

65th JECFA - Chemical and Technical Assessment (CTA) 2005 BEESWAX

Chemical and Technical Assessment (CTA)

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1 Summary

Beeswax (INS No. 901) consists primarily of a mixture of esters of fatty acids and fatty alcohols, paraffinic hydrocarbons, and free fatty acids; minor amounts of free fatty alcohols are also present. Two types of beeswax are marketed: yellow beeswax (C.A.S No. 8006-40-4) and white beeswax (C.A.S. No. 8012-89-3). Yellow beeswax is a yellow or light-brown solid that is somewhat brittle when cold and presents a characteristic odour of honey. White beeswax is a white or yellowish white solid (thin layers are translucent) having a characteristic, but faint, odour of honey.

Beeswax is obtained from the honeycombs of bees (*Apis mellifera L.*, Fam. *Apidae*) after removal of the honey. The combs are melted with hot water, steam, or solar heat. After removing the insoluble impurities, the liquid wax is cast into cakes for further purification to obtain food-grade yellow beeswax. Bleaching the latter with e.g. hydrogen peroxide, sulfuric acid or sunlight, yields white beeswax.

Beeswax consists primarily of five main groups of components, namely:

- 1. Free fatty acids (typically 12-14%), most of which are saturated (ca. 85%) and have a chain length of C_{24} - C_{32} .
- 2. Free primary fatty alcohols (ca. 1%) with a chain length of C_{28} - C_{35} .
- 3. Linear wax monoesters and hydroxymonoesters (35-45%) with chain lengths generally of C_{40} - C_{48} . The esters are derived almost exclusively from palmitic acid, 15-hydroxypalmitic acid, and oleic acid. The variation in total chain length of the ester is mainly the result of the different chain lengths of the alcohol moiety (C_{24} - C_{34}).
- 4. Complex wax esters (15-27%) containing 15-hydroxypalmitic acid or diols, which, through their hydroxyl group, are linked to another fatty-acid molecule. In addition to such diesters, tri- and higher esters are also found.
- 5. Odd-numbered, straight chain hydrocarbons (12-16%) with a predominant chain length of C_{27} - C_{33} . With increasing chain length, the proportion of unsaturated species increases (above C_{33} only unsaturated species are present) and alkadienes and -trienes have been reported at only very low levels.

The composition of beeswax depends to some extent on the subspecies of the bees, the age of the wax, and the climatic circumstances of its production. However, this variation in composition occurs mainly in the relative amounts of the different components present, rather than in their chemical identity (Aichholz & Lorbeer, 1996). In the 1970s, 74 major and 210 minor components were detected and about 40 of these were identified. More recently, over 80 constituents have been separated and identified.

The food applications of beeswax include its use as a component in dietary food supplements (soft gelatin capsules and tablets), glazings and coatings, chewing gum, and as a carrier for food additives (including flavours and colours).

^{*} This document incorporates substantial portions of the submission prepared by Dr. Albert Bär, Bioresco, Ltd., Basel, Switzerland for the 65th meeting (June 2005) of the Joint FAO/WHO Expert Committee on Food Additives.

2 Description

Beeswax (INS No. 901) is marketed as yellow beeswax (C.A.S. No. 8006-40-4) and white beeswax (C.A.S. No. 8012-89-3). It consists primarily of a mixture of esters of fatty acids and fatty alcohols, paraffinic hydrocarbons, and free fatty acids; minor amounts of free fatty alcohols are also present.

Yellow beeswax is a yellow or light-brown solid that is somewhat brittle when cold and presents a dull, granular, non-crystalline fracture when broken; it becomes pliable about ca. 35° . It has a characteristic odour of honey. White beeswax is a white or yellowish white solid (thin layers are translucent) having a characteristic, but faint, odour of honey. The waxes are insoluble in water, sparingly soluble in alcohol, and very soluble in chloroform, ether, and in fixed and volatile oils. They are partially soluble in cold carbon disulfide and completely soluble in it at temperatures of 30° and above. Beeswax has a specific gravity of about 0.95. The following physical properties are characteristic:

Melting range (°C)	62-65 ^{a,b} ; 60-67 ^c
Acid value	17-24 ^{a,b} ; 5-24 ^c
Peroxide value	not more than 5 ^{a,b,c}
Saponification value	87-104 ^a ; 77-103 ^c
Ester value	72-79 ^b

^aCompendium of Food Additive Specifications, FNP 52 Add 13 (2005).

^bFood Chemicals Codex, 5th ed, National Academies Press, Washington, DC, 2003. ^cJapan's Specifications and Standards for Food Additives, 7th ed. (English translation), Ministry of Health and Welfare, Tokyo, 2000.

3 Method of manufacture

Beeswax is obtained from the honeycombs of bees (*Apis mellifera L.*, Fam. *Apidae*) after removal of the honey by centrifugation. The combs are melted with hot water, steam, or solar heat. The liquid product is filtered or centrifuged to remove insoluble impurities and cast into cakes that are further purified by remelting and treatment of the melt with activated carbon, aluminium or magnesium silicates, or diatomaceous earth. The treated melt is finally subjected to pressure filtration to obtain food-grade yellow beeswax.

Bleaching the yellow beeswax with, for example, hydrogen peroxide, sulfuric acid, or sunlight, yields white beeswax. In case of bleaching with hydrogen peroxide, the melted wax is also treated with a bleaching earth or activated carbon to avoid the presence of peroxo compounds in the finished material. The use of chlorinated bleaching agents, such as sodium hypochlorite, sodium chlorite, or chloramine, results in waxes that do not have stable colours and that retain chlorine (Wolfmeier *et al.*, 1996): chlorine bleaches are therefore not likely to be used in the production of food-grade wax.

4 Characterization

Beeswax consists primarily of five main groups of components:

- 1. Free fatty acids (typically 12-14%), most of which are saturated (ca. 85%) and have a chain length of C_{24} - C_{32} .
- 2. Free primary fatty alcohols (ca. 1%) with a chain length of C_{28} - C_{35} .
- 3. Linear wax monoesters and hydroxymonoesters (35-45%) with chain lengths generally of C_{40} - C_{48} . The esters are derived almost exclusively from palmitic acid, 15-hydroxypalmitic acid, and oleic acid. The variation in total chain length of the ester is mainly the result of the different chain lengths of the alcohol moiety (C_{24} - C_{34}).
- 4. Complex wax esters (15-27%) containing 15-hydroxypalmitic acid or diols, which, through their hydroxyl group, are linked to another fatty-acid molecule. In addition to such diesters, tri- and higher esters are also found.
- 5. Odd-numbered, straight chain hydrocarbons (12-16%) with a predominant chain length of C_{27} -

 C_{33} . With increasing chain length, the proportion of unsaturated species increases (above C_{33} only unsaturated species are present) and alkadienes and -trienes have been reported at only very low levels.

The reported concentrations of these main components vary somewhat depending upon the analytical procedures applied (Tulloch, 1980; Brand-Garnys & Sprenger, 1988; Brüschweiler *et al.*, 1989; Aichholz & Lorbeer, 1999, 2000; Carlson *et al.*, 1989; Giumanini *et al.*, 1995; Jiménez *et al.*, 2004).

Crude beeswax contains a large number of minor components (e.g. terpenoids and flavonoids), most of which appear to be plant-derived (Puleo, 1991); the colour of the wax will depend to some extent on the type of flora visited by the bees. Volatile products have also been detected at low levels (Puleo, 1991; Blum *et al.*, 1988; Ferber & Nursten, 1977). Oxygenated compounds produced by the bees are also present, amongst which decanal (ca. 50% of the oxygenated volatiles), 1-decanol, nonanal, octanal, furfural, and benzaldehyde are responsible for the bouquet of the wax (Blum *et al.*, 1988).

The composition of beeswax depends to some extent on the subspecies of the bees, the age of the wax, and the climatic circumstances of its production (Wolfmeier *et al.*, 1996; Aichholz & Lorbeer, 1999, 2000; Fröhlich, 2000). However, this variation in composition occurs mainly in the relative amounts of the different components present, rather than in their chemical identity (Aichholz & Lorbeer, 1996).

GC-MS analysis of beeswax in the late 1970s (Tulloch, 1980) resulted in the detection of 74 major and 210 minor components, about 40 of which were identified. More recently, over 80 constituents were separated and identified by Aichholz & Lorbeer (1999, 2000), and Jiménez et al. (2004) reported 24 previously unidentified compounds, including a new family of unsaturated ethyl esters from C_{16} to C_{34} chain lengths, although C_{20} chain lengths were absent.

Existing specifications of identity and purity for both yellow and white beeswax have established test limits for certain wax-related substances that are not natural components of beeswax, namely ceresin, paraffins, and certain other waxes (FAO, 1992); fats, Japan wax, rosin, and soap (FAO, 1992; Japan, 2000; FCC, 2003); carnauba wax (FCC, 2003); and glycerol and other polyols (FAO, 1992). A revision of the specifications established by FAO in 1992 is being considered at the 65th meeting (June 2005) of the Joint FAO/WHO Expert Committee on Food Additives.

5 Functional uses

The food applications of beeswax include its use as a component in dietary food supplements (soft gelatin capsules and tablets), glazings and coatings, chewing gum, water-based flavoured drinks, and as a carrier for food additives (including flavours and colours).

Food supplements

(a) Soft gelatin capsules

Beeswax is an essential component of the filling of about 40% of soft gelatin capsules sold as food supplements in the European Union (Bioresco, 2005). Due to its particular physical properties, beeswax is the most suitable stabilizer for keeping oil-based capsule contents in suspension.

In certain formulations, such as multi-vitamin capsules, ingredients that are not oil-soluble must be maintained in a homogenous suspension during the production of the capsule to ensure that the correct amounts of the claimed levels of the ingredients are delivered to each capsule. The oil-insoluble material also has to be kept in suspension for the duration of the declared shelf life of the capsule. Inadequate stability or breakdown of the suspension during storage results in the insoluble contents sedimenting on the lower inner surface of the capsule shell. This can form an unsightly dark plaque (or sludge) that may be seen by the consumer as a sign of serious deterioration of the product. The amount of beeswax used in each capsule varies from about 0.7-6.1%, depending on the technical requirements (Bioresco, 2005).

(b) Tablet formulations

Beeswax is also used as a stabilizer and release agent in the production of certain food supplements sold in tablet form. Typical concentrations of beeswax in this type of product range from 0.5 to 3.4% (Bioresco, 2005).

Glazings and coatings

Beeswax may be blended with other ingredients such as oils, fats or other waxes. The EU permits the use of beeswax as a glazing agent for confectionery (including chocolate), in small products of fine bakery ware coated with chocolate, in snacks, nuts, coffee beans, dietary food supplements, and in certain fresh fruits for surface treatment at *quantum satis* levels (EU, 1995). According to Bioresco (Bioresco, 2005), finished food products may contain beeswax at concentrations of up to 500 mg/kg of food. In the USA, beeswax has been affirmed by the FDA as generally recognized as safe (GRAS) for use as a glazing agent at levels not to exceed 50 mg/kg in confections and frostings, 400 mg/kg in hard candy, 1000 mg/kg in soft candy; and 20 mg/kg in all other food categories (USA, 2004).

Chewing gum

Beeswax may be used in the formulation of chewing gum base. The US-FDA has affirmed that this use at a concentration in the final product of no greater than 650 mg/kg is GRAS (USA, 2004).

Carrier for food additives and flavours

In the EU, beeswax may be used as a carrier for colours (EU, 1995). The use of beeswax as a carrier for fruit and honey flavourings in water-based drinks has been proposed for entry into the Codex General Standard for Food Additives (Codex, 2004). Under this proposal, beeswax would be limited to a maximum level of use of 200 mg/kg in water-based, flavoured drinks. The GRAS-affirmed limit in the US is 20 mg/kg (USA, 2004).

Industry information (IOFI, 2005) on the use levels of beeswax as a carrier for fruit flavourings in waterbased drinks indicates that the wax may be mixed with flavour bases at levels ranging from 10-50 g wax/kg flavour base. The concentration of beeswax in the diluted beverage would be between 25 and 100 mg/kg. The use of the wax provides a cloudy appearance to the beverage, which, according to the new information, limits the use of beeswax as a flavour carrier to cloudy beverages such as citrus-based soft drinks, some herbal-based drinks, and "sport" drinks.

Other uses

The US-FDA has affirmed beeswax as GRAS as a flavouring agent and adjuvant, as a lubricant, and as a surface-finishing agent (i.e. a glazing/coating agent) at levels not to exceed 20 mg/kg in all food categories, other than those noted above with higher affirmed levels (USA, 2004). The affirmation of these applications as GRAS does not mean that other applications/levels are not also GRAS – only that the FDA has not affirmed their GRAS status. Substances that are GRAS for an intended use do not require government premarket approval or other government authorization prior to their introduction into the US market place.

Beeswax is also listed as a permitted additive in the legislation of Brazil, although additive functions and levels of use are not specified (Brasil, 2005). The listing relies on the JECFA evaluation of beeswax.

6 Reactions and fate in foods

Natural waxes are water-insoluble, solid mixtures of esters of long-chain fatty acids and long-chain alcohols (wax esters), hydrocarbons, and a variety of other lipophilic compounds including the free components of the wax esters, aldehydes, ketones and terpenoids. Waxes are widely distributed in nature, the commonest site of occurrence being in the surface lipid layer, where they help protect plants and animals against evaporative loss of moisture and noxious influences from the environment (Kolattukudy, 1976). It follows that in relation to food applications, the reactivity of waxes in general, and beeswax in particular, is likely to be negligible.

7 References

Aichholz, R. & Lorbeer, E. (1996). Use of methoxy-terminated poly(diphenyl/1H, 1H, 2H, 2H-perfluorodecylmethyl)siloxane as stationary phase for high temperature capillary gas chromatography and its application in the analysis of beeswax. *J. Microcolumn.*, 8(8): 553-559.

Aichholz, R. & Lorbeer, E. (1999). Investigation of comb wax of honeybees with high-temperature gas chromatography and high-temperature gas chromatography-chemical ionisation mass spectrometry. I. High-temperature gas chromatography. *J. Chromatography.*, A855: 601-615.

Aichholz, R. & Lorbeer, E. (2000). Investigation of comb wax of honeybees with high-temperature gas chromatography and high-temperature gas chromatography-chemical ionisation mass spectrometry. II. High-temperature gas chromatography - chemical ionisation mass spectrometry. J. Chromatography, A883: 75-88.

Bioresco. (2005). Beeswax. Dossier submitted by Dr. Albert Bär, Bioresco, Ltd., Basel, Switzerland to the Joint FAO/WHO Expert Committee on Food Additives, 65th meeting, June 2005, Geneva.

Blum, M.S., Jones, T.H., Rinderer, T.E. & Sylvester, H.A. (1988). Oxygenated compounds in beeswax: identification and possible significance. *Comp. Biochem. Physiol.*, 91B(3): 581-583.

Brand-Garnys, E.E. & Sprenger, J. (1988). Bienenwachs – Neue Aspekte eines klassischen Kosmetik-Rohstoffs. *Zschr. Körperpflegem. Parf. Riechst. Aerosol-Industr.*, 61(14): 547-552.

Brasil. (2005). Resolução RDC no. 43, de 1 de Março de 2005; Diário Oficial: 2 de Março. (http://e-legis.bvs.br/leisref/public/showAct.php?id=15139)

Brüschweiler, H., Felber, H. & Schwager, F. (1989). Bienenwachs-Zusammensetzung und Beurteilung der Reinheit durch gaschromatographische Analyse. *Fat Sci. Technol.* 91(2): 73-79.

Carlson, D.A., Roan, C.-S., Yost, R.A. & Hector, J. (1989). Dimethyl disulfide derivatives of long chain alkenes, alkadienes, and alkatrienes for gas chromatography/mass spectrometry. *Anal. Chem.*, 61: 1564-1571.

Codex (2004). Report of the 36th Session of the Codex Committee on Food Additives and Contaminants, Alinorm 04/27/12, Appendix IX. FAO, Rome.

EU (1995). European Commission Directive 95/2/EC on food additives other than colours and sweeteners. Brussels.

FAO (1992). Beeswax. In Compendium of Food Additive Specifications, Food and Nutrition Paper 52, Addendum 1, Rome.

FCC (2003). Beeswax, White and Beeswax, Yellow. *In Food Chemicals Codex*, 5th ed, National Academies Press, Washington, DC.

Ferber, C.E.M. & Nursten, H.E. (1977). The aroma of beeswax. J. Sci. Fd. Agric., 28: 511-518.

Fröhlich, B.S. (2000). Wachse der Honigbiene Apis mellifera carnica Pollm.: Chemische und physikalische Unterschiede und deren Bedeutung für die Bienen. Diss. Bayerische Julius Maximilians Univ., Würzburg.

Giumanini, A.G., Verardo, G., Strazzolini, P. and Hepburn, H.R. (1995). Rapid detection of highmolecular-mass dienes in beeswax. J. Chromatography, A704: 224-227.

IOFI (2005). Communication (email, 2005-3-14) from T. Cachet, *International Organization of the Flavour Industry* (Brussels), to FAO-JECFA Secretary (Rome).

Jiménez, J.J., Bernal, J.L., Aumente, S., del Nozal, M.J., Martín, M.T. and Bernal, J. (2004). Quality assurance of commercial beeswax. Part I. Gas chromatography-electron impact ionisation mass spectrometry of hydrocarbons and monoesters. *J. Chromatography*, A1024: 147-154.

Japan (2000). Beeswax. In Japan's Specifications and Standards for Food Additives, 7th ed. (English translation), Ministry of Health and Welfare, Tokyo.

Kolattukudy, P.E. (1976). Introduction to natural waxes. In Kolattukudy, P.E., ed., Chemistry and Biochemistry of Natural Waxes. pp. 1-15. Elsevier, Amsterdam.

Puleo, S.L. (1991). Beeswax minor components: a new approach. Cosm. Toiletr., 106(2): 83-89.

Tulloch, A.P. (1980). Beeswax – composition and analysis. Bee World, 61(2): 47-62.

USA (2004). Beeswax (yellow and white). Title 21, United States *Code of Federal Regulations*, Section 184.1973. U.S. Government Printing Office, Washington, DC.

Wolfmeier, U., Schmidt, H., Heinrichs, F.-L., Michalczyk, G., Payer, W., Dietsche, W., Hohner, G. and Wildgruber, J. (1996). Waxes. *In* Ullmann's Encyclopedia of Industrial Chemistry, 28A: 103-122.