is adulterated, the solution will turn clear only at a higher temperature. Some of the details of this test are described by Tulloch<sup>50</sup>. The saponification cloud point is not suited to detect adulteration with carnauba wax, but gas liquid chromatography (GLC) can detect the 6% of free C-32 alcohols (an alcohol with 32 carbon atoms) contained in Carnauba wax. Beeswax only contains very little of these alcohols<sup>51</sup>

## Gas Chromatography and other modern methods

The current quality criteria for pure beeswax according 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 it's reliable determination but are still used today because they are easy to carry out<sup>2</sup>. 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, best combined with MS detection<sup>1,6,8,20-24, 36, 40, 41</sup>.

All beeswax hydrocarbons are of uneven C-number. The presence of hydrocarbon adulterants, like paraffin and ceresin, containing even numbered hydrocarbons can thus be easily detected.

The most common sources are:

- Hydrocarbons from paraffins and microwaxes
- Triglycerides from palm, fat and hardened beef tallow
- industrially produced fatty acids (palmitic, stearic acid); long chain alcohols (C16-C18) and C32-C36 synthetic esters

The Ceralyse laboratory has developed a GC method for the detection of all adulterants.

High-temperature gas chromatography and subsequent chemometric analysis was found to have a superior discriminative power than GC alone<sup>31</sup>

A novel, direct, reagent-free method for the detection of beeswax adulteration by paraffin, microcrystalline wax, tallow and stearic acid using single-reflection attenuated total reflectance mid-infrared spectroscopy was developed, allowing the detection of a minimum of 5% paraffin/microcrystalline wax and tallow adulteration and 0.5% stearic acid adulteration of beeswax to be detected. The upper and lower critical limits for beeswax authenticity were established from the analysis of virgin beeswax and were validated by independent analysis of real sheet and comb beeswax samples using high-temperature gas chromatography with flame-ionization detection. In addition to its simplicity with respect to sample handling, the amount of sample and the time needed are far less than those required in previously described methods, which are based on chemical analysis and chromatographic techniques<sup>30</sup>

FTIR-ATR can be successfully used in paraffin oil adulteration testing<sup>44</sup>

## Contaminants

Beeswax may contain fat-soluble pollutants. Only traces of different environmental pesticides are generally detected. Beeswax is contaminated mainly by lipophylic acaricides applied in beekeeping. Residue levels of different acaricides in the range between 0.5 and 10 mg/kg are found in commercial wax<sup>38,53</sup>.

For its use in cosmetics and pharmaceuticals, beeswax should contain minimal amounts of contaminants. For uses as a food additive there are no specific wax specifications the same MRL as the ones valid for honey should theoretically apply.

Other fat-soluble substances used in beekeeping, such as p-dichlorobenzene, used, against wax moths can also contaminate beeswax<sup>5,52</sup>.

Another potential problem for the quality of beeswax, used for beekeeping is the content of *Penibacillus larvae* spores. Indeed, only heating of wax at 140 °C for 30 minutes will destroy the spores<sup>29</sup>. Heating of pure wax at such high temperatures might cause overheating. Heating under pressure of water-wax mixtures in pressure pots is another possibility to sterilize wax for small scale wax production. In practice only very few wax manufacturers sterilise wax by this procedure. On the other hand, experiments have shown, that only very high contamination with spores might cause American Foul Brood (AFB). In this work it was concluded, that normal contamination with spores of commercial beeswax is not likely to cause AFB<sup>35</sup>

### Preventive measures against contamination

Acaricides cannot be removed from wax by chemical means because of their different chemical structure. The best strategy to improve wax quality is to use non toxic natural organic acids in alternative varroa control<sup>18</sup>. It has been found that residues of synthetic acaricides can be reduced rapidly below the detection limits by exchanging the old contaminated foundations by residue free ones<sup>17, 19</sup>. The contaminants, used for the control of wax moths (e.g. p-dichlorobenzene and naphthaline) can be avoided by using alternative control measures (see table on p. 4). Contaminant free beeswax can be obtained only in organic beekeeping and in countries where bee diseases are not treated with chemicals (e.g. Africa).



**Further reading on beeswax:** <sup>11, 15, 32, 51</sup>

#### References

1. AICHHOLZ, R; LORBEER, E (2000) Investigation of combwax of honeybees with high-temperature gas chromatography and high-temperature gas chromatography-chemical ionization mass spectrometry, II: Chemical ionization mass spectrometry. *Journal of Chromatography.A* 883 (1/2): 75-88.
2. BERNAL, J L; JIMENEZ, J J; DEL NOZAL, M J; TORIBIO, L; MARTIN, M T (2005) Physico-chemical parameters for the characterization of pure beeswax and detection of adulterations. *EUROPEAN JOURNAL OF LIPID SCIENCE AND TECHNOLOGY* 107 (3): 158-166.
3. BEVERLY, M B; KAY, P T; VOORHEES, K J (1995) Principal component analysis of the pyrolysis mass spectra from African, Africanized hybrid, and European beeswax. *J.Anal.Appl.Pyrolysis* 34: 251-263.
4. BOGDANOV, S; IMDORF, A; KILCHENMANN, V; GERIG, L (1990) Rückstände von Fluvalinat in Bienenwachs, Futter und Honig. *Schweizerische Bienen-Zeitung* 113 (3): 130-134.
5. BOGDANOV, S; KILCHENMANN, V; SEILER, K; PFEFFERLI, H; FREY, T; ROUX, B; WENK, P; NOSER, J (2004) Residues of p-dichlorobenzene in honey and beeswax. *Journal of Apicultural Research* 43 (1): 14-16.
6. BOSELLI, E; CABONI, M F; LERCKER, G; MARCAZZAN, L P; SABATINI, A G; BAGGIO, A; PRANDIN, L (2002) Valutazione di produzioni apistiche: gelatina reale e cera, In Sabatini, A G; Bolchi Serrini, G; Frilli, F; Porrini, C (eds) *Il ruolo della ricerca in apicoltura*, Litosei; Bologna; pp 321-329.



7. BRAND-GARNYS, E E; SPRENGER, J (1988) Bienenwachs - Neue Aspekte eines klassischen Kosmetik-Rohstoffes. *Z.Körperpflegemittel-, Parfümerie-, Riechstoff- und Aerosol-Industrie* 61 (14): 547-552.
8. BRÜSCHWEILER, H; FELBER, H; SCHWAGER, F (1989) Bienenwachs - Zusammensetzung und Beurteilung der Reinheit durch gaschromatographische Analyse. *Fat science technology* 91 (2): 73-79.
9. CHARRIÈRE, J D; IMDORF, A (1997) Schutz der Waben vor Mottenschäden. Weiterbildungskurs für Berater. *Mitteilung des Schweizerischen Zentrums Bienenforschung* (24): 1-14.
10. CHUDAKOV, V (1965) *Technology of beeswax (in Russian)*. Mosovksi rabochij Moscow
11. COGGSHALL, W L; MORSE, R A (1984) *Beeswax. Production, harvesting and products*. Wicwas Press New York New York
12. FERBER, C E M; NURSTEN, H E (1977) The aroma of wax. *Journal of the Science of Food and Agriculture* 28: 511-518.
13. FROHLICH, B; RIEDERER, M; TAUTZ, J (2001) Honeybees discriminate cuticular waxes based on esters and polar components. *Apidologie* 32 (3): 265-274.
14. FRÖHLICH, B; TAUTZ, J; RIEDERER, M (2000) Chemometric classification of comb and cuticular waxes of the honeybee *Apis mellifera carnica*. *Journal of Chemical Ecology* 26 (1): 123-137.
15. HEPBURN, H R (1986) *Honeybees and wax, an experimental natural history*. Springer-Verlag, Berlin Berlin
16. HEPBURN, H R; PIRK, C W W; DUANGPHAKDEE, O (2014) The Chemistry of Beeswax *Honeybee Nests*, Springer; pp 319-339.
17. IMDORF, A; BOGDANOV, S; KILCHENMAN, V (2004) Wachsumstellung im Rahmen der Bioimkerei. *Schweizerische Bienen-Zeitung* 127 (11): 15-18.
18. IMDORF, A; CHARRIÈRE, J D; MAQUELIN, C; KILCHENMANN, V; BACHOFEN, B (1996) Alternative Varroa control. *American Bee Journal* 136 (3): 189-193.
19. IMDORF, A; KILCHENMANN, V; KUHN, R; BOGDANOV, S (2002) Wachsumstellung in der Bio-Imkerei. Kontaminationsgefahr durch Rückstände auf den Kastenwänden ? *Mitteilung des Schweizerischen Zentrums Bienenforschung* 49: 1-5.
20. JIMENEZ, J J; BERNAL, J L; AUMENTE, S; DEL NOZAL, J; MARTIN, T; BERNAL, J JR (2004) Quality assurance of commercial beeswax. Part I. Gas chromatography-electron impact ionization mass spectrometry of hydrocarbons and monoesters. *Journal of Chromatography A* 1024: 147-154.
21. JIMENEZ, J J; BERNAL, J L; AUMENTE, S; TORIBIO, L; BERNAL, J (2003) Quality assurance of commercial beeswax - II. Gas chromatography-electron impact ionization mass spectrometry of alcohols and acids. *Journal of Chromatography.A* 1007 (1-2): 101-116.
22. JIMENEZ, J J; BERNAL, J L; DEL NOZAL, M J; MARTIN, M T; BERNAL, J (2006) Sample preparation methods for beeswax characterization by gas chromatography with flame ionization detection. *Journal of Chromatography.A* 1129 (2): 262-272.
23. JIMENEZ, J J; BERNAL, J L; DEL NOZAL, M J; MARTIN, M T; TORIBIO, L (2009) Identification of adulterants added to beeswax: Estimation of detectable minimum percentages. *EUROPEAN JOURNAL OF LIPID SCIENCE AND TECHNOLOGY* 111 (9): 902-911.
24. JIMENEZ, J J; BERNAL, J L; DEL NOZAL, M J; TORIBIO, L; BERNAL, J (2007) Detection of beeswax adulterations using concentration guide-values. *EUROPEAN JOURNAL OF LIPID SCIENCE AND TECHNOLOGY* 109 (7): 682-690.
25. KIRK-OTHMER (2007) *Kirk-Othmer Encyclopedia of Chemical Technology*. John Willey and Sons (5th edition)

26. KRELL, R (1996) *Value-added products from beekeeping*. FAO Food and Agriculture Organization of the United Nations Roma; 409 pp
27. KRIVTISOV, N; LEBEDEV, V (1995) *The bee products (In Russian)*. Editing House, Niwa Niwa, Russia
28. KURSTJENS, S P; MCCLAIN, E; HEPBURN, H R (1990) The proteins of beeswax. *Naturwissenschaften* 77 (1): 34-35.
29. MACHOVA, M (1993) Resistance of bacillus-larvae in beeswax. *Apidologie* 24 (1): 25-31.
30. MAIA, M; BARROS, A I; NUNES, F M (2013) A novel, direct, reagent-free method for the detection of beeswax adulteration by single-reflection attenuated total reflectance mid-infrared spectroscopy. *Talanta* 107: 74-80.
31. MAIA, M; NUNES, F M (2013) Authentication of beeswax (*Apis mellifera*) by high-temperature gas chromatography and chemometric analysis. *Food Chemistry* 136 (2): 961-968.
32. MCLOUD, E S Waxes. *Wattle Bark* 22: 156-173.
33. PETERSEN, I (1999) The Honeycomb Conjecture: Proving mathematically that honeybee constructors are on the right track. *Science News* 156: 60.
34. PIRK, C W W; HEPBURN, H R; RADLOFF, S E; TAUTZ, J (2004) Honeybee combs: construction through a liquid equilibrium process ? *Naturwissenschaften* 91: 350-353.
35. RITTER, W (2003) Early detection of american foulbrood by honey and wax analysis. *Apiacta* 38 (2): 125-130.
36. SABATINI, A G; MARCAZZAN, G L; COLOMBO, R; LERCKER, G (1989) Possibilità di controllo della cera d'api. *Apitalia* 16 (5): 6-7.
37. SCHMIDT, J O; BUCHMANN, S L (1992) Other products of the hive. in: *The Hive and the Honey Bee* (Graham, J.M., Editor) Dadant & Sons, Hamilton, IL. *unknown*: 927-988.
38. SCHROEDER, A; WALLNER, K (2003) The actual situation of varroacides in beeswax: An international comparison. *Apidologie* 34 (5): 1-3.
39. SEELEY, T D; MORSE, R A (1976) The nest of the honey bee (*Apis mellifera* L.). *Insectes Sociaux* 23: 495-512.
40. SERRA BONVEHI, J (1990) Estudio de la adulteracion de la cera de abejas (*Apis mellifera* L.). *Grasas y Aceites* 41 (1): 69-72.
41. SERRA BONVEHI, J; ORANTES BERMEJO, F (2012) Detection of adulterated commercial Spanish beeswax. *Food Chemistry* 132 (1): 642-648.
42. SHKENDEROV, S; IVANOV, T (1983) Les produits de l'abeille. *Zemizdat (Abstract in Honey bibliography)*: 1-238.
43. SHKENDEROV, S; IVANOV, T (1983) Pcelni Produkti, The Bee Products (in Bulgarian). *Zemizdat (Abstract in Honey bibliography)*: 1-238.
44. SVECNJAK, L; BARANOVIC, G; VINCEKOVIC, M; PRDUN, S; BUBALO, D; TLAK GAJGER, I (2015) An Approach for Routine Analytical Detection of Beeswax Adulteration Using FTIR-ATR Spectroscopy. *J Apicult Sci* 59: 37-49.
45. TAUTZ, J (2003) Honey bee dancing causes combs to vibrate 781. *Deutsches Bienen Journal* 11 (1): 16-18.
46. TAUTZ, J; CASAS, J; SANDEMAN, D (2001) Phase reversal of vibratory signals in honeycomb may assist dancing honeybees to attract their audience. *The Journal of Experimental Biology* 204 (21): 3737-3746.

47. TAUTZ, J; LINDAUER, M (1997) Honeybees establish specific sites on the comb for their waggle dances. *Journal of Comparative Physiology.A, Sensory, Neural, and Behavioral Physiology* 180 (5): 537-539.
48. TEMNOV, V (1967) *Technology of the bee products (in Russian)*. Moscow; 192 pp
49. TEMNOV, V A (1967) *Technologia produktov pcelovodstvo. Technology of the bee products*. M. Kolos
50. TULLOCH, A P (1973) Factors affecting analytical values of beeswax and detection of adulteration. *Journal of the American Oil Chemists' Society* 50 (7): 269-272.
51. TULLOCH, A P (1980) Beeswax - Composition and analysis. *Bee World* 61 (2): 47-62.
52. WALLNER, K (1992) The residues of P-Dichlorobenzene in wax and honey. *American Bee Journal* 132 (8): 538-541.
53. WALLNER, K (1999) Varroacides and their residues in bee products. *Apidologie* 30: 235-248.
54. WARTH, A H (1956) *The chemistry and technology of waxes*. (2nd. edition)
55. WEISS, K (1965) Ueber den Zuckerverbrauch und die Beanspruchung der Bienen bei der Wachserzeugung. *Sonderdruck Z.Bienenforsch.* 8 (4): 106-124.
56. [ANON] (2008) *European pharmacopoeia*. Council of Europe Strassbourg (6. edition)