CHAPTER 4 WAX

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4.1 Introduction

The word wax describes a large variety of substances of plant and animal origin, as well as man-made products which are mostly petroleum derivatives. However, natural waxes are not single substances, but a mixture of various long-chain fatty acids and a variety of other constituents, depending on their origin. Each wax therefore has unique physical and chemical characteristics which are exploited in a multitude of applications. In particular, wax from the honeybee has an extremely wide spectrum of useful applications and occupies a very special position among waxes.

Young bees in the hive, after feeding the young brood with royal jelly, take part in the construction of the hive. Engorged with honey and resting suspended for 24 hours together with many other bees in the same position, 8 wax glands on the underside of the abdomens of the young bees secret small wax platelets. These are

scraped off by the bee, chewed and masticated into pliable pieces with the addition of saliva and a variety of enzymes. Once chewed, attached to the comb and re-chewed several times, they finally form part of this architectural masterpiece, a comb of hexagonal cells, a 20 g structure which can support 1000 g of honey. Wax is used to cap the ripened honey and when mixed with some propolis, also protects the brood from infections and desiccation. Together with propolis, wax is also employed for sealing cracks and covering foreign objects in the hive. The wax collected by the beekeeper is that which is used in comb construction. Frame hive beekeeping produces wax almost exclusively from the cap and top part of the honey cells.

For centuries, beeswax was appreciated as the best material for making candles. Before the advent of cheap petroleum-based waxes, tallow (rendered animal fat) was used for cheap candles and for the adulteration of beeswax. Ancient jewellers and artisans knew how to form delicate objects from wax and cast them later in precious metals. Colours of ancient wall paintings and icons contain beeswax which has remained unchanged for more than 2000 years (Birshtein et al., 1976). The wrappings of Egyptian mummies contained beeswax (Benson et al., 1978) and beeswax has long found use in medicinal practices and in creams and lotions. Of all the primary bee products it has been, and remains, the most versatile and most

widely used material.

Figure 4.1 : Wax processed from traditional beekeeping at the honey factory in Kabompo, NW Province, Zambia.

Other waxes derived from plants and animals (data from Brown, 1981 and Tulloch, 1970) include:

***Carnauba* is obtained from the leaves of Copernicia cerifuga, a palm tree found in Brazil It melts at 83-86°C.**

***Ouricuri* is also obtained from the leaves of a palm tree found in tropical America, but it is of lower quality than Carnauba wax. It melts at 84°C.**

***Candelilla* is obtained from a reed-like plant found in Mexico and California. It melts**

at 70^{0}C and has a yellowish colour.

Esparto **is obtained from esparto grass as a by-product of the artisanal paper industry. It produces a high gloss finish with very little rubbing. It melts at 73^{0}C.**

Sugarcane Wax **is a by-product of sugar refining. It melts at 78 to 80^{0}C.**

Ozokerite **is a mineral wax. It is mined.**

Ceresin **is a mixture of purified ozokerite and paraffin wax.**

Ghedda **is the general name applied to waxes from the Asian Apis species.**

Spermaceti **is a very high quality wax obtained from the head of sperm whales. Since there is an international agreement restricting the hunting of these animals, no more spermaceti wax should be used or traded. In most recipes spermaceti can be replaced with beeswax. Synthetic substitutes exist as well.**

Shellac **with a melting point of 74-78^{0}C, shellac is secreted by the Lac insect (Laccifer lacca, Coccoidea) in Asia, and is used for electrical insulation, seals and certain**

polishes.

Chinese insect wax **is produced by Coccus ceriferus and Brahmaea japomca (Coccoidea). It melts at 82-84^{0}C. Other wax producing Coccoidea are Icerva purchasi and Dactylopius coccus whose waxes melt at 78^{0}C and 99-101 ^{0}C, respectively.**

Other wax producing Coccoidea are Icerva purchasi and Dactylopius coccus whose waxes have melting points at 78^{0}C and 99-100^{0}C, respectively.

Many reviews of wax have been published of which some of the more comprehensive are by Bull (1977) Walker (1983a) and Coggshall and Morse (1984), Hepburn (1986) and Crane (1990). An international market review for beeswax was conducted by the International Trade Centre of UNCTADIGATT (ITC, 1978).

Many bee species produce wax but unless otherwise mentioned, only the wax of the honeybee species Apis mellifera will be referred to in this bulletin. Wax from other honeybee species (ghedda wax) is very similar, but has characteristics sufficiently different for it not to be used by the cosmetic industry. Even the wax produced by A. mellifera is not always the same. Thus, the cosmetic industry

generally prefers beeswax from Africa.

4.2 Physical characteristics of beeswax

Virgin beeswax, immediately after being secreted, elaborated and formed into comb, is white (see Figure 4.2). It becomes darker with use inside the hive as pollen, silk and larval debris are inadvertently incorporated. Rendered, but untreated beeswax comes in varying shades of yellow. Pure white beeswax on the market has always been bleached.

Figure 4.2 : Newly constructed white comb in a traditional log hive.

The melting point of beeswax is not constant since the composition varies slightly with its origin. Various pharmacopoeias give a range of 61-66^{0}C or more commonly, 62-65 ^{0}C. Its relative density at 15 ^{0}C is 0.958 - 0.970 g/cm^{3} and its electrical resistance ranges from 5x10^{12} to 20x10^{12} Ohm m (Crane, 1990). Its thermal

conductivity coefficient is *2.5* x d10^{-3} Jcm/s$^{\diamond}$Ccm^{2}. The saponification value of beeswax is 85-100 (Smith, 1951).

Beeswax is an inert material with high plasticity at a relatively low temperature (around 32 ^{0}C). By contrast, at this temperature most plant waxes are much harder and of crystalline structure. Beeswax is also insoluble in water and resistant to many acids, but is soluble in most organic solvents such as ether, benzine, benzol, chloroform, turpentine oil and after warming, in alcohol and fatty oils.

Ghedda waxes from the Asian honeybee species are described as softer and more plastic, but do not have a significantly different melting point (Warth, 1956). The melting point of wax from three Meliponid (stingless bee) species ranged between 64.6 and 66.5 ^{0}C (Smith, 1951 and Phadke et al., 1969). Bumble bee wax has a much lower melting point at 30-40^{0}C and bumble bees therefore mix their wax with pollen in order to improve its structural strength (Alford, 1975). Other insect waxes are normally used for protective body coatings, rather than for structural purposes. They are therefore very different in their composition as well as their physical characteristics and they have much higher melting points.

4.3 The composition of beeswax

Pure beeswax from Apis mellifera consists of at least 284 different compounds. Not all have been completely identified but over 111 are volatile (Tulloch, 1980). At least 48 compounds were found to contribute to the aroma of beeswax (Ferber and Nursten, 1977). Quantitatively, the major compounds are saturated and unsaturated monoesters, diesters, saturated and unsaturated hydrocarbons, free acids and hydroxy polyesters. Table 4.1 lists the proportion of compounds as presented by Tulloch (1980).

There are 21 major compounds, each making up more than 1 % of the pure unfractionated wax. Together they account for *56%* of the wax. The other 44% of diverse minor compounds probably account for beeswax's characteristic plasticity and low melting point (Tulloch, 1980).

Table 4.1:
Composition of beeswax (after Tulloch, 1980). Major compounds are those forming more than 1% of the fraction. The number in brackets indicates the number of compounds making up at least 1 % of the unfractionated, pure wax. The number of minor compounds, those with less than 1% of the fraction, is only an estimate.

Description	% of fraction	Number of components in fractoin	
		Major	Minor
Hydrocarbons	14	10 (5)	66
Monoesters	35	10 (7)	10
Diesters	14	6 (5)	24
Triesters	3	5	20
Hydroxy monoesters	4	6 (1)	20
Hydroxy polyesters	8	5	20
Acid esters	1	7	20
Acid polyesters	2	5	20
Free acids	12	8 (3)	10

Free alcohols	1	5	?
Unidentified	6	7	?
TOTAL	**100**	**74**	**> 210**

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. At 100^0C 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 hydrocarbons (Tulloch, 1980). 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.

Bleaching destroys at least the aromatic compounds of wax. Bleached wax no longer has the pleasant and typical aroma of wax and it can be assumed that it also lacks many of the other minor compounds.

Various plant growth promoting substances, such as myricil alcohol (Weng et al., N-

1979), triacontanol (Devakumar et al., 1986), gibberellin GA_3 (Shen and Zhao, 1986) and a rape oil steroid (Jiang, 1986) have been detected in and isolated from beeswax. Kurstjens et al., (1990) describe at least 11 proteins in the freshly secreted wax scales of A. mellifera capensis worker bees and 13 proteins in the wax combs of A. m. scutellata and A. m. capensis.

The composition of wax from Asian honeybee species is much simpler and contains fewer compounds in different proportions (Phadke et al., 1969, 1971; Phadke and Nair, 1970, 1973 and Narayana, 1970). These ghedda waxes therefore cannot be used as substitutes for Apis mellifera wax in certain recipes. Since little is known about which compounds or mixtures cause the beneficial medicinal and dermatological effects of beeswax, no conclusions can be drawn from the composition data alone. Ghedda waxes are used locally in many of the same ways as Apis mellifera wax is used in other parts of the world. Meliponid waxes, which are less like honeybee wax than Ghedda wax, have been used by Amerindians for many of the same purposes, as honeybee waxes (Posey, 1978).

Beeswax is considered safe for human consumption and has been approved as an ingredient in human food in the USA (USA, 1978). It is inert, i.e. it does not interact with the human digestive system at all and passes through the body unaltered.

However, substances dissolved or encapsulated in wax are slowly released. This property is exploited in many medicinal preparations (see 4.5.10). At the same time these properties can create a problem when wax is stored near toxic chemicals and pesticides or after treatment with various drugs inside the hive. Any fat soluble toxins can be absorbed and then released much later when the wax is consumed as food, used in cosmetics or given to bees in the form of foundation sheets.

4.4 The physiological effects of wax

Most of the effects of beeswax are described in the section on applications (section 4.5). Because it is inert, beeswax has no direct effect on humans or larger animals. However, its indirect effects can be very strong.

If mixed with medicinal drugs or poisonous baits, wax preserves the active materials longer and releases them slowly. It can be used to create thin non-corrosive, non-allergenic protective films on many surfaces from metals to fruits and human skin. Thus it protects against external damage such as corrosion and abrasion as well as against moisture loss. It is a good electric insulator and, when saponified with borax, allows the mixture of very stable and smooth emulsions for cosmetics. Even in small concentrations it improves other formulations in the same way.

A very small anti-inflammatory and antioxidant activity can be observed in beeswax due possibly to some inclusions of propolis or other minor ingredients.

4.5 The uses of wax today

In the past, beeswax had a wide range of uses. Though in many cases beeswax can be replaced with cheaper, synthetic waxes, its very special characteristics, medicinal benefits, plasticity and aroma ensure its continuing use. Many of these characteristics cannot be achieved with artificial waxes. The trend for more natural products in cosmetics may also increase its use. Presently, there is a scarcity of beeswax in industrialized countries, at least seasonally.

In industrialized countries, most nationally produced wax is used by beekeepers for foundation sheets. Approximately one third of imported wax is used for cosmetics, one third for pharmaceutical preparations one fifth for candles and the rest for other, minor uses (ITC, 1978).

In developing countries with traditional beekeeping methods, wax is often wasted. If it is rendered, most is subsequently exported and only relatively small proportions are used by local manufacturers. This, however, depends very much on the local

industry. There are many possibilities for good quality products in local emerging markets and in import substitution. Adj are (1984) listed over 150 uses of beeswax as described also in an old 1954 edition of " The Hive and the Honeybee"

A few examples from the wide range of products in which beeswax can be included, together with a few recipes for small or home-based industrial production are described below. There are many types of synthetic waxes available today, often with superior characteristics for special applications Apart from price and availability however, beeswax has preferred characteristics in a wide range of applications and conditions. There are very few products which consist only of beeswax or in which only beeswax can be used, but the value or characteristics of most other products are enhanced or complemented by its inclusion.

4.5.1 In beekeeping

In countries with frame hive beekeeping, the majority of locally produced beeswax is consumed by beekeepers for the making of wax foundations - the patterned sheets of wax which are given to the bees as a guide for construction of their combs. Bees will not accept foundation made of synthetic waxes such as paraffin wax. Small quantities of paraffin wax mixed with beeswax may be accepted by the bees. Using

such mixed foundation sheets, however, is a severe breach of good beekeeping practices, since it will adulterate all wax rendered from such combs. Non-frame beekeepers use melted wax or strips of smooth wax sheets as guides for bees to start their combs on. Each beekeeper can easily make the strips by dipping wet boards into melted wax (see Figure 4. 3a and 4.4 top right). Patterned sheets are usually made by specialized manufacturers, since the pattern imprinting requires special roller presses. Such presses, until recently, were very expensive, ranging from hand operated roller presses at about US$ 800 each to complete manufacturing lines costing many tens of thousands of dollars (see Figure 4.3 and 4.4). However, since at least 1989 inexpensive presses with moulded plastic rollers have been available for a fraction of the price of metal rollers in Brazil (see Cylindros Alveolador in Annex 1 and Figure 4. 3d). These plastic rollers do not last as long as steel rollers, but they are much cheaper to buy.

In order to reduce damage during hive management and honey extraction in centrifugal extractors, foundation sheets are reinforced with wire either by the beekeeper (frame per frame) or by the manufacturer who embeds the wire into the foundation sheet (see Figure 4.4). Sheets come in different sizes to fit the various sizes of frames. Standardized frame hive equipment within one country and preferably also in neighbouring countries will make manufacturing easier and more

economical. Sheets should always fit the whole width of the frame, otherwise bees will not attach the comb to the frame. This weakens the comb and thus defies the main purpose of the frame. It also reduces the surface area for brood and honey storage by more than 5 %. In most countries foundation sheets are traded by manufacturers against raw wax with a mark-up for labour and equipment cost. Many manufacturers are also suppliers of beekeeping equipment, but also beekeeping cooperatives or large beekeepers sometimes make foundation sheets.

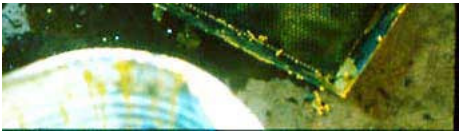
Fledgling beekeeping operations in countries with no tradition of beekeeping always have problems making their own wax foundation, since not enough beeswax is produced. Materials have to be imported or beekeeping started as a topbar operation. It takes a fairly stable frame hive beekeeping industry, i.e. one that is not growing too fast, to supply all foundation needs to its beekeepers because wax production from this type of beekeeping is low (1-2% in weight of honey production as compared to 10-15 % in topbar and traditional beekeeping).

Bottom board and side wall scrapings which contain large percentages of propolis can be processed into cheap wood preservatives (see recipes 4.11.10) particularly for hive equipment, or may be used by beekeepers for baiting swarm traps. However, these scrapings should never be mixed with other beeswax, since they destroy its

quality for other uses.

a)

b)



c)

d)

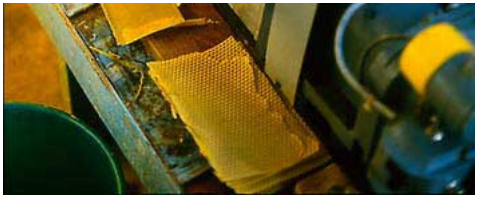
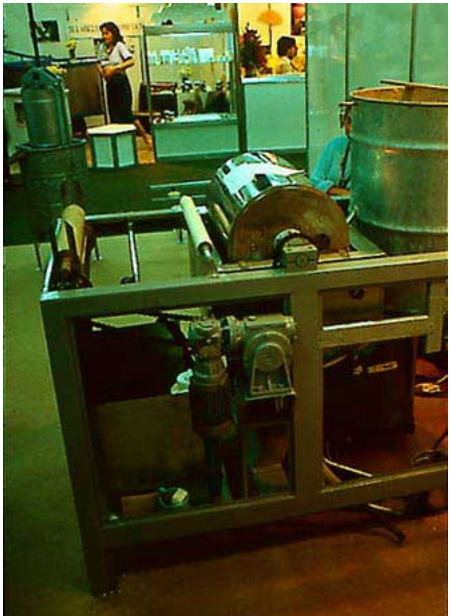


Fig 4.3 a) Melted wax starter strips from unpatterned wax sheets for topbar hives. b) Simple foundation press for single sheets requires more practice and nore wax per sheet; can also be made from gypsum (plaster) using commercial foundation to prepare the plaster moulds.
c) Motorised foundation rollers with moist.
d) Hand-operated, low cost, plastic foundation rollers

a)



c) 

Figure 4.4: Top left: Medium size set up for the production of continuous wax sheets a cooled drum rotating through a liquid wax bath. Top right: Rack and liquid wax ba the production of multiple wax sheets by hand-dipping moist, wooden boards into molten wax. Bottom: Wired frame with wired foundation sheet. All Langstroth ar Dadant size frames should have at least four horizontal wires. Vertical wires can b embedded into the wax sheet by some manufacturers, but either of the wiring meth

usually sufficient.

4.5.2 For candle making

Beeswax, next to the cheaper tallow, was the major raw material for candles until the development of cheaper petroleum products such as paraffin wax, which was introduced during the last century. Since beeswax has a higher melting point than most paraffin waxes (most of which melt between 480 and 68^{0}C) beeswax candles remain straight at higher ambient temperatures. If wick size is correctly proportioned with respect to the diameter of the candle, they are less likely to drip than candles made from other materials. Waxes with a melting point above 88^{0}C do not perform well during burning. The Roman Catholic church requires that its ceremonial candles are made with at least 51 % pure beeswax. A detailed description of candle making is given in the recipe in section 4.11.2.

4.5.3 For metal castings and modelling

Because of its plasticity, beeswax is easily formed and carved. It maintains its shape

well even over very long periods of time as proven by wax sculptures found in ancient Egyptian graves. Its relatively low melting point permits easy and complete removal from casting moulds. The hollow space left in these moulds can then be filled with molten metal. Already in ancient times whether in Asia, the Americas or Europe, craftsmen using this V lost wax method, sculpted small, solid metal figures, jewellery, large hollow sculptures and more recently also bells. Until today, different mixtures of beeswax and other waxes are used to create special forms and surfaces for jewellery and artistic sculptures.

No special preparations are necessary to use beeswax in these applications and in an indirect way, the resulting sculptures or jewellery may be considered a value added product from beekeeping. However far fetched this analogy may be, the lost wax technique is a craft in its own right and requires careful study. It may be undertaken using highly refined plasters like in dentistry, temperature controlled ovens and gas torches, but it is also possible on a very simple level using locally available clays and home-built furnaces. Both are beyond any simple descriptions that can be provided here, but Feinberg (1983) gives details for small-scale manufacturers.

The sculptures of Madam Tussaud's in London are widely known and copied in

many countries. In the museum, famous people are copied in wax and dressed as life-sized figures. A mixture of three parts beeswax and one part of a harder wax are used (Sargant, 1971). Modelling in wax, or ceroplasty is a well developed art used also for scientific models in important collections around the world (Olschki, 1977). During the last century, wax flower modelling was apparently popular in Europe. A bibliography on wax modellers, collections and history has been published by Pyke (1973) and a handbook on sculpting with wax and plaster by Miller (1974).

4.5.4 In cosmetics

The unique characteristics of beeswax give a certain solidity to emulsified solutions, facilitate the formation of stable emulsions and increase the water holding capacity of ointments and creams. These and other characteristics, which only beeswax combines in one substance, make beeswax irreplaceable in the cosmetics industry. Though the desired effects can often be achieved with as little as 1 to 3 % beeswax (Coggshall and Morse, 1984) final proportions are also determined by the relatively high cost of beeswax.

Beeswax not only improves the appearance and consistency of creams and lotions but is also a preferred ingredient for lipsticks, because it contributes to sheen,

consistency and colour stabilization. Other cosmetic applications are found in cold creams (8-12% beeswax content by weight), deodorants (up to 35 %), depilatories (hair removers, up to 50%), hair creams *(5-10%),* hair conditioners (1-3%), mascara (6-12%), rouge (10-15%), eye shadows (6-20%) and others.

Since ancient times, the basic recipe for creams and ointments has consisted of a mixture of beeswax and oil in various proportions according to the desired consistency. Traditionally, vegetable oils were used but they become rancid and limit the period for which such creams can be used. Today, most plant oils have been replaced by mineral oils such as liquid paraffin or preservatives are added. Selective use of vegetable oils from olives, corn, peanuts, jojoba, cacao, palms, coconuts and others still continues, since many of their beneficial effects cannot be provided by synthetic mineral oils.

In order to mix the otherwise incompatible beeswax and oils with water, all of which are essential ingredients of any cream or lotion, an emulsifier has to be added. Borax is the classic emulsifier, available in most pharmacies. Today's "high-chemistry" cosmetics use a large array of other synthetic emulsifiers. The chemical process on which the emulsification is based is the saponification of the acids in beeswax, i.e. the result is technically a soap. The associated cleansing effect is

exploited in so-called cleansing creams, which are very much like simple skin creams.

To remove the free acids from beeswax so that it no longer needs an emulsifier and can be easily mixed with pigments and mineral products, a special process was developed and patented (Brand, 1989). The free acids are removed through reaction with glycidol at 80-120^{0}C in the presence of a basic catalyst.

Recipes for cosmetics, including preparations of depilatory waxes, are presented in Chapter 9.

4.5.5 Food processing

Beeswax has been used in a variety of products and processes from packaging to processing and preservation. It has also been used as a separation agent in the confectionary industry (Ribot, 1960) and in cigarette filters (Noznickli and Likwoh, 1967). Many of these applications could be accomplished with other, cheaper waxes. Since most of these processes involve large scale and complicated production procedures, they are not described here -

A common, simple and small scale application for beeswax is the protection of containers against the effects of acids from fruit juices or honey. Steel drums for storage and shipment of honey have to be treated to prevent corrosion and dissolution of iron. The treatment may involve an expensive food grade paint, a plastic liner made from a food grade plastic film or a thin coat of beeswax.

4.5.6 Industrial technologv

A patent by Enger (1976) describes a material for encapsulating electrical and electronic apparatus for use in high moisture or chemically active environinents. One example consisted of at least 50% (ideally 70% by weight) of silicone, mixed with a fluorocarbon (20% tetrafluorethylene and a natural animal or mineral wax (10% beeswax) and, if necessary, an inert filler. After polymerization or fluorethylene vulcanization with a catalyst and/or heat, the inert product becomes impermeable to ions and fluid.

Another patent describes the preparation of a material for embedding or electrically insulating circuits of high and ultra-high frequency. The mixture of 10-30% ceresin wax, 55-65 % beeswax and 15-25 % ethylcellulose has a high melting point, is very hard at high temperatures, very strong when cold and can be remelted (Franklin,

1951).

A patent for an anti-corrosion rust inhibitor describes the incorporation of one or more different waxes, including beeswax. These waxes are mixed with crystalline polyethylene and polystyrene then heated to more than 200^0C. The residue is removed and after adding liquid paraffin, it is boiled until it is homogeneous. The transparent, creamy liquid not only lubricates saws, just as pure beeswax would do, but protects iron, copper, brass, aluminum, chrome and nickel surfaces (limori, 1975). Other effective coatings contain beeswax; one such is composed of 90% mineral jelly and 10% beeswax (Sanyal and Roy, 1967).

In other formulations, beeswax may be used as a binder, particularly if lubricant characteristics are required (Bera et al., 1971) or if mixtures have to be ingested (see 4.5.10). Pure beeswax was once used for lubricating wire rods during high pressure continuous extrusion of wire (Fuchs, 1970). Beeswax has also been used to decrease viscosity and improve slip casting properties when casting glass under pressure (Bezborodov, 1968). For agricultural pest control, beeswax has been an ingredient of slow release pellets of pyrethrum pesticides (Ahmed et al., 1976). Waxing of the threads on pipes was reported to prevent joints from corroding or locking and simultaneously made them waterproof (Brown, 1981).

4.5.7 Textiles

Textiles and papers can be waterproofed with various products containing beeswax and a French patent is referred to in section 4.11.8. Emulsions containing beeswax for leather treatment have been described in many publications and a basic recipe is provided in section 4.11.7.

Batik is a traditional method of colouring cloth, adaptable to both small and large scale production for artistic and commercial applications. It is based on the principle that wax will protect areas which are not supposed to be stained by the dye in which the cloth will be immersed. By multiple applications, very complex, multi-coloured designs can be achieved (see Figure 4.5). This technique was refined in several Asian countries and is now used around the world. Today, because of its high cost, beeswax has been largely replaced by cheaper alternatives. The wax is used in its pure form and needs no processing before application. Various books about batik have been published in different languages and can often be found in local bookstores.

a)

b)

Figure 4.5 : Batiks from Sri Lanka (top) and Barbados (bottom), both Very popular with tourists, form the basis of a small but profitable local industry.

4.5.8 Varnishes and polishes

A patent was recently registered for a varnish made from dammar resin and beeswax to be used for paintings and for art restoration (Krzyzynski, 1988).

Other recipes for varnishes, sometimes also including propolis are given in section

4.11. If propolis is included, the suitability of the locally available material should be tested. Knopf and Ogait (1961) reported that propolis containing a large percentage of balsam (which has non-drying properties) adversely affected the quality of the varnish. Propolis from different places can exhibit considerable variation in balsam content.

Detailed discussions and recipes for preparations with synthetic wax are presented by Jones (1977) who also lists reasons such as the formation of soft, easily marred films and a lack of availability, why natural beeswax is increasingly being replaced by other waxes in polishes.

4.5.9 Printing

In the old art of etching or engraving, beeswax was used as a protective surface coating. Wax was applied to a heated metal plate. The excess drained off while the remaining wax solidified into a thin film through which the design was drawn. The application of concentrated nitric acid or a mixture (1:8 by volume) of concentrated hydrochloric and nitric acids for a few minutes etched away the exposed metal and left the engraved part ready for negative printing. Today, a liquid asphalt is normally used instead. A US patent (Hughes, 1960) uses beeswax as part of a liquid protective

coating for plastic lithography plates and also for automobiles.

Glass can be etched with hydrofluoric acid after protecting those areas with beeswax which are to remain clear.

All of the acids mentioned are highly toxic and corrosive. Special precautions are required to avoid contact with clothing, skin and eyes.

Various inks, pens, markers and even carbon paper often contain small amounts of beeswax (Polishchuk and Denisova, 1970). One patent (Morishita et al., 1978) for typewriter ink includes a recipe of 1 part Japan wax or beeswax, 1 part Hitaide resin 503, S parts fluorescent granules (pigment) and 0.02 part Emulgen PP 150 (an emulsifier).

4.5.10 Medicine

As a coating for drugs or pills, beeswax facilitates ingestion but retards dissolution of the enclosed compounds until they reach the digestive tract. Beeswax can also be prepared as a mixture with the drug and then functions as a time release mechanism, releasing the drug over a longer period of time.

One such suppository base (a substance which allows slow release of another substance) has been developed on the basis of 5% beeswax, 5% palmitic acid and 90% of Nubon, a semi-synthetic hydrogenated vegetable oil (El-Sabbagh et al., 1988). This was used initially with chloramphenicol. In another preparation, beeswax alone served as the carrier for the drug. On an experimental basis nalidixic acid suspended in beeswax remained longer in the blood of tested animals after oral application than when the acid was administered directly (Lee and Lee, 1987). With another drug, the antihistamine chlorpheniramine maleate, various mixtures of glyceryl monostearate, stearic acid, lactose and higher proportions of beeswax had been successfully tested as a base. Many more examples can be found in pharmaceutical and medical literature. Each drug application requires its own specific modifications of the rudimentary base formulation.

Chewing dark comb (but not the old, black brood comb) without honey, brood or bee-bread is known to be effective against colds. A study by Maksimova-Todorova et al., (1985) has shown that even the wax fractions of propolis have antiviral activities. Older combs contain among many other things a good portion of propolis.

Beeswax can be used to fill capsules with equal amounts of drugs or other ingredients of various granule sizes. The granules of drugs are made adhesive by

coating them with molten wax (about 90g molten wax for 3kg of granules), fat or glycerol, by spraying with liquid paraffin or by mixing them with powdered wax or fat and heating. After thorough mixing the hard capsules are pressed with their open end into an evenly spread layer of the mixture (Iwamoto et al., 1965). This process can also be adapted to making pills with pollen.

A mixture of equal parts melted beeswax and honey is recommended for treating cracked hooves of animals. It should be applied after the cracks have been thoroughly cleaned.

4.5.11 Others

Other products in which beeswax provides some improvement and in which it is a traditional ingredient, include grafting wax, crayons, floor and furniture polish, general purpose varnish, sealing wax, corrosion prevention, protective car polishes and sewing thread
- especially for sail and shoe making.

Again, in many of these products, beeswax can be replaced by cheaper synthetic waxes. The recipes in section 4.11 may be considered as general guidelines for the

manufacture of any of the described products, using either beeswax or other available waxes. The special characteristics derived from the use of beeswax may be of importance in some particular conditions and may bring a better price for the product.

The fact that plant growth stimulators have been isolated from beeswax favours it over synthetic substitutes for use as a grafting wax. An Indian study on A. cerana wax suggests that its triacontanol content may be an economical alternative source for this plant growth stimulator (Devakumar et al., 1986).

Many other applications for beeswax, in cosmetics and pharmaceuticals may benefit also from the presence of minor components which have not yet been thoroughly investigated.

4.6 Wax collection and processing

There are several ways of collecting beeswax. Morse (1965) has experimented with the idea of producing beeswax directly from clustered bees with a caged queen and no foundation. Comb building was prevented by exposing clusters to continuous daylight and wax scales were collected below the cluster. This may be suitable for

certain experimental requirements, but is not economically feasible with the current prices of wax.

More commonly in frame hive beekeeping, wax is rendered from the cappings removed during honey extraction. This produces a very high quality, light coloured wax. Light coloured broken combs provide the next quality of wax, whereas old black brood combs yield the smallest proportion and lowest quality of wax. Scrapings from side walls and the bottom board contain very high proportions of propolis and should not be mixed with better quality waxes. They can be used in swarm traps, for hive wood treatments, or in other preservatives for wood (see recipes in section 4.11.10).

In areas with traditional and topbar hive beekeeping, different qualities of wax can be produced by separating new white honey combs from darker ones or from those with portions of brood. Since whole combs are harvested and crushed or pressed, the proportion of wax per kilogramme of honey (10-15%) is much higher than with frame hive beekeeping, where the yield is only 1-2%.

Before processing, all comb or wax pieces should be washed thoroughly to remove honey and other debris. Crane (1990) even suggests soaking combs in water for

several hours, or up to two days for older brood combs. The first wash, if done with small amounts of water can be used for beer brewing or if no infectious diseases are present for refeeding to the bees.

Several methods of rendering wax are possible and may be adapted to various circumstances. Wax can be separated in solar wax melters, by boiling in water then filtering, or by using steam or boiling water and special presses. If soft water or rain water is not available for these processes, hard water (high calacium content) may be used, but 0.1 % of vinegar should be added to it (Crane, 1990). The different methods are described in further detail in many beekeeping publications, for both small scale, low investment processing and for larger scale operations (Clauss, 1982; Adjare, 1984 and 1990; Coggshall and Morse, 1984; Hepburn, 1986; Gentry, 1988; Graham, 1992 etc).

Wax should never be heated above 85 ^{0}C. If wax is heated directly (without water) or above 85 ^{0}C discolouration occurs. Therefore wax always needs to be processed in water or in a water bath. Wax should not be processed in unprotected steel, iron or copper containers, since it will discolour from reaction with these metals. Direct exposure of wax to hot steam results in partial saponification.

The residues from wax rendering contain sufficient nutrients to be used as poultry food or be turned into good compost. A Polish study measured a crude protein content of 22.12% When added at 4% to the rations of laying hens instead of green forage meal, the residue maintained all growth and health characteristics and improved egg yolk colour (Faruga et al., 1975). With some precautions, the residue can also be included in diets for rearing wax moth larvae (see 8.10.7).

4.7 Buying

A buyer should make sure wax has been stored for a few weeks after processing in water, since newly cleaned wax may contain up to 20% by weight of water. Much of this water will be lost during the first few weeks of storage. Unpleasant surprises found inside larger blocks of wax may be rocks or other heavy materials.

Beeswax should have its characteristic yellow colour and sweet aroma when bought as rendered beeswax. The grey coloured layer at the bottom of inadequately cleaned wax cakes is mostly debris. It should be scraped off and may be reprocessed to extract more wax.

Wax cleaned in a solar wax extractor can sometimes be less aromatic and will be

much whiter, almost the pale white colour of paraffin wax. The aroma of beeswax can be destroyed by overheating and chemical bleaching. Dark coloured beeswax has either been inadequately cleaned or has been processed in unsuitable containers made of iron, copper, brass, nickel, zinc (galvanized steel) or their alloys. The latter discolouration can only be reversed with a special metal binding (chelating) process. White (1966) described using approximately 1.9 g of the sodium salt of ethylene-diamine tetra-acetic acid (EDTA) in a litre of soft (rain) water to process approximately 400 g of wax. The mixture was boiled at 100^0C for one hour, stirring continuously in a stainless steel, glass or aluminum container. After cooling, the bottom layer was scraped off while the clean part was remelted in clean water and cooled.

Adulteration with other waxes is difficult to detect without chemical analyses and physical tests, some of which are described in 4.9.

4.8 Storage

Beeswax should only be stored in its rendered, clean form. Before rendering, it will quickly be attacked by wax moths, which are able to destroy large quantities of wax in short periods of time (see Figure 4.6). Clean wax in large blocks is not attacked by

wax moths. The honey guide of Africa (Indicator minor) is uniquely adapted to digesting wax with an intestinal flora of Micrococcus cerolyticus and the yeast Candida albicans (Friedman et al., 1957). However, the honey guide rarely consumes or steals large amounts of wax while it may destroy wax foundation sheets.

Storage should be in cool dry places and never in the same room with any kind of pesticide. Wax will slowly crystallize over time and as a consequence become harder, but this process is reversible without any damage, just as with crystallized honey. The white bloom, i.e. dust, that sometimes appears on the outside of a wax cake or candle consists of small wax crystals. When melted or pressed with the rest of the wax it reverts to normal beeswax without any residues or impurities. Wax can be stored for very long periods of time without losing its major characteristics as items from Egyptian graves more than 2000 years old have shown.

Figure 4.6: Wax comb destroyed by wax moths before it was rendered into clean wax.

The storage requirements of products made with beeswax are affected by the added ingredients. Polishes containing only mineral or non-vegetable oils can last for years, but cosmetic emulsions, which are mixtures of water and oil have a very limited shelf-life ranging from a few weeks to a few months (and longer if refrigerated). Unless some alcohols, propolis or other preservatives are added, emulsions are an excellent environment for microorganisms to flourish. Clean ingredients, a clean working environment and proper storage are very important to maintain the quality of products and prolong their storage life.

4.9 Quality control

Beeswax, when sold in solid blocks should always both be clean and have the colour and odour characteristics described in section 4.7. Though adulteration is easy (usually with cheap paraffin waxes), its detection is only possible with chemical tests, but it will very likely be detected by any larger buyer long before it reaches an industrial user. Adulteration renders the whole batch useless for most purposes and constitutes a considerable loss to the buyer. Therefore, such practices usually result in a buyer ceasing to buy from the supplier and possibly from the country from which the wax came.

Quality standards for wax are set in most countries according to their pharmacopoeias. A few industries like the Japanese cosmetic industry but also the American Wax Importers and Refiners Association specify their own limits (see ITC, 1978). In addition, for each industrial product in which beeswax is being used, there are other industry standards to be observed. These have to be obtained from the respective industry representations or trade publications. Such standards may vary considerably from country to country and manufacturer to manufacturer.

To detect adulteration, a number of tests may have to be conducted. The simplest is to determine the melting point, by measuring the temperature at which the first liquid wax appears during very slow heating. It should be between 61 and 66 ^{0}C or preferably between 62 and 65 ^{0}C. However, values within this range are not a guarantee of purity.

Determining the saponification cloud point is an officially accepted, sensitive method for determining adulteration. The method is limited to detecting quantities greater than 1 % of high melting (80-85 ^{0}C) paraffin waxes, or more than 6% of low melting (50-55 ^{0}C) paraffins. The test measures the amount of hydrocarbons which saponify (turn into soap) in a specific amount of ethanol and give a clear solution. If

the solution becomes clear at or below 65 ^{0}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 (1973) for the American Wax Importers and Refiners Association and in section 4.11.15. 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} alcohol (an alcohol molecule with 32 carbon atoms) contained in Carnauba wax. Beeswax only contains very little (Tulloch, 1980).

Tulloch (1980) also suggests that GLC can be used to detect adulteration of beeswax with as little as 1 % of petroleum hydrocarbons from low melting paraffins, but not for detecting low levels of high melting paraffin waxes.

Pharmacopoeia list ester values from 66 to 82 but most beeswaxes range between 72 and 80. Tulloch (1980) suggests values of 70 to 80 are most typical. Acid values range from 16.8 to 24 and ratios between ester and acid values are fairly stable and narrow, mostly between 3 3 and 4.2. The ratios can change after excessive heating and can exceed 4.2 with heating to 100 ^{0}C for only 24 hours, while the ester and acid values might remain within set limits. Ester and acid values in waxes from other

Apis species may be significantly different (Ikuta, 1931 and Phadke et al., 1969).

In Africa, adulteration of beeswax with dark and sticky Trigona (Meliponidae) wax has been reported (Smith, 1951). Such wax is of little value in most industrial and beekeeping applications, since the resins are difficult to remove.

For standard testing methods, references can be obtained from Crane (1990), ITC (1978), Apimondia, pharmacopoeias and industry associations.

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4.10 Market outlook

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The cosmetics and pharmaceutical industries have no complete substitute for beeswax. At least small quantities will always be needed to maintain quality and specific characteristics. Beekeepers using frame hive technology are their own best clients and use most of what they produce. Industrial needs are largely provided by

imports from countries with traditional beekeeping techniques. In many other applications, beeswax is replaced with synthetic waxes and compromises in quality are accepted by the manufacturers because of the reduced cost and greater availability of synthetic waxes. Industrial use of beeswax might increase if availability would increase and become more reliable or if prices could drop significantly. The balance between cheap substitutes, the large needs of beekeepers themselves and quality considerations for uses of beeswax has kept prices stable but relatively low for many years, despite scarcity in supplies. Beeswax prices for imports into the USA went above US$4/kg in the early 1980's, but are now fluctuating between US$2.10 and 3.00/kg wholesale for light-coloured wax, occasionally reaching US$6 - 7/kg. Darker wax is 10 - 20% cheaper. Like honey prices, prices for beeswax may vary considerably from place to place.

Markets and prices for products made from beeswax vary widely from country to country. Generally, the best margin between raw material value and end product price may be obtained in cosmetic preparations and jewellery. Most other applications, including pharmaceuticals, except dermatological and traditional medicinal products, are part of a very different industry which requires much larger investments and higher technologies. In these industries beeswax forms only a minuscule part both of the manufacturing process and of the final product.

The refining of beeswax for export is not common at the moment. Most industrial users prefer to buy crudely rendered and filtered wax directly from local sources because their own processing guarantees better quality control. A reliable processor should be able to establish a good enough reputation to also export refined products. Most companies prefer to buy in larger quantities (5-15 tons).

4.11 Recipes

The recipes described below are taken from various sources. They were chosen to highlight principle ingredients and demonstrate basic methods. They are not the only ways of making the product, nor necessarily the best or most economic. Many variations and substitutions are possible. Specific institutions and trade publications may be contacted for more detailed information. This is particularly true for more recent advances, because of the high degree of specialization and enormous volume of new information. Such details go beyond the possibilities of this publication. Instead, it is hoped that a large variety of ideas can be provided to people with special problems which may help them to develop new products adapted to their cultural, economical and technological environment.

Presentation of a recipe does not guarantee that it will fulfil the desired effect, nor

that it will be without side-effects. Anybody using the following recipes should be advised that some of the chemicals are toxic, caustic or damaging to the environment, particularly if discarded improperly. Information should be obtained about the legal requirements concerning use of certain ingredients, precautions to be taken, labelling of finished products and permission to use selected ingredients for the manufactured product.

4.11.1 Bleached wax

Bleached beeswax is preferred for many cosmetic preparations and candles because it permits better colour control of the final product. However, it is lacking in most of the aromatic components.

A non-chemical method for bleaching beeswax is the use of sunlight. The wax is flaked, i.e. cut into small pieces, and exposed to the sun on large trays. It should not be allowed to melt and must be protected from contamination with dirt, dust and other debris. Particularly in tropical climates extra ventilation will be required to avoid melting. Wax left in solar wax extractors will also slowly bleach and slowly turn white.

Berthold (1993) describes a method of bleaching which goes back to the ancient Greeks. The beeswax is flaked and bleached in the sun, then boiled in clean, clear sea water. The scum layer floating on top is skimmed off and the heating repeated. The cooled wax is flaked again and bleached once more in the sun. A final melting in soft fresh water may be necessary to rinse out the salt residues.

Most commercial operations today use chemicals for bleaching wax or special absorbent filters. Among the many possible chemicals are oxalic acid, hydrogen peroxide, orthophosphoric acid, citric acid, sodium dichromat, sodium permanganate, potassium permanganate, ammonium persulfate, benzoyl peroxide and others. After mixing bone charcoal and Fuller's earth or diatomaceous earths into liquid wax and agitating for several hours, impurities are adsorbed and then removed with a filter press.

Berthold (1993) described two practical methods of chemical bleaching. The first one uses oxalic acid, a highly poisonous substance which needs to be handled and stored with care. Glasses and rubber or plastic gloves should always be worn. Water should be kept nearby for washing the skin or face in case of accidents. Spills need to be cleaned up immediately and the acid should be stored in well labelled containers beyond the reach of children. Chemicals should not be spilled or

discarded into open water (drainage ditches, creeks, ponds and lakes). If there is no other way of discarding them, chemicals should be poured into a hole in the ground, far away from wells, and then covered with soil. Stainless steel, fire proof glass or enamel containers need to be used for heating the wax. Containers should only be partially filled so that the mixture will not boil over, particularly if processing takes place over an open flame.

The wax has to be heated above its melting point for at least 10 minutes and stirred in water, to which approximately one tablespoon of oxalic acid has been added per 4 litres of water. Four litres of the above acid/water mix can be used to bleach up to 10 kg of wax in one batch, but the exact proportions should be determined for the local wax and water conditions. Slightly higher concentrations of citric acid are required and the heating will have to be extended. Since citric acid, however, is much less toxic and dangerous, it should be preferred over oxalic acid. To control the progress of bleaching, a small quantity of the wax is ladled or spooned into cold water. If not sufficiently bleached, heating should continue and/or a very small quantity of acid be added. If sufficiently bleached, the wax should be cooled, re-melted in a larger quantity of clean water and moulded into blocks for sale.

In the second method of bleaching described by Berthold (1993), small quantities of

30-50% reagent grade hydrogen peroxide (this is very caustic) is added to the melted wax and water mixture. The temperature is maintained at 65-70 ^{0}C and stirring will expedite the bleaching process. Progress can be checked as in the oxalic acid method. If only low concentration hydrogen peroxide is available, larger quantities will have to be used and the stirring and heating will have to be maintained for longer - up to 30 or even 45 minutes (the concentration of hydrogen peroxide cannot be increased by evaporation). Again, the bleached wax should be re-melted once in clean water to remove all reagents. The exact proportions of hydrogen peroxide, water and the quantity of wax processed, need to be determined by experimentation. As with all recipes, a small batch should be tried first, before processing larger quantities.

Oxalic acid is also used for bleaching wood and is often available in wood stores and hardware stores. Other compounds sold for wood bleaching are unsuitable and cannot be used instead. Pharmacies (drug stores) might stock both oxalic acid and hydrogen peroxide, but these are likely to be of very low concentration. Beauty salons may also stock hydrogen peroxide and chemical supply houses should have both chemicals. If beeswax has to be processed at all, solar bleaching is still the least expensive, least dangerous and least toxic procedure.

4.11.2 Candle makin2

The basic elements of a candle are the solid wax as fuel for the flame and a wick, which serves to bring the molten wax to the flame. Oil lamps work on the same principle, but they need a container to hold the liquid fuel.

The best material for the wick is a fibre which burns with very little ash at low temperatures. Pure cotton thread is the best. Several thin cotton threads should be braided or plaited together until the desired thickness is reached. Twisting of the threads is not recommended, since they might unwind during burning and then create an irregular flame consuming much more fuel. Commercially produced candle wick can often be purchased in speciality shops.

The wick needs to be in the centre of the candle for even burning. The diameter of the wick in proportion to candle diameter is important to maximize the light obtained from the quantity of wax and to prevent wax dripping down the side of the candle. Thicker candles need thicker wicks, but thick candles with a relatively thin wick burn longer and give less light, since the flame is shaded by the remaining edges of the candle. The precise ratio depends on the purpose of the candle and should be determined by experiment.

Beeswax for candles needs to be extremely clean and free of all impurities (propolis or pollen) otherwise the candle will sputter while burning, give irregular light and possibly be splattering hot beeswax. Beeswax purchased from most beekeepers must usually be reprocessed at least once more in clean water.

There are various pigments available from specialty suppliers for colouring wax and some natural dyes will also work. Regular paint pigments are often insoluble in fat or burn incompletely and so should not be used. Normal food colouring does not work very well as it will leave residues, might clog the wick or produce stains. If only applied as a thin outer layer it may be acceptable but special fat soluble pigments give much better results.

Figure 4.7 : A display of homemade candles from West Africa (from left to right).

Candles can be made by various processes (see Figure 4.7). The most suitable techniques for home use or small scale manufacturing involve using candle moulds or wax sheets to roll candles. In all cases, extreme care needs to be taken since beeswax is highly flammable and because of its high heat capacity, can cause severe burns when dropped onto bare skin. Wax for candle making should always be

stained candle moulded in PVC pipe, coloured wax with trimmed tin candle still inside of bamboo mould, 2 candles rolled from wax foundation sheets, decorated

heated in a water bath (see VlProblemsV! below). Stainless steel or glass containers are recommended, but tin cans may be used for small quantities.

Rolled candles

Plain or patterned wax sheets are rolled around a central, wax impregnated wick. The wick has to be soaked in hot wax for a while and cooled in a very straight shape by suspending it with a weight attached at the bottom. The size, height, thickness and length of the wax sheet determines the shape and size of the candle. Frequently, the patterned foundation sheets for beekeeping are used (see Figure 4.7). No special moulds or complicated procedures are involved: it is a very clean and simple process which is easy to carry out.

The sheets are very easy to make. A smooth, wetted, wooden board dipped a few times into molten wax will make two sheets at a time (one on each side of the board). If only small quantities of wax are available, the liquid material can be poured into a flat mould made with a rectangular frame laid on a smooth surface (a wooden board, aluminum sheet or thick glass). The mould or board should be treated with soapy water or diluted honey to prevent the wax sticking to it. It will also be easier to remove the wax if the mould is flexible. A warm mould will

facilitate spreading of small quantities of wax to provide a thin sheet. The mould surface can be sculpted to give the candle surface a special decorative effect like, for example, with beekeeping foundation sheets.

Moulded candles

The most common process for making candles uses moulds to give the wax its final shape. All kinds of patterns can be used; moulded candles do not have to be round. They can be square, triangular, oval, egg shaped, conical, all kinds of other geometric shapes or simply an irregular, carved design. In principle, the mould has to withstand the temperature of the molten wax (up to 100^0C), should not expand or shrink too much with changing temperature and should be easy to remove from the hardened candle.

For round stick candles, the choice of a mould depends on the size of the desired candle and the materials available. Pre-manufactured metal moulds are available from some specialized suppliers, but any round tube of the right internal diameter can be used: galvanized steel, aluminum, polyvinyl chloride (PVC), some types of rubber and bamboo. To facilitate removal of the candle, the PVC or bamboo could be carefully slit on one side. Held together with wire or string during the pouring, it

can be opened a little to remove the candle. A small seam of wax might be left on the candle, but this can be carefully scraped off.

The longer the mould, the more difficult it will be to remove the candle. For solid, one-piece moulds and candles of 2 to 3 cm in diameter, a length of 12 to 15 cm is most practical. If a freezer or refrigerator is available, the moulds and candles may be cooled for a few hours. Cold wax will shrink away from the mould and can be pushed out easily.

The moulds need to be prepared so that the wax will not stick to their surface. Diluted honey or soap can be used as a coating. Silicones are also suitable but Vaseline (petroleum jelly) is not since it will be melted by the wax and will mix into the outer layer. Any coating that is used will have to be wiped off the finished candle with a damp cloth without wetting the wick.

To secure the wick in the centre of the mould, one end is tied to a small stick using a slip khot. The wick is threaded through the mould without touching the coated walls and the stick is placed into two notches cut in the rim of the mould to hold the wick in the centre of the tube. The loose end of the wick is tied tightly to another stick fitting into the notches on the opposite end of the mould. Ensure that

the wick is in the centre of the tube.

One end of the mould is covered with a leaf, foil, clay or stick and placed into sandy ground. The mould should be warmed as much as possible in a stove, near a fire or inside a solar wax melter. Its temperature should be as close to that of the molten wax as possible. A few minutes after all the wax has melted in the water bath, it can be poured slowly into the hot moulds. The hotter the wax, the better is the final result, but it should not be boiling. Wax in the pouring container should not be allowed to cool down too much. Once poured, the mould may be covered so that no dirt enters. Moulds and candles should cool down as slowly as possible, e.g. in a warm room without draughts and direct sunlight.

After about two hours, thin candles (2-3 cm diameter) should have cooled down enough to remove them from the mould. The sticks are removed from both ends, making sure not to pull the wick from the centre of the candle. The mould is opened, refrigerated or the candle pushed out immediately. Any mould coating is carefully wiped off. The wick is cut to a length of 1 cm on the burning end and trimmed and cleaned at the other end. The candle should be stored in a cool, dark place and be wrapped in some clean paper or plastic bag to keep it from getting dusty and dirty. Newspaper should not be used because the print might transfer

onto the candle.

Problems

If the mould cools down too fast or was not hot enough during pouring, the centre of the open end of the candle might sink. It may be refilled with liquid wax immediately after the first pouring has started to solidify and showed first symtoms. The same conditions may also lead to cracked candles. If either occurs, preventive measures include pouring the wax even hotter (but still without boiling it), prewarming the moulds bettered pouring the wax during the warmest time of the day (preferably in the sun) and cooling the moulds slowly in a warm, draught-free place.

If the solidified wax contains small droplets of water, the candle will sputter during burning as with the inclusion of dirt. To avoid this problem, freshly cleaned and processed wax may be heaated for a little longer before dipping or pouring the candles. A period of 5 to 10 minutes close to l00�C should be enough and is said to also improve the non-drip quality of the candle.

The larger the operation becomes, the more important proper control of the

temperature conditions will become.

Odd shaped candles

Odd shaped candles cannot be pushed out of a mould without opening it. they have to be carved individually, or a mould has to be prepared out of at least which, when tied together, has one open end into which the wax can be poured. Therefore they have to be carved individually, or a mould has to be prepared out of at least two pieces which, when tied together has one open end into which the wax can be poured. A simpler alternative is to produce two half caldles in separate moulds and then "glue" the halves toghether with molten wax. Otherwise, the same methods and cautions apply as for stick candles.

The moulds can be made around a clay, wood or wax model with resins, silicone rubber, clay or metal, using techniques similar to those employed in metal casting and dentistry.

Figure 4.8 : Various shaped candles and packaging. A dipped candle is laying on the bottom.

Dipped candles

Very nicely shaped classic candles can be made by repeatedly dipping a weighted or

stiffened wick into a liquid wax bath at *65* ^{0}C. An additional layer of wax is built with each dip. If the temperature of the wax is regulated correctly, this method produces excellent candles, but requires considerable skill and patience. Only very high quality candles and those for special ceremonial purposes are now made this way. Candles have to be immersed fast, left long enough to warm the solid wax and be withdrawn at just the right speed to avoid ripples on the candle and drippings on the bottom. Between dips, candles have to cool for a few minutes. Eason (1991) gives a simple and very clear account on how to dip beeswax candles. Very skilled craftsmen can also pour hot wax over the wick in order to build up thick candles.

Pressed candles

For industrial processes candles can also be pressed, extruded or drawn. To make pressed candles the wax is first powdered by atomizing (by spraying a fine mist) liquid wax during cooling. The powder is then pressed into the desired forms. For extrusion, a hollow tube with a wick in its centre is drawn from a perforated metal sheet and cut into the desired lengths. For drawn candles a continuous wick is intermittently drawn through liquid wax and holes of increasing diameter in metal sheets.

Sculptured candles

In some countries sculptured candles are popular (see Figure 4.8). Thick candles can be sculpted into various artistic shapes, such as animals or ceremonial or religious symbols for birthdays or other special occasions. They can also be decorated with surface materials such as sand and may be painted in different colours. Sculptured casting moulds can be made with silicone rubber so that particular shapes can be produced in larger numbers.

Economics

Although cheap paraffin wax candles are available in most rural areas, the manufacture of beeswax candles can be an additional incentive for beekeepers or for women to get started in beekeeping. In areas with no readily accessible market for beeswax, it is all too often thrown away after honey processing. Under these circumstances, even cheap candles made by mixing paraffin wax with beeswax are an improvement which can provide an additional source of income or avoid extra expenses on lighting. Once larger quantities of wax are saved by beekeepers or beer makers, other markets can be accessed. Beeswax mixed with even the smallest quantity of paraffin or other synthetic wax should never be given back to bees in

the form of foundation sheets or comb starters, because all wax subsequently produced from these colonies will be adulterated.

Further reading

For those interested in more details, the book of Coggshall and Morse (1984) is highly recommended. Other practical details can be found in a variety of publications, mostly bee journals. Some very simple illustrated methods are shown in the Peace Corps beekeeping manual (Gentry et al., 1985; Gentry, 1988) and in an ITDG (1978) publication. The following literature describes particular processes in more detail: the making of reusable and sculptured moulds from silicone rubber (Rigby and Hepburn, 1981), hand dipping of candles (Driesche, 1983), general tricks of the trade (Vinci, 1981; Furness, 1974 and 1986; Coutare and Guzzi, 1989) and supply sources for the UK (Higginbottam, 1974) The basic principles are all the same, but differences usually arise in the material selected for moulds, many of which have been mentioned in these publications.

Figure 4.9 : Special, moulded, carved and painted candles from displays in Germany (Mungersdorff, Koln)

4.11.3 Cosmetics

Only one very basic recipe for making a very simple cream is given here. All other recipes can be found in Chapter 9.

Ingredients (in parts by volume):

1	*Beeswax*	*0.06*	*Borax*
3	*Mineral oil*	*2*	*water*

Heat the wax and mineral oil in a water bath until the wax has melted (70^{0}C). Heat the water to the same temperature and dissolve the borax (approx. ig borax per lOOg of total ingredients). Slowly pour the water phase into the oil phase while stirring vigorously, but not so fast as to incorporate air into the cream. Continue stirring until the mixture has cooled and formed a creamy emulsion. Shortly before it solidifies, aromatic essences can be added. Propolis extract can be incorporated into the liquid phase when the temperature is about 40-50 ^{0}C. If the mixture separates or does not solidijy evenly, reheat it and try again. Patience and experience will lead to success. Store in airtight containers. The cream will keep for many weeks unless short sheij life ingredients such as vegetable oils, tallow or royal jelly have been added.

Most skin creams are used to provide moisture to the skin, keep the skin moist and for replacing some of the oils of the skin. A basic cream therefore contains water, an

oil and a wax to make the mixture creamy and allow even distribution of the water. Since water does not mix with oils or wax, an emulsifier (in this case borax) must be added. The emulsifier changes the acids of the wax into soaps which then mix well with water. The proportions of the ingredients can vary but not more than 6.8% borax, on the weight of wax, should be used. Since borax is not very soluble in the mixture and if too much is added, the cream will have a rough texture (Crane, 1990).

Many different vegetable or mineral oils can be used but the disadvantage of vegetable oils is that they become rancid within a few weeks. Such oils are widely available and some of them have additional beneficial characteristics. Whichever oils are used, they should be as clean as possible usually of higher than food grade. The water that is used should be the best available. Rain or fresh spring water is considered best, but filtered well water or clean pipe water may also be used. Heavily chlorinated pipe water may be harmful and the calcium in hard water reacts unfavourably with beeswax and other cosmetic ingredients. Clean and uncontaminated water is becoming increasingly rare in all parts of the world so special attention should be paid to this important ingredient. Industrial cosmetics are usually made with distilled or de-ionised water.

4.11.4 Grafting wax for horticulture

Mix one part melted beeswax with one part of resin and enough lard or tallow to make the mixture pliable Some finely ground charcoal may be added to protect the wound against sunlight. The mixture may be spread warm or applied in thin strips (Crane, 1990).

Melt equal portions of resin and beeswax in a double boiler or water bath and mix well. After cooling roll the mixture into sticks and store them (individually wrapped) in a cool place. Another recipe recommends a mixture of equal parts resin, beeswax and lard, prepared in the same way.

Since some growth hormones have been discovered in beeswax, the above formulations may actually be better than some commercial preparations.

4.11.5 Polishes and varnishes

Judging by the variation in recipes, it is obvious that there are many ways of preparing a wood finish or polish suitable for particular application. Turpentine is the most commonly available natural solvent for wax, but other oils may be substituted to avoid the rather strong odour of turpentine. Suitable alternatives are orange, lemon or linseed oil, naphtha or other liquid refined petroleum fractions

and to a lesser degree, other refined vegetable oils. The wax content can range from 5 to 50% and occasionally even more. The consistency of the paste or oil may change, but can be corrected with appropriate adjustments in the proportions of each ingredient, e.g. less oil or more wax if it is too liquid.

Paste furniture polish:

Ingredients (in parts by volume) taken from several old and new references:

8	*Turpentine*	*1*	*Liquid soap*
1	*Beeswax*	*4*	*Soft water (rain)*
1	*Pine oil*		

Melt the wax in the turpentine using a double boiler or water bath over low heat. Care is required since turpentine is highly flammable. At the same time, mix die soap in the warm water. when both mixes have cooled a little, or are of similar temperature, pour the water phase into the oil phase and mix thoroughly but gently. Once cooled to less than 50 ^{0}C add the pine oil. while it is solidijying, spoon

or pour the product into wide-mouthed jars or cans which should be sealed immediately. Label the container appropriately. If the wax hardens too quickly or too soon, it may be re-heated.

Aromatic oils (for example, a few drops of lemon oil, pine oil or any other oily aromatic extract) can be added in small quantities to any polish. They should be added when the polish is cool but still soft.

2) ***Ingredients (in parts by volume):***

Melt and mix equal parts of turpentine, linseed oil and beeswax in a water bath. Stir well and when cool spoon into wide mouthed labelled jars or flat tin cans.

Liquid furniture polish:

1) Ingredients (in parts by volume) from several old and new references:

4	*Turpentine*	*1*	*Liquid soap*
1	*Beeswax*	*2*	*Soft water (rain)*

Mix in the same way as the creamy polish. Store in small labelled screw top bottles.

2*) Ingredients (in parts by volume or weight):*

1	*Beeswax*	*1*	*Linseed oil*

Melt and mix in a water bath and store in labelled screw top bottles. The proportions of beeswax and linseed oil can be varied considerably.

Other oils can be added, and also resins which may help to create a slightly harder su~ace film.

If the beeswax/linseed oil mix is boiled until there is some stringy residue forming at the bottom, the clear liquid above can be poured off and used as a varnish.

3) Ingredients (in parts by weight) adapted from Gentry (1988):

4	*Beeswax*	*2*	*Turpentine*
1	*Orange, lemon, coconut or*		

linseed oil

Grate the beeswax into the turpentine. Add one of the oils and mix. The turpentine will dissolve the wax and no heating is necessary. Store in labelled tins or bottles with tight fitting lids.

In order to improve the quality of this and other above polishes, try to get better refined ingredients, particularly turpentine or oils.

Spray polish

All recipes for spray application of beeswax were found to either contain highly toxic chemicals or those which are destructive to the upper atmosphere of the earth and are, therefore not described here.

For optimization of health, environmental hazards and wood preservation, the beeswax/linseed oil polish is best.


GRATIS
25%
mobili
Pure Beeswax
Polish

Figure 4.10: Furniture polish spray with beeswax and a polish paste based mostly on beeswax.

Floor Polish

1) For wooden floors, mix equal parts of beeswax and turpentine. The polish can be used as soon as the beeswax is dissolved.

2) A cheaper product for wooden floors and cement or tiled floors may be prepared as follows:

Ingredients (in parts by volume):

1	*Beeswax*	*1.5-2*	*Paraffin wax*
4	*White spirit, kerosene or diesel fuel*		

Melt the waxes in a water bath, remove from heat for safety and slowly stir in the spirit or juel. The only disadvantage of this polish is the noxious smell of the fuels

after waxing the floor. Many commercial polishes, at least in East Africa, contain these feels as judged by the odour.

3) Ingredients (in parts by weight) adapted from Gentry (1988):

2	*Beeswax*	*1*	*Potash*
3.5	*Soft water (rain)*		

Heat 2.5 parts of water and add the wax to it. Mix the potash with the rest of the water and pour it into the mixture of wax and water. Heat until it becomes a milky fluid. A similar product may be made by using soap instead of potash and less water.

Shoe polish, cream type

Ingredients (in parts by weight) adapted from Minrath (1957):

4.3	*Carnauba wax*	*3*	*Soap, flaked*
3	*Paraffin wax*	*50*	*Water*

or beeswax *q.s.* *Water soluble*
8.5 *Turpentine*

Melt the two waxes in separate containers in a water bath and then slowly add the paraffin wax or beeswax to the carnauba wax. Remove from the heat. when this mixture has cooled down but not yet started to solidift, slowly add the turpentine. Dissolve the soap in the water, heat to boiling, then mix in the pigments and the wax-turpentine solution. Continue stirring until it is cool.

To obtain the right shade of colour, the following equivalents may be added:

Black - Acid Black, Brown - Bismarck Brown G, Red - Crocein Scarlet, Orange-Orange II, and Yellow - Metanil Yellow.

Shoe polish, wax type

Ingredients (in parts by weight) adapted from Minrath (1957):

20 *Paraffin wax or beeswax* *70* *Tupentine*

3	*Carnauba wax*	*q.s.*	*Dyes*
4	*Montan wax*		

Melt the first three ingredients, adding each one after the other has melted, then add the colour. when thoroughly mixed, discontinue heating, remove from the heat source (for safety) and slowly add turpentine while stirring.

To produce the desired shade of colour, the following oil soluble dyes or their equivalent may be incorporated:

Black - Nigrosin, Brown - Bismarck Brown, Red - Rhodamin, Orange - Chrysoidin, and Yellow - Auramin.

If one or the other waxes are not available, they can be replaced with beeswax. The consistency of the final polish may change slightly, but this should not alter significantly the performance of the product.

4.11.6 Cravons

For crayons for drawing on glass or plastic, melt together equal parts of beeswax and asphaltum in a water bath. Add a little lampblack while mixing and allow to cool. Before completely cold, roll pieces into sticks on a smooth su˜ace. Other pigments can be added to provide different colours. Wrap in paper.

Another source (Gala Books, 1971) describes using 4 parts of wax, 1 part of tallow and 1 part of lampblack and, for most other colours, a mixture of 2 parts wax, 1 part tallow and 1 part chrome yellow, prussian blue or 4parts zinc white. Ordinary paint pigments may also be used. These mixes are usually pressed into the right shape. They may also be rolled into sticks and wrapped in paper. Tallow is rendered beef fat and it can be obtained from butcher's shops, slaughterhouses etc.

4.11.7 Leather preserves

1) The recipe recommended by Lloyd (1957) is identical to the first recipe of liquid jurniture polish (4.11.5)

2) Another liquid recipe uses equal parts of turpentine and wax, plus a fat soluble dye. The wax component can be varied according to availability or the final

consistency required of the polish.

3) ***Minrath (1957) suggested 200 g of montan wax, 160 g paraffin wax and 30 g of stearic acid in an equal quantity of turpentine (390g) . Any one or all of the waxes can be replaced by beeswax.***

Melt each wax separately, remove from heat and combine them careflilly, then add molten stearic acid. Once the mixture has cooled but while it is still liquid, add the oil soluble dye. when the mixture begins to solidift, stir in the turpentine.

4) ***Ingredients (in parts by weight) adapted from Minrath (1957):***

20	*Paraffin wax or beeswax*	*70*	*Tupentine*
3	*Carnauba wax*	*q.s.*	*Dyes*
4	*Montan wax*		

Melt the beeswax in a water bath, cool it until it is semi-soft, then add the

remaining ingredients and finally, the aromatic essence. Store in an air-tight container.

Figure 4.11 : The various products sold by the Ruai

Beekeepers' Cooperative in Kenya (from left to right): Honey, saddle soap (similar recipe as furniture polish paste, 4.11.5(1), without aromatic oil), candles, rendered wax, furniture cream polish and honey.

4.11.8 Waterproofing textiles and paper

In order to waterproof paper or textiles, an emulsion has been patented which also provides good air permeability and abrasion resistance. For this purpose, a colloidal emulsion is produced (see 9.4.3 and 9.4.4) by homogenizing melted beeswax (2 parts), fatty acids (3-5 parts) and paraffin wax (15-18 parts) in an alkaline solution of soapy water (Pan and Matsumoto, 1975). The paper or textiles can be brushed with the solution or dipped into it.

4.11.9 Paint

Beeswax has been used in paints since antiquity. The famous mirror wall at Sigiriya, in Sri Lanka was painted with a mixture containing resins, egg white and beeswax,

polished to a very high sheen. It can still be observed after more than 500 years. Some of the wall paintings in Pompeii, Italy, prepared with coloured beeswax are still admirable after almost 2000 years.

A simple mix of 10% resin melted together with beeswax can be coloured according to need with natural dyes or oil soluble pigments and be painted while warm and liquid (Brown, 1989 see 1981). This provides a permanent, waternroof decoration.

4.11.10 Wood preservative

For beekeeping, hive boxes can be weatherproofed by dipping them in hot linseed oil to which 5 to 10% of beeswax have been added. A much cheaper method which is not recommended because it is so dangerous has been described by a beekeeper in Argentina. It involves heating petrol (gasoline) in which old combs and hive scrapings have been melted. The hive bodies can be dipped into the hot fuel or be brushed with it.

Heat petrol ~referably lead free petrol) to 70 or 80^{0}C in an old bucket or steel drum. Be very careflil to keep open flames and sparks under control, keep the container covered and use a large high sided container only halffull. Keep the fire

small. For painting remove the container from the fire so that dripping gasoline does not spill near the flames. Only work in the open air and stay well away from housing.

Immerse at least 2 kg of old comb and hive scrapings per 20 litres of fuel and careftilly stir. After 15 minutes remove from the fire, skim the scum off the su~ace and start painting or dipping. If the liquid has cooled too much (to below 55 ^{0}C) reheat and continue. The proportion of comb can be increased and/or 5 to 10% of linseed oil may be added. Before use, allow the boxes to dry and air for a couple of weeks.

4.11.11 Swarm lure

Worker bees scouting for new home sites in preparation for, or during swarming, apparently react positively to the presence of wax and (to a lesser degree) to propolis. Smearing or melting beeswax inside a bait hive or swarm trap makes it more attractive. Only imitations of the Nasanov pheromone, a volatile attractant (hive odour) secreted by the honeybee workers, are more attractive. A successfully tested pheromone lure is made of equal parts of citral, geraniol, neuronic and geranic acids, preferably enclosed in slow release formulations.

4.11.12 Topical ointment for burns

Ingredients (in parts by weight) adapted from Gentry (1988):

1.8	*Beeswax*	*3*	*Soft water (rain)*
4	*Paraffin*	*0.1*	*Borax*
		1	*Pulverized aloe*

Melt the beeswax in a water bath, add the paraffin, mix until melted and remove from the heat. Mix the borax into boiling water, cool down to the same temperature as the wax, then stir while cooling. when the mixture starts to solidifr, add the aloe.

Instead of pulverized aloe, freshly squeezed aloe juice may be incornorated. Use 3 parts offresh aloe juice for each part of pulverized aloe and reduce the volume of the water by 2 parts. Add the aloe when the wax mixture has cooled below 40^0C. Store in tight, wide-mouthed glass jars. The ointment will keep better if it is stored in a refrigerator. It is better to make very small batches frequently than to make a

large batch occasionally. No information is available on the safe shelf life of this product.

By adding a few drops of propolis extract with the aloe, preservation should be prolonged and healing of wounds may be improved.

4.11.13 Veterinary wound cream

A base cream for treating wounds and skin diseases in animals was described by Vidyaev (1968) as consisting of mineral oil (boiled in order to reduce the water content) to which pine gum resin was added together with beeswax. The mixture was filtered and powdered calcium carbonate added before cooling. No proportions were given in the English abstract, nor were results of application described. However, cream-like consistency can be obtained with proportions copied from the above recipes and resin content may be from 2 to 10%. Addition of propolis extract (at 1-2%) would probably increase the effects of this basic cream.

4.11.14 Adhesive

Beeswax itself, when slightly softened by kneading in ones hands, sticks to many

materials and surfaces. It can therefore be used to temporarily hold light objects together.

The following recipe is referred to as Turners' cement and can be used with a variety of materials, wood, metal and clay pots. Its performance may not compete with other specialized adhesives, but is a cheap alternative when nothing else is available.

Ingredients (in parts by weight) adapted from Brown (1981):

2	*Beeswax*	*1*	*Pitch*
1	*Resin*	*4*	*Fine brick dust*

Melt the beeswax in a water bath and add the resin and the pitch. when everything has melted, stir in the brick dust and leave it to cool. Warm the adhesive before applying it.

4.11.15 Determination of saponification cloud point ((1uoted from ITCg 1978)

Apparatus:

A. lOOml Kjeldahl flask
B. Reflux condenser
C. Thermometer - certified at 63°C

Procedure:

Place 3.0 grams of wax in a 100 ml Kieldahi flask and add 30 ml of a clear ethanolic potassium hydroxide solution (for the preparation of the KOH solution follow the method described below) Connect the flask to a reflux condenser and boil gently for 2 hours. At the end of this period, disconnect the reflux condenser, place the flask in a water bath at 80^0C and insert a thermometer (ASTM designation E1-34C) into the solution. Rotate the flask in the bath while cooling and observe the temperature decrease. The temperature at which cloudiness or globule formation appears in the solution is the Saponification Cloud Point. For more accurate observation of the Cloud Point, place a printed card with broad black letters 1/4 ' high under the flask as it cools. The temperature of the solution when the printing observed through the flask becomes ha~, is to be taken as the Cloud Point.

Preparation of Ethanolic Potassium Hydroxide Solution

Rapidly weigh approximately 35 grams of pelletized potassium hydroxide (reagent grade) and transfer immediately to a bottle which contains 1 litre of pure aldehyde free, 94.9% by volume, ethyl alcohol. Shake the bottle occasionally until all KOH pellets are dissolved. Let stand for 24 hours, and decant or filter rapidly to remove carbonates that have formed. A yellow or brown discolouration of the solution indicates the presence of aldehydes. These can be removed by the following procedure: Add 5 grams of aluminum foil to 1 litre of the ethanolic potassium hydroxide solution and reflux for 30 to 60 minutes. Distill and collect the alcohol after discarding the first 50 ml. Prepare the ethanolic potassium hydroxide anew as described above.

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CHAPTER 5
PROPOLIS

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5.1 Introduction

Propolis is a mixture of various amounts of beeswax and resins collected by the honeybee from plants, particularly from flowers and leaf buds. Since it is difficult to observe bees on their foraging trips the exact sources of the resins are usually not known. Bees have been observed scraping the protective resins of flower and leaf buds with their mandibles and then carrying them to the hive like pollen pellets on their hind legs. It can be assumed that in the process of collecting and modelling the resins, they are mixed with some saliva and other secretions of the bees as well as with wax.

These resins are used by worker bees to line the inside of nest cavities and all brood combs, repair combs, seal small cracks in the hive, reduce the size of hive entrances (see Fig. 5.1) seal off inside the hive any dead animals or insects which are too large to be carried out, and perhaps most important of all, to mix small quantities of propolis with wax to seal brood cells. These uses are significant because they take

Figure 5.1: Honeybees frequently use propolis to reduce the size of the entrance for better defence.

advantage of the antibacterial and antifungal effects of propolis in protecting the colony against diseases. Propolis has been shown to kill the bee's most ardent bacterial foe, Bacillus larvae - the cause of American Foul Brood (Mlagan and Sulimanovic, 1982; Meresta and Meresta, 1988). The use of propolis thus reduces the chance of infection in the developing brood and the growth of decomposing bacteria in dead animal tissue.

The composition of propolis depends on the type of plants accessible to the bees. Propolis changes in colour, odour and probably medicinal characteristics, according to source and the season of the year. Moreover, some bees and some colonies are more avid collectors-generally to the dismay of the beekeeper, since propolis is a very sticky substance which, in abundance, can make it difficult to remove frames from the boxes.

Foraging for propolis is only known with the Western honeybee Apis mellifera. The Asian species of Apis do not collect propolis. Only Meliponine or stingless bees are known to collect similarly sticky resinous substances, for sealing hives and constructing honey and pollen pots for storage. In this bulletin, however, propoli shall refer only to resins collected by honeybees, since almost all of the research has been done on it. There may well be similar traditional uses for resins collected by

Meliponids.

In the natural distribution ranges of Apis mellifera, a multitude of traditional uses are known for this versatile substance. The Greeks and Romans already knew that propolis would heal skin abscesses and through the centuries its use in medicine has received varying attention. The ancient Egyptians knew about the benefits of propolis and in Africa it is still used today, as a medicine, an adhesive for tuning drums, sealing cracked water containers or canoes and dozens of other uses. It has been incorporated in special varnishes such as those used by Stradivarius for his violins (Jolly, 1978).

An excellent review in Spanish on the production, characteristics and uses of propolis was published by Asis (1979 and 1989) another good overview (in English) was APIMONDIA (1978). A brief, more recent review in English is presented by Schmidt and Buchmann (1992).

5.2 Physical characteristics of propolis

The colour of propolis ranges from yellow to dark brown depending on the origin of the resins. But, even transparent propolis has been reported by Coggshall and

Morse (1984).

At temperatures of 250 to 45 ^{0}C propolis is a soft, pliable and very sticky substance. At less than 150 C, and particularly when frozen or at near freezing, it becomes hard and brittle. It will remain brittle after such treatment even at higher temperatures. Above 45 ^{0}C it will become increasingly sticky and gummy. Typically propolis will become liquid at 60 to 70^{0}C, but for some samples the melting point may be as high as 100^{0}C.

The most common solvents used for commercial extraction are ethanol (ethyl alcohol) ether, glycol and water. For chemical analysis a large variety of solvents may be used in order to extract the various fractions. Many of the bactericidal components are soluble in water or alcohol.

5.3 The composition of propolis

In one recent analysis of propolis from England, 150 compounds were identified in only one sample (Greenaway, et al., 1990), but in total more than 180 have been isolated so far. It appears that with every new analysis, new compounds are found.

Propolis resins are collected from a large variety of trees and shrubs. Each region and colony seems to have its own preferred resin sources, which results in the large variation of colour, odour and composition. Comparisons with tree resins in Europe suggest that, wherever Populus species are present, honeybees preferably collect the resins from leaf buds of these trees.

A Cuban study suggests that the plant resins collected are at least partially metabolized by bees (Cuellar et al., 1990). The presence of sugars (Greenaway et al., 1987) also suggests some metabolization by bees, i.e. as a result of adding saliva during both scraping and chewing.

A list of the major classes of chemicals occurring in propolis is given below with references to some recent reviews and analyses from different countries (Table 5.1). The major compounds are resins composed of flavonoids and phenolic acids or their esters, which often form up to 50% of all ingredients. The variation in beeswax content also influences the chemical analysis. In addition it must be said that most studies do not attempt to determine all components, but limit themselves to a class of chemicals or a method of extraction. The selection of the studies presented here is based on the most recent publications with preference given to the most complete studies or to studies from countries where these are the only references.

5.4 The physiological effects of propolis

5.4.1 Unconfirmed circumstantial evidence

The following uses of propolis or its extracts have been found in literature, but without substantiating evidence or reference to scientific studies:
anti-asthmatic treatment in mouth sprays,
support of pulmonary system,
anti-rheumatic (Donadieu, 1979),
inhibition of melanoma and carcinoma tumour cells,
tissue regeneration,
strengthening of capillaries,
anti-diabetic activity,
phytoinhibitor,
inhibiting plant and seed germination (Donadieu, 1979) in general and potato and leaf salad seed germination (Bianchi, 1991) in particular.

Table 5.1:

The major compounds of propolis as analyzed in recent publications.

Class of components	Group of components	References
Resins	45 to 55 % flavonoids	Pápay et al., 1987 - Hungary Bankova et al., 1987 - Bulgaria Nagy et al., 1989 - Czechoslovakia Omar, 1989 - Egypt Greenaway et al., 1990a - UK Greenaway et al., 1990b - Austria, Ecuador, Germany, Israel, UK, USA Wang and Zhang, 1988 - China Mizuno et al., 1987 - Japan
	phenolic acids and esters	Nagy et al., 1985 - Hungary Wollenweber et al., 1987 - West Germany Bankova et al., 1992 - Bulgaria, Mongolia
Waxes and fatty acids	25 to 35 % most are usually from beeswax, but many are of plant origin	Pápay et al., 1987 - Hungary
Essential oils	10 % volatiles	Petri et al., 1988 - Hungary
Pollen	5 % proteins probably from pollen; free amino acids (AA): 16 AA's at more than 1 % of total AA's of which arginine and proline together make up 45.8 %, 8 AA's occur in traces	Gabrys et al., 1986 - Poland

Other organics and minerals	5 % 14 trace minerals of which Fe & Zn are most common, others e.g.: Au, Ag, Cs, Hg, La, Sb;	Scheller et al., 1989 - Poland
	ketones	Bankova et al., 1987 - Bulgaria
	lactones	Cuellar and Rojas, 1987 - Cuba
	quinones	Cuellar and Rojas, 1987 - Cuba
	steroids	Cuellar and Rojas, 1987 - Cuba
	benzoic acid and esters	Greenaway et al., 1987 - UK
	vitamins, only B_3	Greenaway et al., 1987 - UK
	sugars	Greenaway et al., 1987 - UK
General review		Walker and Crane, 1987 - World Asis, 1989 - World Crane, 1990 - World Inoue, 1988 - Japan

5.4.2 Scientific evidence

One of the most widely known and extensively tested properties of propolis is its antibacterial activity. Many scientific tests have been conducted with a variety of

bacteria, fungi, viruses and other microorganisms. Many of the tests have shown positive control of the organisms by various extracts and concentrations of propolis. A synergistic effect has been reported for propolis extract used together with antibiotics (Chernyak, 1971). Whether propolis exhibits bactericidal or bacteriostatic characteristics often depends on its concentration in the applied extract. Sometimes, propolis extracts are more effective than commercially available drugs (Millet-Clerc, et al., 1987). In all cases, the specific conditions and extracts have to be closely considered. Proven effects of propolis on microorganisms are listed in Table 5.2.

Though there is a large variety of effects attributed to propolis, many of the reports are based on preliminary studies. If clinical trials were conducted, they were rarely based on large numbers of patients or rigorous test designs such as the double-blind placebo test (Table 5.3). The majority of the studies were conducted in East European countries. Much practical work and research is also being done in China, but information is difficult to obtain, not least because of the language barrier. Western European and North American medical research has largely ignored this source of milder and widely beneficial material. More detailed studies are warranted to determine the potential benefits from the medicinal use of propolis, particularly for intestinal, dermatological and dental applications.

In addition to the selected studies cited here, there have been over 500 publications in the last 18 years alone. Most were in vitro studies, but clinical trials were also conducted. These can be researched by those further interested in the uses of propolis in the collection of abstracts prepared by IBPA which is available from them.

5.5 The uses of propolis today

5.5.1 In cosmetics

Dermatological and cosmetic applications are at this time probably the most common uses for propolis and its extracts (Lejeune, et al., 1988). Its effects on tissue regeneration and renovation have been well studied. Together with its bactericidal and fungicidal characteristics it provides many benefits in various applications in cosmetics. For some recent specific references on scientific studies, the reader should refer to the section on the effects of propolis (5.4.2). More detailed information on practical application of propolis in cosmetics can be found in Chapter 9.

5.5.2 In medicine

General medicinal uses of propolis include treatment of the cardiovascular and blood systems (anaemia), respiratory apparatus (for various infections), dental care, dermatology (tissue regeneration, ulcers, excema, wound healing - particularly burn wounds, mycosis, mucous membrane infections and lesions), cancer treatment, immune system support and improvement, digestive tracts (ulcers and infections), liver protection and support and many others. Some references to these applications can be found in the list of scientifically proven effects of propolis (Table 5.3) otherwise one might refer again to IBRA's collection of abstracts, Apimondia and the American Apitherapy Society.

Table 5.2:

A list of microorganisms against which propolis or its extracts have been shown to have a positive effect.

Target organism	Comments	Reference
Bactericidal effects		
Bacillus larvae	causes American Foul Brood in honeybees	Meresta and Meresta, 1988
B. subtilis and others		Meresta and Meresta, 1987, 1988

B. subtilis and others		Meresta and Meresta, 1985, 1986
Bacillus de koch	tuberculosos	Karimova, 1975 Grange and Davey, 1990
Staphylococcus species	assiciated with pneumonia	Chernyak, 1973
Staphylococcus aureus	positive synergistic effect with action of 13 antibiotics against 10 strains	Kedzia and Holderna, 1986 Meresta and Meresta, 1988 Dimov et al., 1991
Streptococcus		Rojas and Cuetara, 1990
Streptomyces		Simúth et al., 1986
S. sobrinus, mutans & cricetus	dental caries in rats	Ikeno et al., 1991
Saccharomyces cerevisiae	brewer's yeast	Petri et al., 1988
Escherichia coli		Simúth et al., 1986
Salmonella and Shigella	review	Ghisalberti, 1979
Salmonella	potential use in salmonellosis treatment	Okonenko, 1986
Salmonella	reduction in pathological changes after Salmonella infections in mice	Okonenko, 1988
112 anaerobic strains	inhibitory effect on most	Kedzia, 1986
Giardia Lambia		Olarin et al, 1989
Bacteroides nodosus	reduction of foot-rot in rams	Muñoz, 1989
Klebsiella pneumoniae		Dimov et al., 1991
reduced or no		Brumfitt et al., 1990

bactericidal activity		
general	6 species of bacteria, major (4%) component - flavonoid, Cuba	Cuéllar et al., 1990

Fungicidal effects

Candida albicans	weak effect by ethanol extracted propolis (EEP) no effect by aqueous extracted propolis (AEP)	Valdés et al., 1987
	better effect in vitro	Petri et al., 1988
	in comparison with 10 antibiotics EEP had best effect in synergism with natamycin and flucytosine	Holderna and Kedzia, 1987
Aspergillus niger		Petri et al., 1988
Botrytis cinerea	in vitro EEP is fungicidal, but in vivo with strawberries has insignificant effect	La Torre et al., 1990
Ascosphaera apis	chalkbrood pathogen in honeybee colonies	Kedzia, 1986 and Ross, 1990
6 fungi infectious in humans	antifungal properties vary with different samples of propolis	Millet-Clerc et al., 1987
Plasmopara viticola	ineffective, greater leave damage by P. viticola with 1% propolis treatment	Hofmann et al., 1989

general	antifungal activity increased in presence of propylene glycol	Millet-Clerc, et al., 1987 Milena, et al., 1989
Antiviral effects		
Herpes	Herpes 1 and 2 in vitro anti-herpes ointment patent	Sosnowski, 1984 Popescu et al., 1985
Potato virus	EEP is effective, AEP less so	Fahmy and Omar, 1989
Influenza	reduced influenza mortality in mice with oral and injected propolis extracts	Maksimova-Todorova et al., 1985 Neychev, et al., 1988 Serkedjieva, 1992
Newcastle disease		Maksimova-Todorova et al., 1985
general	review	Benkova et al., 1988 König and Dustmann, 1989
Nematodicidal effect:		
Ascaris suum	in intestines of guinea pigs, assessed to be effective through immunostimulation	Benkova, et al., 1989

Table 5.3:

Medicinal and other effects described for propolis or its extracts.

Application	Comments	Reference
Allergen	some allergic reactions may be due to pollen content, but the majority of reactions have been shown to be related to pentenyl esters and phenylethyl esters of caffeic acid	Hashimoto et al., 1988 Hausen and Wollenweber, 1988
Irradiation protection	of mice against gamma radiation after intraperitoneal injection of EEP	Scheller et al., 1989a
	free radical scavenger	Scheller et al., 1990
Anti-tumour (cancer)	review of anti-cancer, anti-viral, endocrinological and allergic activity of caffeic acid and derivatives extracted from propolis	König, 1988
	review, Ehrlich carrinoma	Scheller et al., 1989e
	cytotoxicity on cultures of human and animal tumour cells	Grunberger et al., 1988
	cytotoxic and cytostatic effects in vitro against hamster ovary cancer cells and sarcoma-type tumours in mice	Ross, 1990
Ulcers	patient histories	Gorbatenko, 1971
	patient histories	Makarov, 1972

	beneficial for stomach ulcer cures, but not for ulcers of the duodenum	Gueorguieva and Vassilev, 1990
Leprosy	leprosy	Grange, 1990
Mammalian tissue regeneration	stimulation of various enzyme systems, cell metabolism, circulation, collagen formation; improved healing of burn wounds	various reviews
	as a result of arginine presence	Gabrys et al., 1986
	accelerated epithelial repair of skin wounds in rats, but not in dental sockets after tooth extraction	Filho and Carvalho, 1990
Anaesthesia	in strong concentrations, raw or extracted, review	Crane, 1990
	anaesthetic, anti-inflammatory, anti-bacterial, anti-fungal effect	Túthné and Pápay, 1987
	anaesthetizing ointment for dentistry	Sosnowski, 1984
Dental care	less caries in rats	Ikeno et al., 1991
	subsidiary treatment for gingivitis (gum infections) and plaque (deposit on teeth)	Neumann et al., 1986
	pulp gangrene antiseptic (50 % EEP)	Gafar et al., 1986
Other medicinal applications	stimulation of immune response in mice	Manolova et al., 1987

immune system improvement in 2 cases of alveolitis fibroticans with a preparation containing EEP, Esberitox N and a calcium-magnesium preparation	Scheller et al., 1989c
bronchitis, best results with inhalation of EEP together with propolis tablets and application of dolomite	Scheller et al., 1989b
in rats and mice, a concentrated EEP dose at 100-500 mg propolis per kg body weight, reduces blood pressure, produces a sedative effect, protects the liver against tetrachloride, the stomach against ulcers, forms and maintains serum glucose, but has no diuretic, anti-bleeding or anti-sclerotic activities	Kedzia et al., 1988
strengthening capillaries	Budavari, 1980
vaso-motor catarrh treatment with propolis ointment	Zommer-Urbanska et al., 1989
Legg-Calve-Perthes illness (hip joint disease in humans) by intra-articular injection of AEP	Przybylski and Scheller, 1985
liver protection against alcohol (ethanol) in rats	Giurgea et al., 1987 and 1989
liver protection against tetrachloride in rats	

		Coprean et al., 1986
Veterinary applications	improved weight gain and reduced diarrhoea in milk-fed calves with 5 ml of 20% EEP in morning and evening	Gubicza and Molnar, 1987
	mastitis, successful treatment even with antibiotic resistant infections	Meresta et al., 1989
	coccidiosis in rabbits with 3 % EEP orally	Hollands et al., 1988
	Eimeria (intestinal parasitic protozoa) in rabbits with 2-3 % EEP orally for 4 weeks	Hollands et al., 1984
Antioxidant	as a result of synergism between individual ingredients	Yanishlieva and Marinova, 1986
	oxidation at different speeds in different propolis types depending on presence of non-saturated compounds; with less contamination by wax, more non-saturated compounds are present	Omar, 1989
	in the presence of polyunsaturated fatty acids in animal feed, EEP is better than vitamin E	Okonenko et al., 1988
	stabilizing sunflower oil against oxidation	Yanishlieva et al., 1986
	as an anti-hypoxic in form of lyophilized phenolic polysaccharides	Tikhonov and Mamontova, 1987

	as food preservative in various reviews, but without reference to scientific studies	various
Pesticides	effective in vitro tests against strawberry pest Botrytis cinerea, but no statistical differences for in vivo tests	La Torre et al., 1990
Phytoinhibitor	inhibiting plant and seed germination	Donadieu, 1979 without research references
	inhibiting germination of potato and leaf salad vegetables	Bianchi, 1991 without research references

Direct external application of ethanol extracts or concentrated ointments (with up to 33% propolis) have given good results in veterinary use for wound healing and sores. Plastic surgery too, is using propolis extracts for improved wound healing and reduced scar tissue development.

5.5.3 Traditional use

In Europe and North Africa, the special wound healing properties of propolis were already known to the Egyptians, Greeks and Romans and in ancient times. In records of the 12th century, medicinal preparations with propolis are described for treating mouth and throat infections, as well as caries. Propolis probably has been

more commonly used in wood preservatives or varnishes than may be suggested by the single, frequently cited reference to Stradivarius (Jolly, 1978).

In sub-Saharan Africa, propolis is still used today in herbal medicines and the more mundane applications mentioned earlier such as waterproofing containers and wood, adhesive, bow string preparation and for tuning drums.

5.5.4 Food technology

The antioxidant, antimicrobial and antifungal activities of propolis offer scope for applications in food technology. One special advantage is that, unlike some conventional preservatives, the residues of propolis seem to have a generally beneficial effect on human health. However, only very few studies have been done on the possible side-effects of increased consumption of propolis. Individually, some of the components identified in propolis can be very damaging to human health.

Mizuno (1989), registered a patent which includes propolis as a preservative in food packing material.

Extension of frozen storage life of fish by 2-3 times is cited including Donadieu

(1979), but without reference to original studies. propolis is permitted as a preservative for frozen fish. by various authors, In Japan, the use of Addition of only 30 ppm (parts per million) of propolis to the rations of laying hens increased egg production, food conversion and hen weight by S to 6% (Bonomi, et al., 1976). Ghisalberti (1979) reports additional weight gains for broiler chicken of up to 20% when 500 ppm of propolis was added to their diets.

5.5.5 Others

The search for new uses of propolis continues. Sangalli (1990) mentioned use of propolis for post-harvest treatment and conservation of fruits. Applications in pesticides and fungicides are still in the testing phase. However, for many of its traditional uses propolis is being replaced by more readily available, sometimes more effective but often also more toxic alternatives.

Beekeepers use propolis, melted together with wax or in an ammonia solution (Anon, 1982) to apply to the inside of hives or swarm traps to attract swarms. Adequate ventilation and aeration after painting with the ammonia solution are both necessary. Rubbing propolis or painting it (after melting with wax from old combs) works as well or better and avoids the use of noxious and toxic ammonia.

The current trend to return to environmentally safer and less energy intensive production methods in many developed countries, the increased buying power of consumers and growing markets for more expensive products may lead to considerable growth in the use and new applications of propolis, particularly in cosmetics and food technology.

5.6 Formulation and application methods for human and animal use

5.6.1 Raw propolis

Unprocessed propolis can be used in chunks, or it may be frozen and broken or ground to fine powder. Large pieces of pure propolis can be chewed, but it should only be consumed in small quantities, since it may cause stomach upsets. Smaller pieces and powders can be taken in capsules or mixed with food or drinks.

5.6.2 Liquid extracts

Most commercial uses of propolis are based on preparations made from primary liquid extracts. The raw material is rarely suited for direct inclusion in final products. Similarly, for most private or small scale uses, raw propolis is usually treated with a

solvent and only the resulting extract is used.

A large variety of organic solvents might be applied but only a few are non-toxic and can be used safely for internal and external applications with humans and animals. The most commonly used is ethanol. A knowledgeable pharmacist or cosmetic chemist can select a few other non-toxic solvents for special applications. In some instances, reduction or elimination of the solvent is necessary and either (on an industrial scale) by lyophilization, (freeze drying) or vacuum distillation and (in small-scale production) by evaporation or distillation.

5.6.3 Additives and tablets

Propolis or its extracts can be taken with, or be used as an additive to other medicinal, dietetic and cosmetic preparations. Ethanol extracts can be directly mixed with most foods, medicines or cosmetics. Less frequently, aqueous (water) or glycol extracts are used. Propolis extract paste can easily be included in tablets or sweets.

5.6.4 Injection

For experimental purposes with animals, special extracts of propolis were injected subcutaneously or intramuscularly. Results were positive and injectable extracts for humans may become feasible in the near future.

5.7 Extraction methods

There are a few basic extraction methods which can be varied by using different solvents. The selection of the solvent depends on the final use of the extract and on technical feasibilities. Most active ingredients seem to be soluble in propylene glycol and ethanol. Fewer ingredients are soluble in water, but even water extracts show at least some bactericidal and fungicidal effects, as well as wound healing properties. Acetone extracts have been used for production of shampoos and lotions. Once the specific chemicals or chemical groups and their biological effects are better understood, better and more specific extracts can be prepared for equally specific applications.

The antimicrobial action of alcohol extracts is influenced by the extraction method, e.g. the duration of the soaking period or the amount of heating The concentration of the alcohol used and nature of stirring during extraction seem to have less of an influence (Obreg6n and Rojas, 1990). Debuyser (1984) reports extractions with a

70% solution of alcohol as the most active, without stating what kind of activity is being referred to. In general, it can be said that the longer the propolis is soaked in alcohol the more ingredients will be dissolved. Soaking beyond two or three weeks however, does not seem to increase the extent of extraction.

In scientific and non-scientific literature alike, the method for determining propolis concentration in the extract is not always specified. A scientific method should consider the ratio of the dry weight of dissolved matter to the weight of the solvent (A) or quantify ppm (parts per million) of active ingredients. However, a more practical way appears to be using the ratio (by weight) of total propolis placed into the solvent to the weight of the solvent (B). The latter method is certainly less precise, because of the incomplete dissolution of propolis, and the final concentration therefore depends very much on the extraction method, the solvent and the quality of the propolis. Thus, for standardization, in addition to concentration, a description of the solvent, the temperature and the duration of extraction is required. However, the practical method (B) results in less active ingredients for the same concentration determined according to the scientifically measured concentration (A). Standardization will also require measurable parameters for control as for example, certain stable compounds which are extracted in proportions similar to the total concentration of active ingredients (for

other standards see section 5.11). A quantitative standardization is needed for future commercialization of propolis and its extracts.

Five and ten percent solutions using the latter method (B) i.e. the ratio of the total weight of propolis to the weight of the solvent, are most commonly used in small-scale production. Frequently however, the weight of alcohol is assumed to be equal to that of water, i.e. 1 ml of alcohol is assumed to weigh 1 g. Yet, absolute ethanol weighs approximate 20% less than the same volume of water These weight differences can also result in large differences in concentrations of active ingredients. Fortunately, the exact dosage of propolis is not usually of great importance. However, commercialization requires dealing with precise values. No uniformity exists yet in cosmetic applications either, since many recipes are based on propolis extract paste and others on liquid extracts of various concentrations. Cosmetic applications however, often contain not more than 1 % of the preferred propolis extract which can mean as little as 0.05 % to 0.06% of the active ingredients.

A few extraction methods for commercial use of propolis are described below. Additional solvents may be used in order to extract special components. Medicinal and food technology processes or studies are almost always conducted with ethanol

or aqueous extracts. Glycol extracts are practical for many cosmetic applications because of their improved dissolution in water based emulsions.

Preuaration for extraction

The propolis should be prepared by removing coarse debris and excessive wax. It should then be broken into small pieces or ground to a fine powder. If the propolis is too sticky to be broken up, it should be placed in a refrigerator or freezer for a few hours. Alternatively, pull the pieces into thin sheets or strips in order to increase the contact surface between propolis and alcohol, to promote dissolution.

Choice of the correct solvent is very important if the product is to be used for human consumption. Normally, only ethanol or exceptionally, glycol (as in method 4) should be used. Other alcohols may be used only if their internal and external physiological interactions are sufficiently known and safe.

So-called denatured, rubbing or methyl alcohol should not be used. If the extracts are intended for external application only, rubbing alcohol may be used in some cases, but different countries use different chemicals to make pure alcohol unpalatable for drinking or internal consumption. Similarly, there are different types

of denatured alcohols intended for different purposes. If cheap alcohol is used, care should be taken that the chemicals used for denaturing it are compatible with the planned end use. Chemicals added to denature alcohol may interact negatively with other ingredients so reducing their beneficial effects and may cause irritations, burns or even poisoning. There have been fatal accidents caused by extracts of propolis prepared with unsuitable alcohol.

For most preparations intended for internal use, gin, rum, cachasa, arrak or other clean, locally distilled liquors can be used. These liquors usually contain less than the optimal 70% of alcohol but for home processing, they produce acceptable results. However, for high quality commercial product, particularly for cosmetics or medicines, high quality laboratory grade or drinking alcohol (ethanol) should be used. 70% ethanol has given the best results in several studies which tested the extracts for their bactericidal and fungicidal effects.

Alcohols of different concentrations extract different compounds and influence the solubility of dried extracts. Thus, extracts made with higher concentrations of alcohol, when dried, are predominantly soluble in organic solvents and oils. But dried extracts from extractions with a very low concentration of ethanol are much more water-soluble. Sosnowski (1984) in a patent application described dried

filtrates from 10-25 % alcohol extracts which are completely soluble in water.

In some, if not most countries, special laws apply to the manufacture of products containing alcohol. Information should be sought and a licence should be obtained, if necessary. For production and use within the home, most countries do not require a special licence.

Materials required

The basic requirements for small-scale processing are a large capacity bottle which can be tightly closed, a scale (more sensitive if working with smaller quantities) and a strainer (special filter paper, several layers of clean cotton cloth or cotton balls) - A refrigerator or freezer is useful, but not essential. A heat source is necessary to evaporate the solvent but it is better to use a distillation apparatus, vacuum drier or freeze drier (see also equipment for royal jelly).

Method 1: Ethanol Extracted Propolis (EEP) - the simplest method for extracting propolis

The exact concentration of the desired extract should first be decided. The initial

concentration of propolis to be extracted should not exceed 30%, due to less efficient or less complete extraction at higher concentrations. The correct quantity of propolis is weighed and the right volume of alcohol measured. It would be easier to weigh the correct quantity of alcohol since alcohol is much lighter than water. The specific gravity of pure ethanol is 0.794 as compared to 1.00 for water. For reasons of simplicity one can assume that one litre of 100 % alcohol weighs 800 g, 11 of 70% alcohol approximately 860 g, 11 of 50% alcohol approximately 900 g, and so on. Other alcohols and solvents have different specific gravities and quantity measures will vary accordingly. Therefore, weighing both the propolis and the solvent is the preferred method.

Pour the alcohol and propolis into a container, seal the top and shake briefly. Repeat the shaking once or twice a day, but otherwise leave the mixture in a warm dark place for at least three days. To achieve the best results, the propolis should be extracted for one or two weeks. Soaking for more than one week, according to some authors and for two weeks according to others, provides no additional benefits.

Some producers boil the alcohol and propolis mixtures for eight hours in order to dissolve all the resins. If the propolis contains wax, most of this will be dissolved by

heating or must be removed prior to extraction. For a high quality product, however, heating should be avoided.

After one or two weeks, the liquid is filtered through a clean and very fine cloth, paper filters or cotton ball. The cloth may be folded into several layers to increase its effectiveness. A second filtration may be advantageous and if the extract can be refrigerated to less than 4 ^{0}C but not freezing, for several hours or a day until filtration, better results are achieved. The filter should also be cooled prior to use. The remains of the first filtration can be washed or soaked in alcohol again.

The filtrate should be a clear liquid, free of particles and dark brown or slightly reddish in colour. It should be kept in CLEAN, dark, airtight bottles. If dark coloured bottles are not available, the bottles should be kept in a cool dark place or wrapped with a cloth, paper or straw, to keep out light.

Ingredients for a 10% extract:

Propolis	1 part	or	100 g	or	1 kg
Alcohol	9 parts		900 g		9 kg

or any multiple thereof.

Ingredients for a 5% extract:

Propolis	1 part	or	100 g	or	1 kg
Alcohol	19 parts		1900 g		19 kg

or any multiple thereof.

Since solvents are relatively expensive, consideration should be given to preparing a more concentrated first extract (< 30%) The final extract can be diluted or further concentrated depending on its intended use. Most extracts are used with reduced solvent content, i.e. very high propolis concentration. Starting with a concentrated solution will therefore require less evaporation, however, as also extracts all compounds less efficiently.

Higher concentration of the extracts can be achieved by simply leaving the extract in an open large mouth container, suitably protected against dirt, dust and insects for a while. Most of the alcohol will evaporate at room temperature in a few hours. For

further drying and recuperation of the alcohol, see method 6 and 7.

Method 2: Quick extraction

For this extraction, finely broken pieces or powdered propolis are placed in a large filter or cloth bag and pure alcohol (over 95 % ethanol) is poured through the filter. This may be repeated several times. The resulting extract should be stored as described in method 1.

The extraction is much less effective with lower concentrations of alcohol. The extract, once finished, can later be diluted with water. However, concentration of active ingredients can hardly be compared to extracts achieved with method 1, because of the lesser degree of extraction.

No references could be found for a quantitative comparison of the effectiveness of this method with method 1. Since extraction efficiency increases with time in method 1, it may be assumed that for some applications method 2 is of limited use, particularly when the desired active ingredients are less soluble. Method 2 may be used with sediment from the filtration in method 1.

Method 3: Glycol extracted propolis (GEP)

This method is similar to method 1 and differs only in the solvent used. Instead of ethanol, glycol (propylene glycol) is used. However, the concentration of propolis should not exceed 10% and extraction is more efficient under partical vacuum (Sangalli, 1990) The disadvantage of glycol as compared to ethanol is the need for higher temperatures during evaporation of the solvent, which adversely affects many of the volatile compounds of the propolis extract.

Glycol is usually cheaper than drinking quality alcohol, because of lower taxes, but it may be more difficult to obtain in some countries. Some cosmetic producers prefer glycol extracts to ethanol extracts for certain preparations. Glycol extracts mix more easily with some lotions, particularly those with a large water phase. They are also easier to use with nasal or oral sprays, since the glycol evaporates slower and it is not toxic for external applications. However, it must always be taken into consideration that glycol is considered safe for human consumption, i.e. internal use only up to 1.5 g of glycol per day per adult (Sangalli, 1990).

Method 4: Aqueous (water) extracted propolis (AEP)

Aqueous extracts can be obtained by soaking propolis for several days or boiling it in water. The yield of active ingredients is lower than with alcohol, but aqueous extracts have been shown to exhibit bactericidal and fungicidal effects. All other processing, filtering etc., are the same as those in method 1.

Method 5: Oil extracted propolis (OEP)

Extracts prepared according to this method described by Marchenay (1977), and cited by Debuyser (1984) are less adaptable to commercialization, but present some simple ways of preparing inexpensively, small quantities of extract for internal as well as external application.

Mix 10 g of cleaned propolis with 200 ml (about 200 g) of olive or almond oil, or with 100 ml of quality linseed oil (refined food quality) or with 100 g of butter. Other edible oils can be substituted for the ones mentioned here.

Heat gently in a water bath for approximately 10 minutes to not more than 50 ^{0}C, stirring continuously. Filter and store the extract in well sealed containers in the dark. Refrigerated storage is recommended.

Method 6: Propolis paste

This method is the same as method 1 until the filtered liquid extract is obtained. The liquid is then partially evaporated to provide a product with paste-like consistency. The paste is well suited for mixing with various emulsifiers for applications in cosmetics.

Evaporation can be achieved by gently heating the extract in an open container over low heat. Alcohol is very flammable, so appropriate precautions should be adopted around open flames and abundant ventilation should always be provided.

A simple distillation apparatus, like the one used for preparing local distilled liquors, would allow the collection of most of the expensive alcohol for reuse. The most sophisticated and least damaging evaporation would, however, be accomplished with low pressure vacuum evaporators or freeze driers. If quality control is exercised, the propolis extracts in this paste form may become easier to market and should sell for a considerably higher price.

Method 7: Dry propolis extract

Dry extracts are those with a solvent content of less than 5 %. They are obtained from extracts according to methods 1, 2 or 3, followed by evaporation, freeze drying or spray drying (Sangalli, 1990). The last two drying methods require relatively expensive laboratory equipment (see Suppliers List in the Annex).

Drying does not result in powders is the propolis was extracted with highly concentrated alcohol. Instead, the residue is a sticky elastic paste. To achieve a dry powder which would be easier to use in most pharmaceutical or cosmetics applications, one of the following methods should be used. The problem is that the following methods may compromise the extraction process and have not been tested for their biological effectiveness, in contrast to extracts from Method 1.

Method 8: Water-soluble, dried powder ethanol extracts

Propolis is prepared and extracted as described in method 1 but using a 10-25 % ethanol solution, though many other solvents are mentioned in a patent application (Sosnowski, 1984). After 1 to 10 days at 0 to 37^{0}C (preferably towards the warmer temperature limit) with periodic agitation, the solution is filtered for the first time through Whatman No. 1 filter paper, or a double layer of very fine cotton cloth. The filtrate is cooled as much as possible (without freezing) for 24 hours and is then

filtered again, cold, through a Whatman No.50 filter paper. A third and final filtration may be carried out cold or at room temperature with a 2 ~m filter. Finally, the solvent is removed by evaporation or freeze drying.

For extraction methods like this one and others, where the final product is a paste or powder, the initial proportions of propolis and solvent are not very important. Much larger quantities of propolis can be used for quicker extraction, e.g. 500 g propolis in 1000 ml solvent. However, sufficient active ingredients usually remain in the filter residues to justify another, longer extraction with clean alcohol.

A few recipes using the dried powder are mentioned at the end of this chapter. No scientific publications or studies were cited by Sosnowski (1984) concerning the efficacy or biological activity of this extract, though he claims that the antioxidant properties of the propolis extract from concentrated ethanol or diluted ethanol are the same.

Method 9: Free-flowing, non-hygroscopic propolis powder

For those who have access to the appropriate equipment and chemicals, propolis extracts can be made easier to handle and more heat stable by complexing with

Bcyclodextrin. The result is a free-flowing, non-hygroscopic powder (Szente and Szejtli, 1987).

Method 10: Water soluble derivatives (WSD)

Water-soluble propolis extracts are important for some medicinal and cosmetic applications. Dimov et al., (1991) published a method patented by Nikolov et al., (1987) which produces a dry powder of lysine-complexed propolis extracts, known as the Water Soluble Derivatives (WSD). A translation of the Bulgarian Patent was provided by Dr.Ivanovska:

100 g of propolis are extracted three times with boiling methanol for one hour, using 800 ml of methanol each time. The extracts are filtered hot, stored overnight at 4 ^{0}C and filtered again. The precipitates, i.e. the filter residues of the cold filtration, are washed with cold (4 ^{0}C) ethanol and filtered. Both filtrates are combined and evaporated to dryness, giving 60 g of a resinous, brown product. 10 g of this dry product are gradually stirred into 150 ml of an 8% L-lysine solution at 50-60^{0}C. This solution is freeze-dried, resulting in 22 g of a dry, yellow-brown powder.

WSD 's are still being tested for their antibiotic characteristics. They were found to induce non-specific protection against gram-negative bacteria, i.e., Klebsiella i,neumoniae, Proteus vul~aris, Escherichia coli and Pseudomonas aeruginosa (Dimov et al., 1992).

Elaboration of any of the above-mentioned extracts often includes evaporation of part or all of the solvent. If concentrated extracts are required, it is better to use concentrated ethanol for extractions since it evaporates at a lower temperature than the other solvents mentioned. Thus, the risk of destroying some of the active ingredients through heat damage is reduced. This is important, even though some of the active compounds are thermostable (resistant to heat) since the synergistic forces of all the ingredients in propolis are not yet fully understood.

For large-scale operations, evaporation under low pressure (partial vacuum) or by freeze drying are preferred because any damage due to heating can then be avoided. However, a Hungarian study showed some antibacterial activity was still present in steam-distilled essential oils from propolis (Petri et al., 1988).

Other solvents can be used to extract propolis, for example many alcohols, ether, acetic acid, acetone, benzene, 2% sodium hydroxide and ammonia (common

household cleaner) (Anon, 1982). These solvents should not however be used if the extract is intended for consumption by humans or animals.

5.8 Collection

The average production of propolis per colony per year has been described as 10 to 300g (Ochi, 1981 and Andrich et al., 1987) but the production depends on the bees, the climate, the forest resources and the trapping mechanism. According to personal observations, it may occasionally be considerably higher. If there is any selection by queen breeders and beekeepers, it has been against heavily propolizing bees, since they make work in the apiary more difficult. Bees which produce larger quantities of propolis could be selected if required.

Contamination of propolis with wax, pieces of wood, paint and other debris should be avoided. The cleanest collection methods employ special traps placed on top of a hive, below the covers (see Fig. 5.2 to 5.5) or next to lateral walls inside the hives. Thus bees do not mix as much wax with the propolis and no contamination occurs during harvesting. Trap harvesting is also faster and may be more productive.

Traps are basically screens or special plates with small holes which simulate cracks

in the hive walls (see Figure 5.2). Bees try to seal the holes and thus fill the trap with propolis. The most economic trap design is an inner cover with a large hole, covered with regular nylon fly screen, secured in place by the points of nails and a perforated frame (see Figure 5.5). However, to avoid contamination with wax, the screen should not touch the top of the frames. The total area exposed by a screen may have to be varied according to the bees and local conditions. Trap harvested propolis usually fetches a better price because of its cleaner and therefore of better quality.

Light, and in particular air circulation are important to stimulate propolis use. Accordingly, traps placed on top of hives should be covered but the hive cover needs to be propped opened slightly to increase air circulation and to allow in some light (see Fig. 5.4). In tropical regions it may be necessary to prevent the entry of too much rain. Also, when using a type of bee sensitive to disturbances or likely to abscond, the lid should not be opened too far otherwise bees might escape. Newly established colonies should be given some time to establish themselves before they are used for trapping.

Propolis is removed from traps by cooling the plastic sheets or fly-screens for a few hours in a refrigerator or freezer. Once cooled, the propolis becomes brittle and can

be removed from the screens by simply flexing and brushing them, pulling over a table edge or by using a special high pressure air device designed by Pechhacker and Huettinger (1986). The trap is then ready for re-use.

Before the advent of recent trap designs, most propolis was collected by scraping the "bee glue" off walls, frames, entrances and covers. Marletto (1983) noted that the propolis collected from the cover or top frames was usually cleaner than that collected near the entrance. Even contaminated scraped material can be used and purified by repeated extraction and filtering.

In order to avoid contamination with too much wax, scrapings from frames or bottom boards and lids should be kept separate from each other and from propolis collected with traps. Chunks and pieces should never be combined into large balls. Enquiries should be made with potential buyers to see how they prefer propolis. Large pieces often have to be ground or broken into smaller chunks first.

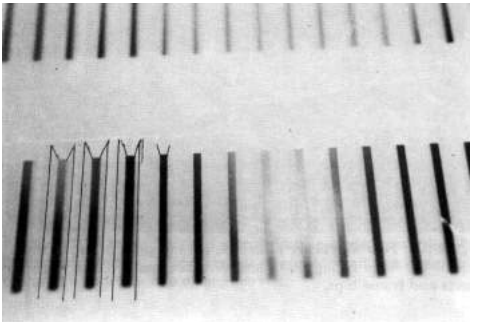

Figure 5.2*:* Flexible, 3 mm plastic sheets with rows of slots, 2 mm side on one side and 4 mm on the other

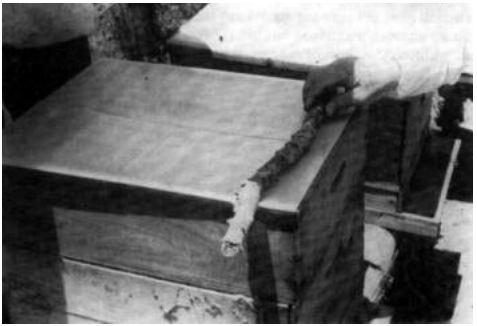

Figure 5.3: Four sheets are placed on the top super with the wider side of the holes facing down and with bee space (1 cm) between sheets and frame tops.

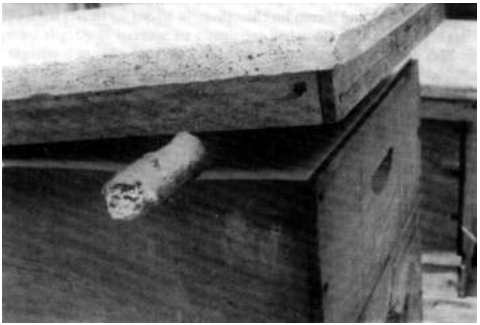

Figure 5.4: The cover is left open a little to increase ventilation and let light in.
This stimulates the bees to seal the slots with propolis.

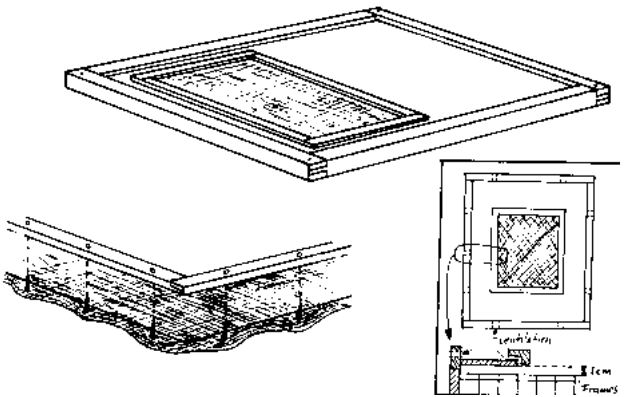

Figure 5.5: A simple design of a propolis trap made from nylon, fly or mosquito screen. The screen is removable and can be quickly

replaced with a new one during harvest.

For better quality propolis, some authors recommend collection after the major nectar flow (Donadieu, 1979. This may be true in temperate climates where bees are preparing for over-wintering and therefore collecting more propolis. In tropical climates, no studies are available which demonstrate seasonal variation, or its absence. It is possible that at the beginning of the rainy season, propolizing will be more active. Internal traps may be more advantageous, but some experimentation is required. Tropical races of A. mellifera have also been reported as producing very little propolis.

5.9 Buying

Unprocessed propolis should always be acquired in the form of chunks or small pieces and never lumped into larger pieces or balls. Some buyers prefer large chunks and others like smaller pieces, but preference for the latter is usually related to trap collected propolis, since small scrapings often have a high level of contamination. Quality criteria are described in section 5.11.

Buying quality propolis extracts is difficult, because the brownish colour of alcohol extracts does not reveal the quantity and quality of the propolis nor the care taken in extracting it. Even chemical analyses can only provide a quantitative judgement with regard to the major compounds (for a simple antioxidant activity test see 5.16.13) and biological activity tests are slow and expensive. Extracts should therefore be bought only from producers whose methods and commitment are well known. For evaluating products derived from propolis, (5.16.13) tests and analyses become inevitable as well as a reliable and responsible manufacturer.

5.10 Storage

In general, propolis is fairly stable, but proper storage is important. Propolis and its

extracts should be stored in airtight containers in the dark, preferably at less than 10^0C-12^0C and away from excessive and direct heat. For similar reasons, very old propolis from the hive should not be mixed with fresher propolis. Over 12 months of proper storage, propolis will lose very little or none of its antibacterial activities. Alcohol extracts may be stored even longer.

Lyophilization (freeze drying) of extracts has been described as a method which preserves the antibacterial characteristics, but nothing has been written about effects of long-term storage of such materials. This method may gain importance for larger scale use and certain formulations, but it is possible that some of the synergistic characteristics of propolis may be lost during lyophilisation.

The shelf-life of propolis containing products depends very much on their composition and has to be determined for each case. The more the other components of a product are susceptible to decomposition, the shorter will be the shelf-life of that product. This is the reason for compromises that are necessary in the selection of artificial and/or natural and traditional ingredients, preservatives and larger production for extended markets. However, propolis and its extracts function as a mild preservative due to their antioxidant and antimicrobial activities and thus may actually prolong the shelf live of some products.

5.11 Quality control

Since propolis comes in many colours, odours and composition, it is very difficult to give precise guidelines. Most fresh propolis has a pleasant resinous odour. Wax content and visual contamination should obviously be as low as possible. Old propolis becomes very hard and brittle and may also be very dark. However, frozen or recently frozen propolis is also very brittle.

Official quality standards exist for propolis in various East European countries, but most standards refer to the cleanliness or adulteration of the raw product and sometimes, its extracts. Maximum and minimum limits for certain chemical groups are set, but few standardised tests are available to determine the biological activities of various components. Tikhonov et al., (1978) describe the average contents of the principal ingredients as possible standards for raw propolis (Table 5.4). Official quality standards exist in Romania and the former USSR (Crane, 1990).

Franco and Kurebayashi (1986) suggested methods for quality control and Hollands et al., (1988) for testing coccidiostatic effects. Vakikonina et al., (1975), Petri et al., (1984) and Bianchi (1991), describe the discoloration of a 0. iN potassium permanganate solution as a reliable test for the antioxidant effect of propolis and

its extracts, and the detection of some adulterants (see 5.16.13). Bacteriological tests can be carried out and the results compared with those from samples of known purity and origin, but these tests apply to only a small proportion of all the various beneficial activities of propolis. None of these tests have yet been widely accepted as providing a reliable evaluation of the overall quality of propolis or its extracts. Most likely, only a range of tests will ever give a reliable evaluation of the numerous diverse characteristics of propolis.

Because of its recent manipulation and harvesting by bees, fresh trap-collected propolis is of the highest quality and the least contaminated, if collected on a regular basis. Plant origin however, may be important for certain applications and therefore propolis collected in a certain region or during a certain season may be preferred.

Table 5.4:

Quality standards for propolis as suggested by Tikhonov et al (1978) and upper and lower limits as established by Russian Regional Standards (RSFSR, 1977).

	Tikhonov et al,	RSFSR
Extractable substances	21.93 +/- 2.22%	
Oxidizability value	17.08 +/- 5.52%	< 22.0%
Resinous-balsam substances	46.18 +/- 1.15%	
Waxes	27.11 +/- 7.68%	< 30.0%
Polyphenols	14.66 +/- 2.34%	> 20.0%
Plysaccharides	2.26 +/- 0.32%	
Mechanical impurities	9.76 +/- 1.81%	< 20.0%
Iodine number		> 35.0

After incorporation into other products, testing for propolis becomes even more complicated and overall product quality becomes important. Since there is a wide variety of products in which propolis can be included, the standards for each type of

product need to be considered. In section 5.16.13 a method is given to evaluate propolis antioxidant quality in other products.

One easy way to determin a different kind of quality, particularly poor qualaity as a defect, is the homogeneity of products containing propolis extracts (see Figure 9.9). Without good equipment, a good and stable emulsion is difficult to obtain. Hand-mixed emulsions tend to be stable for shorter periods of time only. Separation after brief or inappropriate storage is unacceptable to consumers and also affect performance of the product. Thus special care needs to be taken to ensure the compatibility of the extraction method and ~e ingredients of the end product. Suitable emulsifiers and better mixing techniques, i.e higher speed, longer time, warmer temperatures and different mixing sequences would have to be determined by testing (see Chapter 9).

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5.12 Market outlook

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It should be noted that the opinions expressed here are not based on extensive market surveys, but enquiries among a relatively few buyers and producers.

The market for raw material and secondary products containing propolis will probably continue to grow as they find more acceptance in medicinal uses and as more cosmetic manufacturers realize their benefits and marketing value. Improvements in the productionof water-soluble formulations of the active ingredients should further facilitate their wider use. Presently,, the demand is higher than supply in most countries. Unstructured and unorganized marketing, however, does not create much of a price advantage for the producer.

The difficulty of establishing uniform rules and quality control standards is probably a further impediment to market development. Concerns of importers or buyers about product effectiveness may be avoided by early collaboration with well established and reliable laboratories or researchers. Many of them will probably be glad to analyze and perhaps even test good samples of well documented origin.

International prices for raw propolis are going down. Having reached levels as high as US$160/kg or even US$300/kg, less than 20 years ago (Crane, 1990) prices of some buyers in 1992 are as low as US$4-12/kg. In several countries prices of US$30

could still be obtained in 1991. Some producers say there is a market for already fractionated extracts, i.e. extracts which are separated into various groups of components. These fractions are purchased by pharmaceutical companies and their market is most likely to increase. Though these special extracts bring a much higher price, producing them requires a well equipped chemical laboratory and trained staff for processing.

There is an opportunity produce for and develop local markets. The kind of products made and the extent of a local market will depend partly on the base ingredients available and the ability of entrepreneurs to adapt their products for local acceptance and use. Once quality standards of the large consumer nations are reached, exports may become feasible. Gaining market experience now, while competition is still relatively low will provide an advantage in the future when competition and quality control become more stringent. This should be true for raw materials as well as for manufactured products.

5.13 Caution

Hausen et al., (1987) cited almost 200 cases in which people have shown allergic reactions to propolis. In some cases of direct contact with propolis, this may have

also been a result of contamination by other bee products such as pollen or bee hairs. However, extracts and products containing propolis extracts have been shown to cause allergic reactions as well (Hausen, et al., 1987, Hausen and Wollenweber, 1987 and Ko~nlg, 1988) mostly in the form of contact dermatitis. Hashimoto et al., (1988) identified caffeic acid and its derivatives as the major allergenic agents.

Therefore, with all preparations intended for human or animal luse, small quantities should be tried during the first days, slowly increasing to the full dosage (half for children) in order to test for the compatibility of the preparatino or allergic reactions. Equally, termination of medical treatments prescribed by a physician should be gradual, slowly reducing the daily dosage.

Prolonged chewing of large amounts of raw propolis may lead to nausea and stomach upsets. Donadieu (1979) recommended chewing one gram at a time, three times a day.

5.14 Patents including propolis

Since many of the formulations prepared with propolis are made by or for the pharmaceutical and cosmetic industries, they and their production processes are

often protected by patent rights.

The following are a few patents which include propolis as an ingredient. Copies of patents can usually be obtained through the patent office of the country in which the patent has been registered. The addresses of the USA, European and World patent offices are listed in Annex 2. Those of other national offices can be obtained from the country's consulate or embassy.

Pharmaceutics

Anti-inflammatory (topical)	Busciglio, 1988
Antibiotic ointment (dermatitis)	Iwasaki, 1990
Anti-inflammatory and cell growth inhibitor	Nakanishi et al., 1989
Tissue regeneration agent (veterinary)	Dubaj et al., 1988

Propolis-stabilized vitamin C (Tablets of 91.5% glucose, 5% vitamin C and 3.5% ethanol exract of propolis)	Dubovsky et al., 1988
Drug for muscle hypoplasia in piglets	Musci et al., 1989

Cosmetics

Deodorant	Vol'Fenzon et al., 1989
Deodorant mouthwash	Cho et al., 1988

Other

Germicide, insecticide for food packaging	Mizuno, 1989a, b

Extraction methods

WSD - Water Soluble Derivatives	Nikolov et al., 1987

5.15 Information sources

Pharmaceutical, cosmetic, dermatological, medical and most beekeeping journals in different countries occasionally publish articles on propolis composition, uses and recipes for products. As a single source of information the IBRA has compiled a bibliography of all propolis-related articles which have appeared until recently in the Apicultural Abstracts. The American Apitherapy Society is collecting case histories of medical uses and continuously updates its database on research and other related publications.

Figure 5.6: Various products containing propolis (from left to right): extracts of various concentrations, revitalizing cream, extracts with dropper, caramels, soap, shampoo and night cream.

5.16 Recipes

As with all other recipes in this book, no guarantee is given that they will work under all conditions or that they will be effective for what their authors have claimed. They are meant as a basis for experimentation and adaptation to local conditions. When preparing a new formulation, notes of all environmental conditions, exact ingredient mixes and a precise description of every step in the process should be kept. These notes will allow the repetition of a successful trial and help avoid repeating those which have failed.

Propolis extracts or their dried residues (pastes or powders) are said to be beneficial if included in normal formulations of all kinds of creams, ointments, lotions, shampoos, lipsticks, anti-cellulite and anti-wrinkle preparations, mouth and nasal sprays etc. As a general guideline, propolis can be added to a product at 1 to 3 % by weight in the form of a 50% propolis-ethanol solution, i.e. 0.5 to 1.5% of extracted propolis. Up to 10% of less concentrated solutions are recommended by some authors which represents essentially similar amounts of extracted propolis dry weight. Only a few applications will benefit from much higher concentrations.

If the final product is an oil or fat-based product, a propolis solution prepared with highly concentrated ethanol will blend well with the final product. Glycol or less concentrated ethanol may be used for extracts that will be added to products which

contain some water. For additional cosmetic recipes see Chapter 9.

5.16.1 Ointments

1) Simple Vaseline-based ointment

Ingredients (in parts by weight) after D. and G. Barral (1992):

1 *Propolis extract*

9 *Vaseline or other petrolatum*

Prepare a propolis extract in 96% ethanol to a concentration of 10% propolis (method]) then reduce the solvent to obtain 30% propolis content by weight. Mix the extract with a small quantity of the Vaseline. Once the mix is homogeneous or well emulsified the rest of the Vaseline can be added slowly. If not mixed well the propolis extract will separate and leave dirty looking droplets in the cream (see also Fig. 9.9). Slight warming in a water bath will improve mixing. Using an emulsifier or electric mixer makes mixing easier.

The propolis extract may make up to 10% (by weight) of the final ointment. 10% of lanoline can also be melted with the Vaseline (using a water bath) following the same procedures as for the propolis.

2) Simple ointment based on vaseline or animal fat

Propolish cream (in parts by weight) after Savina and Romanov (1956):

This cream can be used for application on cuts, abscesses and festering wounds in animals and external ulcers and burns in humans.

10	*Vaseline or animal fat*
1	*Propolis*

Bring the vaseline or fat to boiling point, cool to 50-60 ^{0}C, add propolis, heat to 70-80^{0}C, stir for 10 minutes and cover for 10 minutes. Filter through one layer of thin cloth into clean container and seal. ft is ready as soon as it has cooled, but will not stsore for very long, particularly if animal fats are used.

3) Simple oil-based ointment

Ingredients (in parts by weight) after Proserpio and Martelli (1986):

2	*Propolis ethanol extract, 20% (EEP, method 1)*
1	*Beeswax*
7	*Lanolin*
10	*Butter of palm, cacao, kerat* *or similar*

Melt the beeswax in a water bath, slowly stir in the melted lanolin and mix well. while the mixture is cooling mix in the butter. The propolis extract is best mixed with a small amount of butter and added to the rest of the mixture once the latter has cooled to less than 40 C.

5.16.2 Oral and nasal spravs

D. and G. Barral (1992) recommend preparing a 2 to 10% propolis solution in

propylene glycol (Method 3). For flavour, an extract of some herbs in glycol or ethanol canbe prepared and filtered. Regalis, anis, eucalyptus and mint are among the many suitable herbs that can be used.

The two alcohol extracts are mixed using only a small quantity of the plant extract, according to taste. The alcohol solution can be further diluted before bottling in small mechanical sprays (vaporizers). Glycol is preferred over ethanol in this recipe because of its slower evaporation after application. A caution about excessive use of the glycol based spray should be included on the label (see Method 3 for reasons).

5.16.3 Suntan lotions

Select a suntan lotion and add sufficient propolis-glycol extract to make up 2-5% in propolis dry weight.

For basic suntan lotion formulations see the recipes in Chapter 9.

5.16.4 Propolis syrups or honeys

For syrups to be taken orally use the propolis in ethanol extract and mix it with a glucose/fructose syrup (e.g. honey or inverted sugar syrup). A sugar mixture is

reported to work better than a syrup made from a single sugar. The alcohol acts as a preservative.

Mixing propolis extract with a slightly diluted honey should work even better, since they complement each other's function. To find a water-soluble extract with all the curative values of raw propolis would be best. One of the previous methods (7-10) could be tried.

The propolis extract, however, can also be mixed with undiluted honey. To make the mixing or emulsification easier, only a small quantity of honey should be taken and mixed with the extract. Once this mixture is homogeneous, it is easily mixed with the rest of the honey. Store this product in dark or opaque containers.

5.16.5 Propolis tablets

This basic formula can also be used to incorporate pollen, where most of the sugar can be replaced with it; but a 10 to 20% sugar (honey) content should be maintained. Unless the tablets can be coated with wax or a similar barrier, the use of honey ~hould be limited because of its hygroscopic nature. Thikonov, et al., (1991) describes another recipe for a sublingual tablet with propolis.

Ingredients (in parts by weight) after Bianchi (1990):

1	*Gum arabic*
1	*Water*
1	*Propoli paste (from an aqueous EEP)*
10	*Powdered sugar*
q.s.	*Flavouring (not essential)*

In a small container, mix the water with the gum arabic until a homogeneous mass is obtained. while stirring, slowly add the propolis extract and mix well. Then slowly add the powdered sugar and mix continuously. Add the flavouring if required.

Prepare a suiface for rolling out the dough, thinly cover it with powdered sugar and roll out the dough to a unijorm thickness. when the thickness is that of the desired tablets cut the dough with metal, glass or plastic rings of the desired

diameter or shape. Unite the leftover dough, roll it out again and continue cutting pills until the dough is finished.

Dry the pills, suitably protected from dust, in the open air or in an oven or solar drier. The temperature should never exceed 40^0C. Store the product in clean, dark containers.

To protect against various infections and inflammations of the mouth and throat, particularly after tooth extraction, one pill may be slowly dissolved in the mouth 3 or 4 times a day. The exact size of the pill is not that important, since no precise dosage of the propolis is necessary. This medication should not be taken without consulting a doctor.

5.16.6 Propolis shampoo

Propolis shampoo has been described as having anti-dandruff properties. Formulations for other shampoos can be found in Chapter 9. Propolis extract prepared from diluted alcohol (less than 25 %) or glycol, can be mixed with many readily available shampoos. When mixed with alcohol, depending on the gel agent, some shampoos may loose viscosity.

Figure 5.7: Anti-dandruff shampoo with propolis.

Ingredients (in parts by weight) after Lejeune et al., (1984):

1	*Propolis extract*
20	*Texapon N40 (alkyl sulphate by Henkel, see Annex 2)*
3	*Comperlan KD (copper diethanolamide by Henkel)*
2.5	*Sodium chloride*
0.1	*Lactic Acid*
3	*Vegetable oil, preferably ricinus (castor) oil*

Add demineralized water or boiled rain water to make up 100 parts.

A 1 % propolis extract in 96% ethanol was found most cost-effective and compatible with other ingredients. The Henkel products are added to obtain a

pleasant viscosity which might also be obtained using other emulsifiers and natural gels if the alcohol is eliminated from the propolis extract. The oil is needed for protection of the scalp and hair.

Dissolve the sodium chloride in 20 parts of water, filter the solution and add the lactic acid. The oil phase is mixed after heating the Comperlan in a water bath to 40 ^{0}C. First add the Texapon and then the oil to the Comperlan. Mix careflilly and slowly to avoid the formation of too much foam. After, also the propolis extract is added the two liquids (oil and water phases) can be united and the volume is made up to 100 parts with water. The resulting shampoo is a clear brown colour with a pleasant aroma and it can be stored in dark bottles for at least 12 months.

5.16.7 Anti-dandruff lotion

This simple lotion is easy to prepare and, if stored in dark bottles away from heat, can be used for at least 12 months.

Ingredients (in parts by weight) after Lejeune et al., (1984):

1 Propolis (50% EEP)

5	*Sodium laurylsulphate*
37	*Ethanol (96 to 100%)*
57	*Rain water, boiled*

A 10% propolis extract is prepared according to method 1 and solvent reduced the to provide a 50% extract of propolis by dry weight.

Mix the propolis extract with 37 parts ethanol and the laurylsulphate with 57 parts of boiled rain water. Then mix the two solutions together.

If the propolis extract contains less than 50% dry weight, appropriate calculations can avoid solvent reduction and later addition of the same solvent, i.e. add Sparts of 10% EEP and only 32 parts of ethanol. On the other hand the exact concentration of propolis is not very important as long as the lotion contains at least 0.5% of propolis by weight. The alcohol content of the lotion should be about 45% by volume.

5.16.8 Propolis toothpaste

The antibacterial, wound healing and circulation improving characteristics of propolis can be used for daily tooth and gum care. Rather than making your own toothpaste, it is easier to add propolis to an existing formulation. For home use simply take a tube of toothpaste, open it at the folded end and spoon out the contents. Mix the contents well with 3 to 10% of propolis paste (method 6) refill the tube and close up the end again.

For small-scale commercial production find a supplier of the base formulation and add your own propolis extract, or ask a larger manufacturer to formulate and pack the paste for you with your own label.

Proserpio and Martelli (1982b) recommended the following base formulation for a toothpaste. Other toothpaste formulations can be found in Chapter 9.

Ingredients (in parts by weight):

2.5 Propolis extract (10% EEP, method 1)

25.0 Boiled and cooled water
1.0 Carboxymethylcellulose (emulsifier)

25.0 Glycerol

1.5 Flavours and sweeteners

40.0 Calcium phosphate

2.0 Silica powder

2.0 Sodium laurylsulphate

1.0 Clear mineral oil

The propolis can be extracted with ethanol or, alternatively, glycol. Borax can be used as the emulsifier, but it is harmffil to consume borax in appreciable quantities and its inclusion in products that might be consumed is illegal in the USA and some other countries.

Once the components are well mixed they should be packed as soon as possible. Tubes are the preferred containers for toothpaste, but (if consumers will accept

them) alternative packaging could be soft squeeze bottles with a spout that can be closed.

5.16.9 Anaesthetic propolis paste

The major application for the paste is in dentistry. Propolis is supposed to give this paste anaesthetic and regenerative effects. It also contributes to antimicrobial and analgesic properties. Alternatively, the propolis extract can be mixed with ready-made benzocaine creams at a rate of 30% of a 50% propolis-ethanol solution. These pastes generally contain no water, so the propolis should be added in the form of a high-percentage alcohol extract.

The propolis solution should be prepared in advance to the right concentration. For this purpose the original extract prepared at a 10 to 30% propolis concentration should be evaporated until a 50% concentration is reached.

Ingredients (in parts by weight) after Sosnowski (1984):

10 Lanolin

10	*Unbleached beeswax*
10	*Petrolatum (or Vaseline, the trade name for a petrolatum)*
2	*Ethyl aminobenzoate*
3	*Clove oil*
15	*Propolis (50% EEP)*

Melt the beeswax and mix it with the petrolatum in a water bath, continue stirring during cooling and slowly mix in the lanolin. when the mixture has cooled to about 40 ^{0}C, start stirring rapidly while mixing in the propolis extract, followed by the other ingredients.

5.16.10 Creams

Propolis extract can be mixed with most creams. Moisturizing, rejuvenating or curative creams can be improved by adding 1 to 5 % (dry weight) propolis extract; many commercial preparations contain much less than this. Some extracts require ernulsifiers and others can be mixed directly depending also on the basic

formulation of the cream. The antibacterial, antifungal, stimulating and rejuvenating effects of propolis are particularly welcome in certain skin and hair-care preparations. Pharmaceutical creams with propolis extract can be used by humans and for animals.

For basic cream recipes see Chapter 9.

5.16.11 Facial masks

1) Facial masks are intended either to moisturize or to cleanse and tighten ghe skin. The following recipe is for a cleansing mask and the propolis is said to help rejuvenate the skin.

Ingredients (in parts by weight) after Sosnowski (1984):

50	*Filler (this may be Fuller's earth, china clay, kaolin, bentonite or a mixture of any of them)*
44.0	*50% glycerol solution*
5.7	*50% propolis solution*

q.s.	*Perfume or essential oils*

Mix the glycerol and the propolis extract (made with high percent alcohol) well, heating slightly if necessary. Mix with the filler and the petyume. Other beneficial plant extracts in alcohol may also be added in small quantities.

2) A simpler cleansing mask for oily skin (modified from Krochmal)

The ingredients (in parts by volume) for this mask should not be mixed until immediately prior to use, since they do not contain preservatives and will spoil rapidly.

4	*Fuller's earth (or substitute)*
1	*Rose water*
1	*Lemon juice*
2	*Honey*

1 5 to 10% propolis extract

The propolis extract here should have been prepared with diluted ethanol (less than 25%) or glycol, so that it is more water-soluble, or one of the powdered formulations should be used. The rose water can be prepared by dispersing a few drops of rose oil in water or by preparing a cold infusion tea) from a few rose petals in clean water. Other water or alcohol based petyumes or aromatic extracts can be used.

5.16.12 Micro-encapsulation

Several authors have described the encapsulation of propolis extracts as a mechanism for prolonged, slow release. Micro-encapsulated propolis could also be used in food as a preservative against bacterial decay.

Pepeljnjak et al., (1981) has shown the prolonged antibacterial effect of propolis enclosed in soft gelatine capsules. Encapsulation techniques in general are highly advanced, but simple methods requiring less expensive technology are possible. Further details can be found in Kondo (1979)

5.16.13 Ouality tests for antioxidant activity

A very simple home test has been suggested in a Canadian bee newsletter (CHRA, 1988): "To know whether your propolis is still active, put half a tea spoon of ground propolis into a small cup of fresh milk and let the milk sit at room temperature for four days. If the milk is still fresh after that time, your propolis is O.K."

A more accurate, but still simplified method for testing containing propolis is described below (after Bianchi, 1990):

Ingredients required:

200 mg	*Propolis*
5 ml	*Ethanol*
100 ml	*distilled water (boiled and cooled)*
1 ml	*20 % sulphuric acid*
1 drop	*0.1N potassium permanganate solution*

Apparatus required:

1	*Scale, precise to at least +/- 10 mg*
2	*250 ml Erlenmeyer flasks or other clean glass containers*
1	*Filter paper, cotton balls, cotton cloth or coffee filter*
1	*2 ml pipette and syringe or medicine stopper for drop application*
1	*50 ml beaker or other clear, clean glass container of small diameter*
2	*Medicine stoppers*
1	*Stopwatch or watch which indicates seconds*

For raw propolis:

1) Place 200 mg of finely broken propolis into the Erlenmeyer flask and add 5 ml of ethanol.

2) Leave for one hour then add 100 ml of boiled and cooled distilled water, mixing all well.

3) Filter everything

4) From the filtrate (the clear liquid) take 2 ml with the pipette orthe syringe, transfer it into the 50 ml beaker and add 1 ml of the 20% sulphuric acid. Mix for one minute, then add one drop of the permanganate solution.

5) Watch the colour of the liquid closely; the liquid should turn colourless, i.e. no longer pink, within 11 seconds. If discolouration takes longer, the propolis is of lower quality, i.e. has less antioxidant activity.

For propolis extracts:

The reaction time for discolouration depends on the quantity of dissolved propolis in the reagent (test liquid). Therefore, for different concentrations of extracts the times will be different. The initial quantity mixed with the distilled water can

(accordingly) be adjusted to a standard dry weight of propolis extract which then can be compared with a similar solution or raw propolis of known origin.

Mix 2 ml of a 10% ethanol extracted propolis solution (method 1) with 100 ml of boiled and cooled distilled water and follow the above test from step 3. Discolouration should occur within 20 seconds.

For propolis paste:

To 100 mg of paste add 5 ml of ethanol and then 100 ml of distilled water (boiled and cooled). Follow the above test from step 3. Discolouration should occur in less than 20 seconds.

For other propolis containing preparations:

For preparations with approximately 3 to 10% of propolis dry weight per weight of the preparation the following test should work. Always try a standard product first for comparison, i.e. the same product containing a known quantity of guaranteed fresh propolis.

To 2 g of a product containing 3 to 10% of propolis on a dry weight basis, add 10

ml of ethanol and mix well until it is dissolved. Add 100 ml of boiled and cooled distilled water. Mix and if necessary filter and then proceed with step 4. Discolouration should not take longer than 50 seconds.

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CHAPTER 6
ROYAL JELLY

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6.1 Introduction

Royal jelly is secreted by the hypopharyngeal gland (sometimes called the brood food gland) of young worker (nurse) bees, to feed young larvae and the adult queen bee. Royal jelly is always fed directly to the queen or the larvae as it is secreted; it is not stored. This is why it has not been a traditional beekeeping product. The only situation in which harvesting becomes feasible is during queen rearing, when the larvae destined to become queen bees are supplied with an over-abundance of

royal jelly. The queen larvae cannot consume the food as fast as it is provided and royal jelly accumulates in the queen cells (see Figure 6.1). The exact definition of commercially available royal jelly is therefore related to the method of production: it is the food intended for queen bee larvae that are four to five days old.

The differentiation between queen and worker bees is related to feeding during the larval stages. Indeed, all female eggs can produce a queen bee, but this occurs only when, during the whole development of the larvae and particularly the first four days, they are cared for and fed "like a queen". Queen rearing, regulated by complex mechanisms within the hive, induces in a young larva a series of hormonal and biochemical actions and reactions that make it develop into a queen bee. A queen bee differs from a worker bee in various ways:

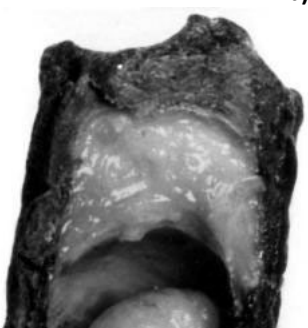
in its morphology: the queen develops reproductive organs while the worker bee develops organs related to its work such as pollen baskets, stronger mandibles, brood food glands and wax glands.

in its development period: on average the queen develops in *15.5* days while worker bees require 21 days.

in its life span: the queen lives for several years as compared to a few months for the worker bee,

and its behaviour: the queen lays up to several thousand eggs a day while workers lay eggs only occasionally. Unlike workers, the queen never participates in any common hive activities.

a) **b)**



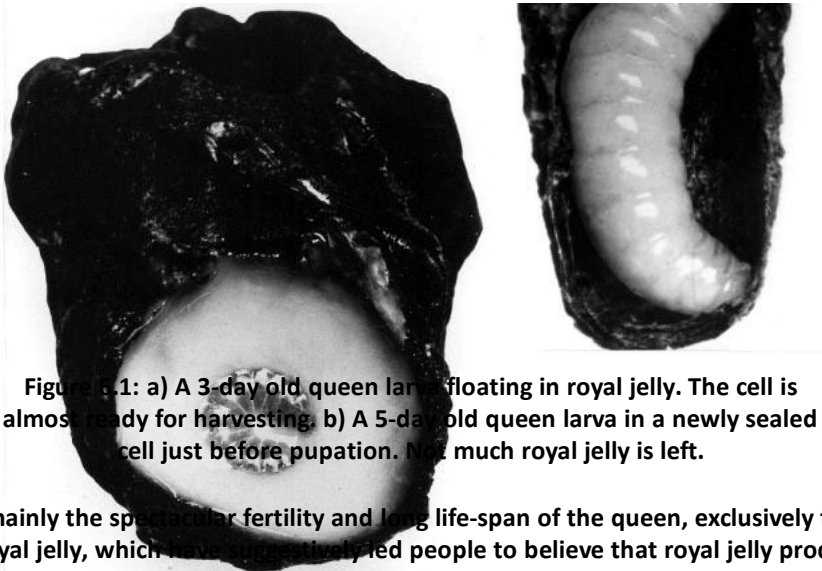


Figure 1.1: a) A 3-day old queen larva floating in royal jelly. The cell is almost ready for harvesting. b) A 5-day old queen larva in a newly sealed cell just before pupation. Not much royal jelly is left.

It is mainly the spectacular fertility and long life-span of the queen, exclusively fed on royal jelly, which have suggestively led people to believe that royal jelly produces similar effects in humans. In the early *1950's,* articles began to appear, particularly in the French beekeeping press, in praise of the virtues of royal jelly, referring to

research conducted in several hospitals. Chauvin (1968) however, was unable to find the source of such information and therefore considered it unfounded.

The myth of royal jelly started with an amazing biological phenomenon on the one hand and commercial speculation on the other, which, on the basis of initial results obtained by entomologists and physiologists, exploited the suggestibility and imagination of consumers willing to be seduced by the fascination of this rare and unknown product was exploited. In fact, royal jelly was so rare and so little known that it was impossible to verify its actual presence in many products claiming its content.

In the years immediately following its first marketing, royal jelly quickly became widely known and consumed and the increasing demand motivated experts to refine production techniques and led more and more beekeepers to specialize in this activity. At the same time, research on quality control of the commercial product and identification of its biological and clinical properties found growing support.

Consumption of royal jelly has been growing ever since, even without its benefit to human health having ever been scientifically confirmed. The Western medical

establishment has always been wary of the effects claimed for this product and in most cases refuses to consider it, largely because of the way royal jelly was initially promoted. In spite of a vast number of publications praising its virtues and the apparently abundant bibliography, there is still a serious lack of scientific data on the clinical effects of royal jelly.

6.2 Physical characteristics of royal jelly

Royal jelly is a homogeneous substance with the consistency of a fairly fluid paste. It is whitish in colour with yellow or beige tinges, has a pungent phenolic odour and a characteristic sour flavour. It has a density of approximately 1.1 g/cm^3 (Lercker et al., 1992) and is partially soluble in water. Aqueous solutions clarify during basification with soda.

Viscosity varies according to water content and age - it slowly becomes more viscous when stored at room temperature or in a refrigerator at *5^{0}C.* The increased viscosity appears to be related to an increase in water insoluble nitrogenous compounds, together with a reduction in soluble nitrogen and free amino acids (Takenaka et al., 1986). These changes are apparently due to continued enzymatic activities and

interaction between the lipid and protein fractions. If sucrose is added, royal jelly becomes more fluid (Sasaki et al., 1987). Such changes in viscosity have also been related to the phenomena which regulate caste differentiation in a bee colony (see also 6.4.1).

Certain debris in royal jelly, is a sign of purity as, for example, the ever present fragments of laarval skin (see also 6.8). Wax fragments too, are encountered more or less regularly, but their presence is largely dependent on the collection method. Stored royal jelly often develops small granules due to precipitation of components.

6.3 The composition of royal jelly

Numerous chemical analyses of royal jelly have been published over the years. Only recently though, have highly refined technologies given detailed analyses of the unusual composition and complexity of this somewhat acidic substance (pH 3.6 to 4.2).

The principal constituents of royal jelly are water, protein, sugars, lipids and mineral salts. Although they occur with notable variations (Table 6.1) the composition of royal jelly remains relatively constant when comparing different colonies, bee races

and time.

Water makes up about two thirds of fresh royal jelly, but by dry weight, proteins and sugars are by far the largest fractions. Of the nitrogenous substances, proteins average 73.9% and of the six major proteins (Otani et al., 1985) four are glycoproteins (Takenaka, 1987). Free amino acids average 2.3% and peptides 0.16% (Takenaka, 1984) of the nitrogenous substances. All amino acids essential for humans are present and a total of 29 amino acids and derivatives have been identified, the most important being aspartic acid and glutamic acid (Howe et al., *1985).* The free amino acids are proline and lysine (Takenaka, 1984 and 1987). A number of enzymes are also present including glucose oxidase (Nye et al., 1973) phosphatase and cholinesterase (Ammon and Zoch, 1957). An insulin-like substance has been identified by Kramer et al. (1977 and 1982).

Table 6.1:

Composition of royal jelly (form Lercker et al., 1984 and 1992)

	Minimum	Maximum
	57%	70%

Water	57%	70%
Proteins (N x 6.25)	17% of dry weight	45% of dry weight
Sugars	18% of dry weight	52% of dry weight
Lipids	3.5% of dry weight	19% of dry weight
Minerals	2% of dry weight	3% of dry weight

The sugars consist mostly of fructose and glucose in relatively constant proportions similar to those in honey. Fructose is prevalent. In many cases fructose and glucose together account for 90% of the total sugars. The sucrose content varies considerably from one sample to another. Other sugars present in much lower quantities are maltose, trehalose, melibiose, ribose and erlose (Lercker et al., 1984, 1986 and 1992).

The lipid content is a unique and from many points of view, a very interesting feature of royal jelly. The lipid fraction consists to 80-90% (by dry weight) of free fatty acids with unusual and uncommon structures. They are mostly short chain (8 to 10 carbon atoms) hydroxy fatty acids or dicarboxylic acids, in contrast to the fatty

acids with 14 to 20 carbon atoms which are commonly found in animal and plant material. These fatty acids are responsible for most of the recorded biological properties of royal jelly (Schmidt and Buchmann, 1992). The principal acid is 10-hydroxy-2-decanoic acid, followed by its saturated equivalent, lO-hydroxydecanoic acid. In addition to the free fatty acids, the lipid fraction contains some neutral lipids, sterols (including cholesterol) and an unsaponifiable fraction of hydrocarbons similar to beeswax extracts (Lercker et al., 1981, 1982, 1984 and 1992).

The total ash content of royal jelly is about 1 % of fresh weight or 2 to 3 % of dry weight. The major mineral salts are, in descending order: K, Ca, Na, Zn, Fe, Cu and Mn, with a strong prevalence of potassium (Benfenati et al., 1986).

The vitamin content has been the object of numerous studies, from the moment when the first research (Aeppler, 1922) showed that royal jelly is extremely rich in vitamins. Table 6.2 indicates the results obtained by Vecchi et al., (1988) with regard to water-soluble vitamins. Other authors report averages close to the minimum values of Table 6.2 (Schmidt and Buchmann, 1992). Only traces of vitamin C can be found.

As far as the fat-soluble vitamins are concerned, it was initially thought that, given

the enormous fertility of the queen bee, royal jelly would contain vitamin E. But tests have shown that it does not. Vitamins A, D and K are also absent (Melampy and Jones, 1939).

During the first studies, much emphasis was placed on the search for sex hormones in royal jelly. The first positive tests were later proven wrong. Melampy and Stanley (1940) showed no gonadotropic effects on female rats and Johansson and Johansson (1958) clearly demonstrated the absence of any human sex hormones. Recently though, with much more sensitive radio-immunological methods, testosterone has been identified in extremely small quantities: 0.012 ~g/g fresh weight (Vittek and Slomiany, 1984). In comparison, a human male produces daily 250,000 to 1 million times the amount present in one gram of fresh royal jelly (Schmidt and Buchmann, 1992). No biological effect has been demonstrated for such small amounts.

Table 6.2 Vitamin content of royal jelly in μ g per gram of fesch weight (Vecchi et al., 1988)

	Thiamine	Riboflavin	Pantothenic Acid	Pyridoxine	Niacin	Folic acid	Inosito

Minimum	1.44	5	159	1.0	48	0.130	80
Maximum	6.70	25	265	48.0	88	0.530	350

Numerous minor compounds, belonging to diverse chemical categories, have been identified in royal jelly. Among these are two heterocyclic substances, biopterine and neopterine at 25 and 5 ijg/g of fresh weight respectively. These compounds are found in the food of worker bee larvae too, but at about one tenth of these concentration (Rembold, 1965). Other substances identified include several nucleotides as free bases (adenosine, uridine, guanosine, iridin and cytidine) the phosphates AMP, ADP, and ATP (Marko et al., 1964), acetylcholine (1 mglg dry weight, Henschler, 1954) and gluconic acid (0.6% of fresh weight, Nye et al., 1973).

In all popular and scientific literature, there is a fraction of royal jelly described as "other, as yet unknown". This phrase not only emphasizes the incomplete state of analytical knowledge about the product, but also the lack of understanding of the biological activities (proven or presumed) of royal jelly. Up to now, despite many efforts, most of these activities have not been proven definitely, nor have they been

attributed to any of the known components.

6.4 The physiological effects of royal jelly

6.4.1 On honevbees

The effect of royal jelly on honeybee larvae, for which it was originally intended as food, is briefly described since in addition to being a fascinating biological phenomenon, it is also the basis of the royal jelly "myth".

In the 1950's, in the wake of new discoveries in the medical field of such wonder drugs as penicillin, hormones and vitamins became "popular" and were seen by many as the simple answers to many biological questions. The elusive "hormonal" effect of royal jelly on honeybee larvae led to the belief that its almost miraculous action on bee larvae could be similar on humans.

By deduction these "hormonal" effects were not only responsible for the caste differentiation between worker and queen bee, but also for the enormous fertility of a queen genetically equal to a worker bee, distinguished apparently only by the food it ate. The same applies to the queen's longevity, unique for an adult insect.

Though it is known that royal jelly is a necessary food for the queen's survival and productivity, it is not known which royal jelly fractions are essential, which ones can be replaced and what constitutes minimum or optimum requirements for a queen. Almost all the attention has been focused on the immature stages of development.

Numerous studies were carried out to discover hormones or other substances powerful enough to induce all the necessary changes and give the queen such "superior" qualities. Indeed, the initial studies led to the belief that a "queen determinator" did exist and was an extremely unstable substance (as elusive as eternal life). It appeared to be so unstable that one day after secretion, it was already ineffective. However, the results of other studies did not confirm this hypothesis.

In an attempt to identify the queen determinator, all the components of royal jelly, particularly the more unusual ones or those with known biological activity or present in greater quantity have been tested. In the late 1980's the mystery had still not been solved and a number of contrasting hypotheses had produced equally convincing explanations. Rembold et al. (1974) ware thought to have been close to identifying a specific substance with queen determinator activity which they had isolated; other researchers proposed a differentiation mechanism based on the

different proportions of nutrients in the food of worker and queen bee larvae. Weiss (1975) and Asencot and Lensky (1975) believed it was the sugar content of larval food (higher for the young queen bee larvae) that was supposed to cause the differentiation into queens.

More recently, Sasaki et al. (1987) proposed yet another hypothesis incorporating the many contrasting results from other researchers and suggested the "correct" viscosity of royal jelly was a key factor together with higher consumption, but even this theory still has to be substantiated with proof. In other words, it is still not known how royal jelly works nor what is responsible for its amazing effects.

However, if parallels are still being drawn between honeybees and royal jelly, and humans and royal jelly, then they should serve to emphasize the complexity and interdependence of different therapies and factors such as who is taking what, when and how much. Eating royal jelly, or rubbing it into the skin will not make anyone younger or live for a thousand years. On the other hand, using it to supplement and support other diets, activities or medicines may have synergistic effects which cannot be explained by a list of compounds and their individual effects. Tests of such a hypothesis in clinical and scientific trials are needed. There is plenty of circumstantial evidence, reviewed in the following section, that leads us to

believe that royal jelly might be highly beneficial to mankind.

6.4.2 Unconfirmed circumstantial evidence

Royal jelly was initially advertised for its rejuvenating effects (De Belfever, 1958). The activities most frequently reported in advertisements and constantly confirmed in the declarations of those who have taken royal jelly are indicated in Table 6.3, citing the contents of one of Europe's most widespread and popular publication on the subject (Donadieu, 1978). Royal jelly, taken orally for 1-2 months by swallowing or letting it melt under the tongue in doses of 200-500 mg a day, is said to act as a tonic and stimulant, with a euphoric effect on healthy humans.

In addition to these indications, users declared that royal jelly had solved most of their health problems. In many cases these were chronic or recurring disorders, for which other treatments did not lead to the desired results, so that the effects obtained by taking royal jelly have been considered "miraculous".

It must be emphasised that these claims are unconfirmed by any scientific studies or documentation. There is no proof that the effects are exclusively or even mostly attributable to royal jelly.

People who have taken royal jelly said that they soon experienced a feeling of general well-being, i.e. an effect on their physical output (resistance to fatigue), intellectual performance (greater learning capacity and better memory) and on their mental condition (greater self-confidence, feeling of well-being and euphoria). In other words, royal jelly appears to act as a general stimulant, improving immune response and general body functions.

Table 6.3:
A list of properties, benefits and improvements attributed to royal jelly quoted from personal case histories and non-scientific literature.

Internal Use	External Use
Tonic	Skin conditions
Stimulant - physical performance, better memory, learning capacity and self-confidence	Epithelial stimulation and regrowth

~~General health improvement~~ Anorexia	~~Anti-wrinkle~~ Sebaceous secretion (fat secretions of skin glands) normalized
Increased appetite	
Skin conditions	
Sexual desire and performance	
Influenza	
Increased resistance to viral infections	
High blood pressure	
Low blood pressure	
Anaemia	

~~Arteriosclerosis~~	
Cholesterol levels	
Chronic and incurable disorders	

6.4.3 Scientific evidence

Royal jelly is neither toxic when injected into mice and rats at high dosages of up to 3 g per kg body weight per day (Hashimoto et al., 1977) nor mutagenic, as tested on DNA of Salmonella typimurium (Tamura et al., 1985).

Takahashi et al., (1983) reported cases of allergic contact dermatitis in 2 out of 10 patients subjected to patch tests. In the context of allergic reactions it needs to be mentioned that intramuscular or intraperitoneal injections, the most common form of royal jelly administration in early years, have been completely abandoned (even under strict medical supervision) because of the risk of serious allergic reactions (Dillon and Louveaux, 1987) Today, royal jelly is most often administered orally and externally (in cosmetics).

In vitro studies have confirmed that lO-hydroxydecanoic acid in royal jelly has antibiotic activity. The antibiotic effectiveness is thermostable, i.e. is not destroyed by moderate heating, but it decreases with improper or long-term storage. Antibiotic action has been proven against the following microorganisms: Escherichia coli, Salmonella, Proteus, Bacillus subtilis and Staphylococcus aureus (Lavie, 1968; Yatsunami and Echigo, 1985). It shows one quarter of the activity of penicillin against Micrococcus pyrogens and is also fungicidal (Blum et al., 1959). In vitro, antiviral effects have been described (Derivici and Petrescu, 1965) and better resistance to viral infections has been observed in mice.

This same antibiotic action of fatty acids is neutralized by raising the pH above 5.6. Since injection into blood, muscle or the peritoneal cavity will raise the pH to 7.4, and the pH is above 5.6 in the intestines, the therapeutic value of the anti-bacterial activity of fatty acids is likely to be negligible for any internal applications, but will remain effective for topical use.

In studies on the internal effects of royal jelly with live animals or humans the jelly is usually administered either by mouth or by injection. The latter allows better assessment of hormonal activities ascribed to royal jelly but carries a substantial risk of allergic reactions.

Oral administration

Positive effects on reproductivity, though not necessarily due to hormone-like action, have been reported at least for chickens, quails and rabbits. Rabbits reacted to a normal diet supplemented with 100-200 mg of royal jelly per kilogramme of body weight with increased fertility and embryonic development (Khattab et al., 1989). Japanese quail reached sexual maturity sooner and laid more eggs after supplementation of diets with high doses (0.2 g) of lyophilized (freeze-dried) royal jelly (Csuka et al., 1978). Bonomi (1983) increased egg production, fertility and hatching in laying hens by using 5 mg royal jelly per kg of feed, but Giordani (1961) found no histological changes in male or female reproductive organs or weight gain with higher doses of 10 to 40 mg per day.

Growth rates of mice slightly increased with a dosage of 1 g of royal jelly per kg of feed, but decreased with higher dosages (Chauvin, 1968). Bonomi (1983) reported weight increases in chicken, partridges and pheasants with a supplement of S mg royal jelly per kg of feed and Salama et al. (1977) reported weight increases in rats when 10, 20 or 40 mg were injected directly into their stomachs. The administration of 0.02 g of royal jelly to calves less than 7 days old gave a weight gain of 11 - 13 % during the following 6 months in comparison with untreated controls (Radu-

Todurache et al., 1978). They also mentioned that the treated calves showed lower mortality and higher resistance to infection.

Figure 6.2: Dark glass bottle with fresh royal jelly and

miniature spatula for oral administration (human consumption).

Injections

Intravenous injections cause slight vasodilation (temporary enlarging of blood vessels) and have a hypotensive effect (lowering blood pressure); both due to acetylcholine in royal jelly (Jacoli, 1956; Shinoda et al., 1978).

Injections of royal jelly solutions induced higher blood sugar levels than oral applications (Chauvin, 1968). No hypoglycemic (insulin-like) reaction could be shown in rats (Fujii et al., 1990). Afifi et al. (1989) reported weight increases in guinea pigs after injection of 100-300 ing royal jelly per kilogramme of body weight. Small doses injected into cats raised haemoglobin and erythrocyte counts and repeated doses of up to 10 mg/kg of body weight stimulated motor activity and weight gains in mice. Repeated higher doses of 100 mg/kg in mice, however, caused weight loss and impaired cerebrocortical (brain cortex) cellular metabolism (Lupachev, 1963).

Animal tests

In other studies human diseases were simulated in animals in order to identify the mechanisms of royal jelly action. Thus it is known that royal jelly can reduce blood plasma levels of cholesterol and triglycerides (Cho, 1977) and cholesterol and arterial cholesterol deposits in rabbits when these disorders were induced experimentally (Carli et al. 1975). Nakajin et al., (1982) stated that although royal jelly has no effect on lipid levels in blood plasma in normal rabbits, it can reduce the cholesterol content in the blood of animals fed on a diet which induced high levels of blood cholesterol.

Vittek and Halmos (1968) found that royal jelly promoted bone healing in rabbits. The healing of skin lesions was accelerated and anti-inflammatory action was shown for rats by Fujii et al. (1990).

Other researchers tested royal jelly and some of its compounds on tumour cell cultures, showing the inhibitory action of lO-hydroxydecanoic acid (Townsend et al., 1960) and certain dicarboxylic acids. However, they also showed that the same acids could induce tumours in mice when royal jelly is mixed with the culture medium (several mg/ml at less than pH 5) prior to injection into the test animals (Morgan et al., 1960). Wagner et al., (1970) found no significant effects of prolonged survival in mice irradiated against experimentally induced tumours and treated with royal jelly

(20 mg/kg of body weight) as compared to control mice which did not receive any royal jelly. More recently, Tamura et al., (1987) have shown tumour growth inhibition in mice with prophylactic and therapeutic oral administration of royal jelly. Inhibition of rapid-growth cancers (leukaemia) was insignificant but it was noticeable on slow-growing, solid tumours (Ehrlich and Sarcoma strains).

Human tests

Studies of the effects of royal jelly on humans are extremely numerous, particularly in Eastern Europe. A few early studies were presented in Russian by Braines (1959, 1960 and 1962). Most studies however, arc difficult to evaluate for the scientific value of the reported information. Although many are presented as scientific publications, they often lack details on test methods, use parameters difficult to quantify (well-being, euphoria and rejuvenation) do not entirely exclude effects from other concurrent treatments, or use subject numbers too small to exclude accidental effects or natural variation. Of all the works consulted and selected for this chapter, of which a few are summarized in Table 6.4, not one is totally without criticism. The information presented therefore must be considered only as an indication of possible effects requiring further clinical testing.

The mechanisms of royal jelly's activity is not known and none of the numerous hypotheses have been confirmed. An early explanation (Johansson and Johansson, 1958) claiming high vitamin content as a contributory factor can be refuted on the grounds that the same effects should then be achievable with vitamin supplements or a glass of milk, which contains amounts of vitamins similar to the usual dose of royal jelly. Beneficial effects on intestinal flora through selected anti-microbial action can mostly be excluded due to pH. The action of some compounds on endocrine glands, or becoming part of enzyme systems or directly affecting intermediate metabolism has been suggested by Bonomi (1983).

Table 6.4.:
A list of some effects of royal jelly on humans.

Applications	Description	References
Premature bebies and those with nutritional deficiencies of	8-100 mg orally, improvement of general condition; increase in weight, appetite, red blood	Malossi & Grandi, 1956 Prosperi and

various origins	cells and haemoglobin	Ragazzini, 1956 Prosperi et al., 1956 Quadri, 1956
Elderly (70-75 years), anorexic, depressed and low blood pressure patients	20 mg injected every second day, improvements on all accounts 20 mg taken orally every second day, improvements as above	Destrem, 1956 Destrem, 1956
Psychiatry	Improvements of asthenia, nervous breakdown, emotional problems and counteraction of side effects of psychoactive drugs	Telatin, 1956
Chronic metabolism	Mixture or royal jelly, honey and ginseng,	Borgia et al., 1984

	improvements in weight gain and psychological conditions, but changes of blood characterisics	
Stimulating metabolism	Stimulating effects comparable to that by proteins, effect assumed to be due to activity of enzymatic complexes	Martinetti and Caracristi, 1956
Wound healing	5-30 mg/ml injected into burn blisters, improved regrowth of skin	Gimbel et al., 1962

6.5 Uses and marketing of royal jelly

Royal jelly can be sold in its fresh state, unprocessed except for being frozen or

cooled, mixed with other products, or freeze-dried for further use in other preparations. The fresh production and sale can be handled by enterprises of all sizes since no special technology is required. In its unprocessed form it can also be included directly in many food and dietary supplements as well as medicine-like products or cosmetics. For larger industrial scale use, royal jelly is preferred in its freeze-dried form, because of easier handling and storing. Freeze-dried royal jelly can be included in the same products as the fresh form. The production of freeze-dried royal jelly requires an investment of at least US$ 10,000 for a freeze-dryer, sufficient production volume and an accessible market for the raw material or its value added products. The discussion below describes some of the value added products in which royal jelly has been included in the past.

Since the assumed benefits of royal jelly have not been sufficiently proven, statements in advertisements and on package labels should be very careful to avoid suggestions which are not well-founded. Any kind of fraudulent or exaggerated statements and claims are in the long run more damaging than any short-term benefit that may be derived from, for example, an increase in the price of a product. Products containing royal jelly should be specially marked or packaged in order to distinguish them from similar products without it.

6.5.1 As dietary supplement

Royal jelly belongs to a group of products generically described as "dietary supplements" These are products which are consumed not for their caloric content nor for pleasure, but to supplement the normal diet with substances in which it might be lacking. In reality, however, the use of royal jelly is not so much linked to its high content in "noble" substances, but to its assumed stimulant and therapeutic value. However, it cannot be defined as a medicine because the data required for classification in this category are lacking. If it were declared a medicine, its use would become dependant on medical prescriptions and the production and marketing of royal jelly-based products would become the exclusive domain of the pharmaceutical industry.

Figure 6.4: A package of 10 vials each with 166 mg of freeze-dried royal jelly (the equivalent of 500 mg fresh royal jelly) on a glycine base (filler or support) and 10 vials with 5 ml of a glucose flavoured solvent (water) preserved with ascorbic acid. The contents of the two vials have to be mixed before use.

A large amount of royal jelly is sold and consumed as it is harvested. In its unprocessed, natural state it is preferred by most producers, because it does not require any special technology and by consumers because of its unaltered "naturalness". The fact that its taste is not very pleasant, instead of deterring consumers appears to enhance its image as a "medicine". For those who do not appreciate this particular medicinal aspect, royal jelly can be mixed with a little

honey, sugar syrup or water, or it may be encapsulated.

Unprocessed royal jelly is usually packaged in small, dark glass bottles of sizes that correspond to the duration of a "treatment" e.g. 10, 15 or 20 g. A tiny plastic spatula is usually included for the "correct" dosage of 250 - 500 mg (see Figure 6.2) Special isothermal packaging (usually a moulded polystyrene box) is sometimes used to make the product look even more precious and protect it perhaps from brief temperature fluctuations. In Italy, in the past, it was also sold in special glass syringes, allowing more precise dosages and giving greater protection against oxidation.

Producers also sell pure royal jelly in its original queen cells after having removed the larvae and sealed the cells. The cells may be sealed with another wax queen cell cup, with liquid wax or by squeezing the ends of the cell together. The queen cells thus prepared can be packaged in small plastic boxes or glass jars together with a small spatula. The main disadvantage of this type of packaging is that the royal jelly does not keep well (two weeks in a refrigerator or a few months when frozen immediately) and only sells well directly from the producer to the consumer. On the other hand such sales can be extremely profitable and are also attractive to consumers who can be sure that the product is untreated and fresh. Given the

normal variation in content of queen cells the net weight must be given for the smallest possible quantity (e.g. minimum content 250 mg/cell).

Royal jelly sold in any of the above forms must always be kept at or below 5~ C during storage, during transportation and in the retail store. Empty packages can be displayed while full containers are stored in a refrigerator.

6.5.2 As ingredient in food products

A mixture of royal jelly in honey (1-3 % royal jelly) is probably the most common way in which royal jelly is used as a food ingredient. Among the advantages of this product are that no special technology is required and the honey masks any visible changes in the royal jelly. The final product is pleasant-tasting and it provides the beneficial effects of both products. One teaspoon of the mixture typically contains 100 - 300 mg of royal jelly, about the dosage of royal jelly that is most commonly recommended. Nothing is known however about the preservation of royal jelly in such a mixture. It should, therefore, be kept refrigerated.

Another food frequently enriched with royal jelly in some European countries is yogurt, which has an acidity similar to royal jelly and also requires refrigeration.

Yoghurt is already a popular food for health-conscious consumers who often appreciate its further enrichment with royal jelly. The higher price that is usually charged reflects what the market will bear rather than the extra production costs, i.e. the market value added to such a product by the royal jelly is higher than the cost of the jelly and extra production costs.

Sometimes, vitamin supplements and fruit juices are enriched with freeze dried royal jelly. Royal jelly is widely used in beverages in Asia.

Royal jelly is also sold in a jelly made of honey, sugar, jam and pectin. Though simple enough to produce, there are no data available on the durability or residual efficacy of royal jelly presented in this way.

6.5.3 As ingredient in medicine-like products

This category of products resembles medicines as far as their form of presentation is concerned, but in other respects these products are no different from the dietary supplements and foods described in the two preceding sections. However, they require more advanced technology for production and packaging and make higher demands on product stability as well as quality control. For the same reasons, many

of these applications use freeze dried royal jelly. Unfortunately, the pricing of these products does not always reflect the quality of the product and many are grossly overpriced.

In medicine-like formulations royal jelly is generally included for its stimulatory effects. However, it is also used to solve specific health problems. A variety of formulations are available, often containing ingredients otherwise used to alleviate particular afflictions. As has been seen in an earlier section, there is no solid scientific base for any such uses. Advertising or other popular information should therefore be treated with great caution and royal jelly should never be used as a substitute for other treatments unless the treatment has been approved by a competent physician.

Whether royal jelly is the only active ingredient, or is mixed with others, the basic forms of presentation remain the same and are adapted to the desired applications or consumer preferences. Dosages may be presented in any of the following ways (see Figure 6.3):

- as a single dose package of dry royal jelly with separate solvent,
- as a single dose of mixed pulverized ingredients with or without solvent and in

tablet or capsule form,
- as a single or multiple dose liquid solution for oral administration or injection

Single-dosage packages generally have to use a filler to bring the dose of the active ingredient (royal jelly or the ingredient mix) to a volume that can be easily handled by the consumer. An envelope containing only 250 mg of freeze-dried royal jelly would look very empty and the powder it contained might easily be lost. Sugar, salt, aromas, citric acid, glycine, a.o. may all serve as fillers (see Figure 6.4). As well as being mere fillers, they often render the product more pleasant to taste. Additional ingredients mixed with royal jelly are often other food supplements like plant extracts (ginseng), yeasts, pollen extracts and others.

Most packages provide the dry phase in a separate package, envelope or vial and a solvent in an appropriate container. Not only does this separation allow more effective treatment of the liquid phase (such as pasteurization or sterilization) but it also improves storage life and therefore facilitates shipping and marketing. Some refined packaging contains the dry phase in a special lid which upon opening releases the powder into the solvent.

In tablet form, the principal excipient is usually a powdered sugar plus a binding

agent such as gum arabic (for simple recipes see 5.16.5 and 6.11.7). For larger production, tableting machines are necessary which can sometimes be purchased second-hand at reasonable prices. Hard and soft gelatine capsules can be used for similar formulations. The hard capsules can be filled by hand on a small scale or by machine on a more industrial level (see also Figures 3.10 and 3.11), but soft capsules and gelatine drops need expensive equipment and are usually manufactured only by larger enterprises or under contract by large enterprises for third parties.

Another form of presentation is in vials with a liquid solution of royal jelly. These are simple to prepare and can use fresh unprocessed royal jelly, but they present preservation problems both with regard to microbiological activity and the long-term stability of the royal jelly. The addition of a little alcohol or propolis extract increases protection against microbial growth. Such preparations are distributed widely and are now being imported mostly from Asia by Europe, the USA and some Latin American countries . One of the more common formulations contains honey, royal jelly and an alcohol extract of ginseng (see Figure 6.10). Since these products are not regulated as food or as medicines, they are not required to list all ingredients, particularly the different preservatives which are necessary in these liquid formulations.

The production of injectable royal jelly preparations must be left to qualified laboratories in order to avoid problems with contamination and toxicity. There are patents that protect the production of royal jelly extracts for human use (by injection), but up to now there is no actual production or use for these "medicines", at least in Western Europe.

The medicinal or pseudo-medicinal use of royal jelly is much more popular in Asia and Eastern Europe, where rules on medicinal formulations and applications are very different from those in Western Europe and North America. In Africa, very little use of royal jelly has been reported, either as a food supplement or as medicine.

6.5.4 As ingredient in cosmetics

Except in Asia, probably the largest use of royal jelly is in cosmetics. Royal jelly is included in many dermatological preparations, but mostly in those used for skin refreshing, and skin regeneration or rejuvenation. It is also used in creams or ointments for healing burns and other wounds. It is usually included in very small dosages *(0.05* to 1 %) but it is likely that it deteriorates relatively quickly. No precise data on loss of effectiveness are available. The freeze-dried form of royal jelly is usually preferred because of ease of handling. A royal jelly/lactose paste mixed at

0^0C is said to stabilize royal jelly (Rubinsstein, 1954). The paste can then be added to cosmetic preparations. More information and recipes can be found in Chapter 9.

6.5.5 Others

The only other known uses for royal jelly are in animal nutrition. In particular, royal jelly has occasionally been used (fresh or freeze-dried) to stimulate race horses. For experimental purposes it is also used as a food for rearing mites and insects.

6.6 Royal jelly collection

Royal jelly is produced by stimulating colonies to produce queen bees outside the conditions in which they would naturally do so (swarming and queen replacement). It requires very little investment but is only possible with movable comb hives. Expert personnel are required, who are able to devote considerably more time than is commonly required for the production of other bee products. Without this prerequisite it is possible to only occasionally collect the contents from cells of natural swarms - and this amounts to no more than a gram or two per hive.

A well-managed hive during a season of 5-6 months can produce approximately

SOOg of royal jelly. Since the product is perishable, producers must have immediate access to proper cold storage (e.g. a household refrigerator or freezer) in which the royal jelly is stored until it is sold or conveyed to a collection centre.

The most rational and economic methods for large scale production are variations of the Doolittle method of queen rearing. Usually, the starter colony is omitted and cell cups, with transferred larvae, are directly introduced into the finisher colonies. Strong queenright colonies are preferred, in which the queen chamber is separated from the cell rearing chamber by a queen excluder. The only required adaptation is to shorten the cycle in the finishing colonies (3 days versus 10) before cells are removed for harvesting (Figure *6.5).* For occasional and small scale production any other queen rearing method can be used. However, there are many queen rearing methods which differ only in hive design and the use of starter and/or finisher colonies. For more details, it is recommended that the reader consult a regular beekeeping text or better, one specialized in queen rearing. Recommended English texts are Laidlaw, 1979; Laidlaw, 1992 and Ruttner, 1983.

a) b)

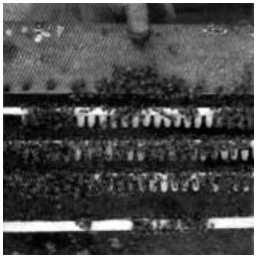


Figure 6.5 : a) Special frame with queen cells for queen rearing or royal jelly harvesting. These cells have already been sealed and are too old for collection of royal jelly. However, queens may be raised from these cells if they are introduced into queenless hives. b) Queen cells of the right age for royal jelly harvesting.

The basic requirements are movable comb hives, preferably some queen excluders, queen cups (made from wax or plastic), a transfer needle, a spoon or suction device to remove royal jelly, dark glass vials and a refrigerator. Special hive modifications may facilitate the work according to personal preferences, and centrifugal extractors for royal jelly may be used for large scale production. Feeding with sugar syrup (1:1 in sugar/water) increases cell acceptance, even when flowers are available.

Individual queen cells should not contain less than 200 mg of royal jelly. Low cell content means that there are too many cells for the finisher colony or that the colony is not in a condition to provide for queen rearing. There are racial differences in productivity and specially selected strains can be obtained. However, importing queens may not guarantee higher production in a different environment and carries a considerable risk of importing new or resistant diseases, thus reducing productivity and economic feasibility.

Mature queen cells, i.e. those with larvae four days old (3 days after grafting), must be brought quickly into the extraction room. The open, narrow part of the cells is cut to facilitate and speed up collection. Then the larvae are removed with a pair of soft forceps, taking care not to harm them and contaminate the jelly. The royal jelly is extracted by emptying each cell with a small spatula, by sucking it up with a

special mouth operated device, with a pump operated device or by centrifugal extraction (see Figure 6.6). Following extraction, the cells are immediately ready for another rearing cycle.

The royal jelly must be filtered using a fine nylon net (nylon stockings are excellent) to eliminate fragments of wax and larvae. Metal filters should not be used. The jelly should be placed into dark glass vials or food-grade plastic containers, avoiding any excessive exposure to air. It should be refrigerated immediately. Any material or equipment contacting royal jelly - including hands - must be clean and disinfected using heat or pure alcohol. The laboratory must be kept impeccably clean and extraction should never be done outside or in sunlight.

The commercial production of royal jelly requires a methodical approach, good organization and precise timing. Constant attendance is essential as one day off can eliminate two days of production. In order to have a weekly day of rest (e.g. Sunday) no queen cells would be introduced on Thursday, which means that there will also be no collection on the following Wednesday.

These techniques are suitable for both small and quite large enterprises. Depending on the intended market, the approach can be either one of low cost or one in which

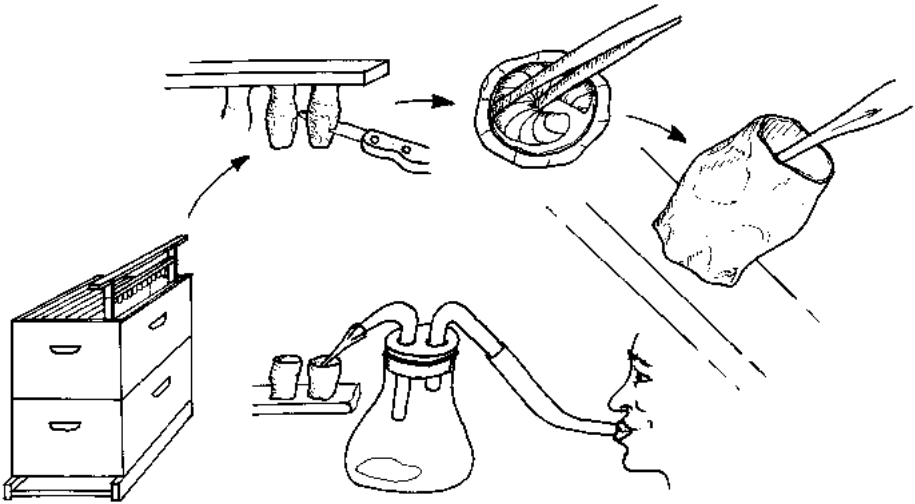
all collecting, processing and distribution takes place in highly controlled environments. The latter will result in a product which is better suited for industrial use (see also section 6.11.1).

6.7 Storage

Royal jelly has a limited shelf-life. Early beliefs in the extreme instability of royal jelly activity, based on the alleged rapid loss of the "queen determination" factor (see 6.4.1) have not been confirmed. Since neither the mode of activity nor the actual effects of royal jelly are known, there are no data available on changes in its biological effectiveness on humans after long term storage.

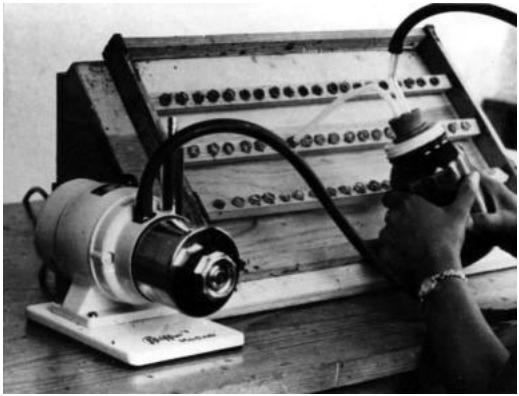
Information is, however, available on changes in composition due to long term storage, such as a higher acid titre, a large unsoluble protein fraction, less free amino acids, less glucose oxidase and others (Takenaka et., 1986 and Karaali et al, 1988). Such changes make it appear likely that also biological activity is influenced by storage. Refrigeration and freezing delay and reduce the chemical changes. Although freeze-dried jelly is the most staable form of royal jelly, some changes still take place.

a)



b)

Figure 6.6: a) The steps for removing



royal jelly from a queen cell and a diagram of a simple suction device for the collection of royal jelly from queen cups. b) A small vacuum pump can be adapted for the collection of larger quantities of royal jelly. Note that all the queen cells have been cut down in size to facilitate removal of the larvae and the royal jelly.

On the basis of the above, we can conclude that refrigeration of royal jelly at 0~ to 5 ^{0}C is a minimum precaution. Still better is storage, whenever possible, at temperatures below -17^{0}C, which is attainable in most household freezers. Since royal jelly is an emulsified product and not cellular tissue, freezing presents no particular problem and common household freezers can be used.

As there are no criteria for establishing "safety" limits for product activity, storage and shelf-life should be kept as brief as possible. For products sold in Europe, the average recommended storage time after production is 18 months under refrigeration. For products stored at - 170 C, storage can be extended to 24 months. After defrosting and packaging, the product should not be stored in a refrigerator for more than 12 months.

Freeze-dried royal jelly and royal jelly based products are generally stored at room temperature, sometimes for several years. Freeze-dried royal jelly is certainly more stable than the fresh product, but it was reported that only during the first two months of storage at room temperature no signs were observed of any deterioration (Okada et al., 1977). Therefore, also in this case cold storage is recommended to minimise changes and products should be kept on the shelf for as short a time as possible.

The storage recommendations for fresh and dried royal jelly are valid in the same way for all wet or dry products to which royal jelly has been added. Contrary to many recommendations on packages, these products should be stored in the same manner as the pure, fresh jelly.

In 1956, a French patent was granted for a method of stabilizing royal jelly by mixing it with an easily assimilable, adsorbent substance such as a carbohydrate or protein.

A homogenised paste of 10 g fresh royal jelly with 100 g of lactose, mixed at 0^0C was proposed by Jean *(1956).* However, no evaluation or verification of increased shelf-life is available. Such support substances, often sugars but also glycine are frequently used to increase the volume of single doses of freeze-dried royal jelly, to make handling easier for both packers (weighing of very small quantities is both difficult and imprecise) and customers.

Like all other bee products, royal jelly has its own microbiological protection and presents few microbiological storage problems when it is in its natural state. This protection however is not absolute and certain hygiene precautions must be observed during production (section 6.6) and storage. Hygienic working conditions and clean containers are a minimum requirement, and airtight containers should be used to provide additional protection not only against contamination but also against oxidation.

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6.8 Quality control

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Analytical techniques are sufficiently advanced to permit identification of pure, natural royal jelly and to reveal possible adulteration. They can also be used to determine the quantity of royal jelly used in combination with other products.

The analysis of royal jelly is generally based on the quantitative determination of the three principal categories of compounds (lipids, sugars and proteins), its water content and of other significant indices such as pH and total acidity. Lipids are the most important compounds in determining the authenticity or adulteration of royal jelly, since several of them are not found in any other natural products. The qualitative and quantitative analysis of the lipid fraction also makes it possible to determine the amount of royal jelly in a multi-component product (Pourtallier et al., 1990). Among the biologically active components, the vitamin content can give an indication of the corresponding (assumed biological) activity of royal jelly. The most important indicators and limits are presented in Table 6.5. For methods of analysis, the respective publications should be consulted. Apparently, there are no legally

established standards or international agreements. Nakamura (1985) reports the standards required by the Japanese "Fair Competition" regulations and approved by the Fair Trade Commission of Japan (see Table 6.5).

In addition to scientific analysis, there are some simple tests that can be used to indicate whether royal jelly is of good quality. Royal jelly generally darkens with age due to oxidation, although some fresh royal jellies may already be quite dark. Experience makes it possible to distinguish the appearance, smell and taste of a well-preserved or fresh royal jelly from one that is neither. Other simple tests are listed below.

The appearance of a solution and the presence of exuviae (larval skin fragments):

1 g of royal jelly is diluted in approximately 20 ml of distilled water. An opalescent solution with suspended material results (Nakamura, 1985). Then a concentrated solution of caustic soda is added drop by drop until the solution becomes clear. The alkaline solution thus obtained is (more or less) dark yellow green, more rarely yellowish pink or pink (Chauvin and Louveaux, 1956). Fragments can be seen suspended in the liquid which may be decanted and filtered. Under a microscope, the filtered residues should be identifiable as larval exuviae or exuviae fragments.

Table 6.5

Quality control methods and proposed limits for pure, natural royal jelly

	Pourtallier et al., 1970		Pourtallier et al., 1990		Lercker, et al., 1
Compounds	Methods	Limits	Methods	Limits	Methods
Water content, %	Freeze drying	60 - 70	Freeze drying	64 - 68	Freeze drying
Lipids, % dry weight basis	Selective extraction with methanol	12 - 18	Selective extraction with ethyl ether, followed by qual. & quant. GC of fatty acids	9-12.5	Qual. & quant. HRGC o fatty acids
10-hydroxy-2-decanoic acid					as above, in % of free ac
10-hydroxydecanoic acid					as above, in % of free ac
Proteins, % of dry weight	Selective extraction with methanol	35 - 45	Selective extraction with methanol	36 - 42	Total N, automatic Kjeld method
Sugars, % of dry weight	Titration of reducing sugars	20 - 33	Qual. & quant. gas chromatography (GC)	38 - 43	Qual. & quant. HRGC
Fructose, % of total sugars					as above
Glucose, % of total sugars					as above
Sucrose, % of total sugars					as above
Total acid, meq %	Titration	110 - 150			

pH	in aqueous alcohol solution of 0.4 %	4 - 4.2			
Riboflavine µg/g					
Thiamin µg/g					
Niacin µg/g					
Folic acid µg/g					

GC = Gas chromatography HRGC = High resolution gas chromatography

Boiling test

Royal jelly boiled with a small piece of potassium hydroxide will emit the smell of ammonia.

Mercury chloride reagent test

A white sediment is formed when the mercury chloride reagent solution is added.

Iodine solution test

A red-brown sediment is formed when the iodine solution is added (Nakamura, 1985).

Pollen analysis

Microscopic analysis of the pollen content can be used to determine the origin of the royal jelly. This is a simple procedure, but it requires a great deal of experience in determining the pollen species and interpreting the results (Chauvin and Louveaux, 1956 and Ricciardelli D'Albore and Bernardini, 1978).

6.9 Caution

No toxic effects have been observed in royal jelly for external use, as food or for injection. Allergic reactions however, as a result of contact or injection, may occur. As with all other potential allergenic substances, small quantities should be tried for a few days before using full doses. In case of allergic reactions, its use should be suspended immediately.

Since none of the claimed therapeutic or other effects of royal jelly have been proven with certainty, any advertising or package labelling should, for legal as well as ethical reasons, be truthful and should not raise unjustified consumer expectations. In the long-term this will improve consumer confidence and ultimately, sales.

From the production and organizational point of view, the temperatures to be maintained during storage are the most restricting factor. It is therefore essential that production and marketing are extremely well-planned and appropriate storage facilities are available at the producer, distributor and retail level.

6.10 Market outlook

No official market statistics are available, only estimates (Nardi, 1986). China is unanimously recognized as the world's largest producer and exporter of royal jelly. Its estimated annual production is in the order of 400 to 500 tons, nearly all exported to Japan, Europe and the USA. China accounts for approximately 60% of world production. Other countries in the Far East (Korea, Taiwan and Japan) are also important producers and/or exporters. In the rest of the world, royal jelly is produced mainly in Eastern Europe and,

At the time of writing (April 1993) the international wholesale price of royal jelly, based on that of China, the largest supplier, was US$ 50-80 per kg. Local prices in different countries can still vary considerably and be much higher (the price in Argentina in 1992 varied between US$ 100 and 180/kg). Comparing these figures to the one reported by Inoue and Inoue almost 30 years ago (1964, US$ 180 to 400 per

kg, in various countries) there has clearly been an enormous drop in price in real terms. Even without international competition, the decline in price was already obvious by the late 1950's in countries where the use of royal jelly started. The greater availability worldwide (particularly due to increasing Asian production) and the fact that the properties of royal jelly have not yet been determined conclusively, are probably the two main reasons for this drop in price.

In its processed form as tablets, capsules or vials, the equivalent of 1 kg of royal jelly may cost the consumer of some products as much as US$ 3,300. The price margin is similar to that of dried and processed pollen.

Japan has probably the highest domestic consumption of royal jelly (180 tons, Inoue, 1986) a large part of which is imported from other Asian countries. Outside Asia, the main markets for royal jelly are in the European and North American cosmetics industry and to a lesser extent, in the health food market. If therapeutic and other beneficial properties of royal jelly can be established scientifically, this market for royal jelly products (see Figure 6.7) with all its "value added", has the potential to explode.

Figure 6.7 : A variety of products containing royal jelly (from left to right): freeze-dried royal jelly with separate solvent in individual dosages, soap, individual liquid dosages, yoghurt, night and day cream, fresh royal jelly and shampoo with royal jelly.

The Asian market is potentially very large and with proper marketing should have tremendous value. In Asia, consumer preferences and traditions differ from those prevailing in Europe and North America and have facilitated marketing and increased production. Local cosmetic industries in particular, have very great potential for growth once quality and marketing (most of all packaging) approach the levels of Western competitors. The use of royal jelly in cosmetics has led to some very successful products. In one case (in Thailand) a business originally based on cosmetics with royal jelly and other bee products was so successful that it grew into a multimillion dollar enterprise.

While these successful companies became large operations, there is still plenty of room for small, local businesses (beauty parlours, vendors, pharmacies and others) to formulate articles containing bee products and in particular, royal jelly. These need to be adapted and selected according to local consumer preferences and customs. The need for high quality packaging and intelligent marketing, cannot be over-emphasised.

To conclude, a statement by Inoue and Inoue (1964) which unfortunately is still valid after 30 years, can be quoted: "We believe that the demand for royal jelly will increase again if, and only if, a reliable therapeutic value for humans can be established by further scientific research, and as a result official recognition is obtained from the Ministry of Health". The same might be said for its "added value" products.

6.11 Recipes

The proportions of royal jelly in a dietary product are usually adjusted to provide a dose equivalent to 200 to 300 mg fresh weight of royal jelly. Preparations such as soft gel capsules (also called gelatin drops or pearls) and those with freeze-dried granules (juice concentrates) which require higher and more expensive technologies, are not usually manufactured by small enterprises, but hired out to large companies specializing in this kind of work.

While the composition of the products can be varied and different formulations be tested, selected formulas need to be precise to allow consistent product quality between batches and the correct product consistency, where this is required.

The larger the production grows, the more important become hygiene, quality control, storage capacity and quick distribution and sale. Processes and ingredients may have to be adjusted slightly to accommodate larger scale production. Care should be taken however, not to alter or destroy the natural characteristics of the raw materials.

Certain types of packaging such as some automatic-mixing vials, blister packages for pills and capsules, and plastic and metal foil lined cartons or papers also require more expensive technology, but alternatives can be employed. For all preparations, the final presentation is very important. Unfortunately, presentation has sometimes become more important than the quality of the packaged product.

6.11.1 Freeze-dried (lyouhilised) royal iellvy

Freeze-dried royal jelly is a very hygroscopic powder. It is obtained by evaporating the water content from the frozen product in a vacuum. This is the drying process which best maintains the original characteristics of the product: it retains the volatile components which would be removed by evaporation at higher temperatures and does not damage nor denature the thermolabile components.

Freeze-drying requires special equipment, ranging from a simple laboratory freezedrier (see Figure 6.8) to large industrial plants (see Figure 6.9). Though the small laboratory models are normally used for analysis only, small volumes of royal jelly can be processed adequately with this size of equipment. Prices range from approximately US$ 10,000 for thc smallest drier system to several hundred thousand dollars for larger, industrial systems.

For drying, the royal jelly is first diluted with some clean water. This leads to a more regular and complete loss of water, particularly if large quantities are freeze dried in one batch. No such preparation is necessary if royal jelly is dried directly in the sales vial. During the final drying phase, in order to achieve more complete removal of residual water, the substrate can be warmed very slightly, but never above 35 ^{0}C.

Figure 6.8 : 4.5 Benchtop freeze-drier system (Courtesy of Labconco, advertised through Cole Parmer Instrument Company).

Figure 6.9: Industrial size freeze-drier in room with controlled environment (Courtesy of Ghimas SpA).

After freeze-drying, the royal jelly becomes extremely hygroscopic and must be protected from the humidity of the environment by storage in an airtight container. Larger processors handle freeze-dried royal jelly only in controlled atmospheres, i.e. air conditioned rooms with very low humidity. Depending on the final use of the dried royal jelly, a carrier base or stabilizer is added at this point, as described in section 6.7. This reduces the hygroscopicity of the dried product.

Freeze-dried royal jelly marketed directly to the consumer is usually presented in separate vials one or more for a liquid solvent and others containing the dry phase.

This is the best solution for conservation without chemical preservatives. The liquid phase can be pasteurized and packed aseptically, without damaging the heat sensitive royal jelly (see also Figure 6.4).

Ingredients for one dose:

Liquid phase (6-10 ml)	***Dry phase***
5-8 g honey	*170 mg freeze-dried royal jelly*
q.s. water to fill vial	*130 mg glycine or other stabilizing support*

A typical package contains 10 glass vials with the sterilized water-honey solution. The dry phase is packed in separate, metal or gel capsules, which themselves are often packed in individual glass vials. If necessary, the proportion of stabilizing support can be increased to reach a volume or weight which is easier to process.

6.11.2 Honey with royal jelly

For this type of product both liquid and fast crystallizing honeys can be used. Preparation of creamed honeys with royal jelly is described in Chapter 2. If the moisture content of the honey is sufficiently low (<16%) there is no visible alteration even when the product is stored at room temperature, but there are no data available on the stability of the royal jelly components and in any case, consumers should be advised to store the mixture in a refrigerator (Contessi, 1990).

The honey must have a very low moisture content, since the added moisture of royal jelly (0.6 to 0.7 g of water per gram of royal jelly added) could cause the honey to ferment. If, for example, 3 % of royal jelly is mixed with the honey an additional 2% of moisture is added. To avoid such a problem, freeze-dried royal jelly could be used instead. Moreover, in honeys that are not dense, e.g. those with a higher moisture content, the royal jelly tends to separate from the honey and rise to the surface. The honey and royal jelly mix can be packaged in the same way as pure honey, since it has the same physical characteristics, but it is preferable to package it so as to differentiate it visually from pure honey (in a glass jar or bottle of different colour or shape, or in a tube or straw with an additional carton etc).

To prepare the mixture, the procedure described in section 5.16.4 is used, i.e. the royal jelly is blended into a small amount of honey and this pre-mix is then stirred

into the rest of the honey. Royal jelly may be added to creamed honey before crystallization.

Similar honey-based products can be prepared by adding other products of the hive (pollen and/or propolis extract). In these cases, physically stable products are obtained only when crystallized (creamed) honey is used.

6.11.3 Yoghurt with royal lelly

Yoghurt, like royal jelly, has a low pH and requires cold storage, so a minimum of problems are encountered in storing and selling mixtures. A commonly used mixture is 2 g of royal jelly per kilogramme of yogurt, so that in a standard 125 g jar (one serving) there are 250 mg of royal jelly. Royal jelly is added to the yoghurt after fermentation and is thoroughly blended by homogenization. Except for industrial homogenizers, homogenization is best achieved by making a small pre-mix, followed by final blending of thc pre-mix with the whole batch.

6.11.4 Jellies and soft caramels

Ingredients (in parts by weight):

20-25	*Water*
up to 75	*Sucrose, glucose, honey or fruit purees*
1-1.5	*Pectin*
1	*Royal jelly*
q.s.	*citric acid, natural aromas*

The pectin should be dissolved in cold water before boiling it (see also sections 2.12.13 and 2.12.18). The ratio between sugars and honey can be varied, according to cost, flavour or other considerations. The total water content ranges between 20 to 25% and the quantity of pectin or other gum determines the final consistency. To the above base recipe, a number of other, aromatic agents can be added, such as fruit puree, essential oils and plant extracts.

These gelatinous caramels can be produced manually by pouring the solidijying jelly onto a flat table or metal tray or into moulds of different shapes. The royal jelly should be added just prior to the pouring at a temperature as low as possible.

Once cooled and semi-hardened, small cubes are cut out and covered with fine sugar crystals or powdered (icing) sugar. The cubes are then individually heat-sealed into clear plastic bags or packed in clear plastic boxes and labelled. Similar formulas are marketed by various producers.

6.11.5 Liquid preparations

The following four products were selected as examples because of their form of marketing, as well as their distinct, but typical formulation. Packaging is often in small (single) doses, which is fairly expensive and may require special bottling equipment. Separation of the dry and liquid phases is partly for better conservation of the active ingredients, but probably just as important, it makes for special consumer appeal. Presenting it in this new form as if it were a medicine and requiring the consumer to actively participate by "mixing his/her own preparation" creates an important appeal for some markets and adds to ever increasing product diversity in what has become a highly competitive market.

Even considering the expensive packaging this is a very popular and profitable form of marketing royal jelly. Since these products only form a very small market, very little official quality control is exercised and consumer confidence is easily misused.

Frequently, though not stated in formulations or ingredient lists, preservatives such as ascorbic acid or alcohol are added. The liquid phase always presents a preservation problem.

1) Ingredients for one dose:

300 mg *royal jelly (fresh)*

Honey and water to fill a 50 ml vial

A typical package contains ten 10 ml dark glass vials; each vial contains one dose. This formulation is not very stable unless all the ingredients have been pasteurized. Heating would however destroy much of the assumed beneficial activity of royal jelly. Ascorbic acid is frequently added for a more extended but still limited shei{life.

2) Ingredients for one dose:

Liquid *Dry phase*

phase		
200-300	*mg royal jelly (fresh)*	*120 mg micro-encapsulated cod liver oil*
3.3 g	*Acacia honey*	
6.7 g	*Fructose*	
q.s.	*Vanilla essence*	
q.s.	*Citric acid (as preservative) water to fill 10 ml*	

Liquid and dry phases are maintained separately until use. The cod liver oil is contained in a special capsule from which it is released at the moment of use.

3) Ingredients for one dose:

4.0 g	*Honey*
0.5 g	*Ginseng extract*
0.3 g	*Royal jelly (fresh)*
q.s. to 10 ml	*Water (boiled or distilled)*

A typical package contains 10-12 heat-sealed glass vials (see Figure 6.10). The top of a vial is easily broken off and small straws are provided to drink the liquid directly from the vial. Other types of sterile seals can be employed to make use of cheaper and more common bottling equipment. Preservation is a particularly difficult problem, as the liquid should not be sterilized by heat. Chemical preservatives are needed. The alcohol in the ginseng extract is often sufficient.

4) Ingredients (in parts by weight):

40	*Honey*

10	*Royal jelly (fresh)*
q.s. to 100	*Water*

This product is fermented like mead, but the fermentation is stopped at a low alcohol content. Royal jelly is added after the fermentation. It is marketed as a special type of mead and bottled in dark, multi-dose bottles of 250 ml capacity.

Figure 6.11: Individual doses of a liquid formulation (3) presented in heat sealed glass vials and attractively packaged. The vial top can be broken off easily and

6.11.6 Dried juice concentrate

Ingredients for one dose:

fructose

dried fruit juice powder
0.17 g freeze-dried royal jelly (equivalent to 0.5 g of fresh jelly)

A dried fruit juice powder, fructose (to taste) and the dry royal jelly are mixed. The dry powder is packed in plastic and aluminum lined paper envelopes in individual doses of approximately 4 g for one glass of reconstituted fruit juice. The production of good quality dried fruit juices requires expensive equipment. Pre-manufactured powders made from many different fruits may be purchased and enriched thus requiring only packaging equipment.

6.11.7 Tablets

Ingredients (in parts by weight) modified after Karaali et al., (1988):

10	*Freeze-dried royal jelly*
30	*Mannitol*
5	*Lactose*
8	*Gum arabic (binding agent)*

2	*Magnesium stearate (binding agent)*
1.5	*Sodium citrate (preservative and flavouring)*
q.s.	*Food dyes and other flavours*

A single tablet might contain 565 to 580 mg of ingredients, i.e. 100 mg of royal jelly.

Mannitol and lactose can be replaced by other powdered sugars. Glycine and the binding agents can be substituted with Agar Agar, pectin, gelatin, various gums, or beeswax. The sodium citrate can be replaced by citric acid. Flavours and dyes can be permitted natural plant extracts. Liquids (including water) should be added sparsely to obtain a thick gel, or an almost dry mass if the tablets are to be pressed. As with encapsulated formulations, freeze-dried royal jelly can be added to many herbal formulas.

6.11.8 Capsules

All the ingredients must be dry and in the form of a fine powder. They must be thoroughly mixed - the last ingredient to be added should be the royal jelly. Mixing and the final filling of the capsules should ideally take place in a room with very low humidity. For small quantities, a plastic bag provides a controlled atmosphere and can be shaken sufficiently. There are small electric ball mixers available which are well suited for medium to commercial quantities.

Final encapsulation into hard gelatin capsules can be done manually or with machines of varying capacities (see also 3.11.8). Dry powders are easiest to fill, but moist pastes such as those prepared with honey, can also be filled into capsules.

Formulations for soft gel capsules require oil based extracts, mixtures and expensive technology and are outside the scope of this bulletin.

Some possible powder mixes are (weights and proportions are only guidelines since no exact dosages are required):

1) Ingredients (in parts by weight):

1 *Freeze-dried royal jelly*

2-4 Pulverized glucose, fructose or lactose. Be-collected pollen or dried propolis extract can be used to partially replace the sugars

2) Ingredients (in parts by weight):

6 Gingko biloba leaves

4 Ground Kawakawa root

2 Melilotus tips

8 Oyster shell powder, ultra fine

6 Freeze-dried royal jelly

All need to be pulverized (dry powders), mixed and encapsulated, 300-350 mg per capsule.

3) Ingredients (in parts by weight - all dried):

7 Gingko biloba leaves

3 Carrots

3 Rosehips

1.5 Ginseng root

as ultrafine powders:

7 Selenium yeast

4 Wheat germ

3 Freeze-dried royal jelly

Again, all need to be mixed well before encapsulation. Exact proportions are not important for product consistency, but ingredient choice and quantities should be based on herbal characteristics. Other herbal formulations may be enriched with

royal jelly and/or pollen, propolis etc. However, preparations with herbal extracts or herbal powders should be handled with caution and mixtures should only be designed by people with sufficient experience in herbal medicines.

6.11.9 Cosmetics

Royal jelly can be easily added to any creams or lotions, usually at a concentration of 0.1 to 1 % fresh or 0.03 to 0.3% freeze-dried royal jelly. The formulations generally do not have to be changed and thus any agreeable recipe can be adapted. Since royal jelly is already an emulsion, it can also be added to any existing cream providing the cream is not solely oil-based. Mix the royal jelly with a small quantity of the cream first and then add this mixture to the rest. For detailed recipes see Chapter 9.

[2] This chapter was written in Italian by Dr. Lucia Piana, translated by Lorenza Manzi and edited by R. Krell

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CHAPTER 7
VENOM

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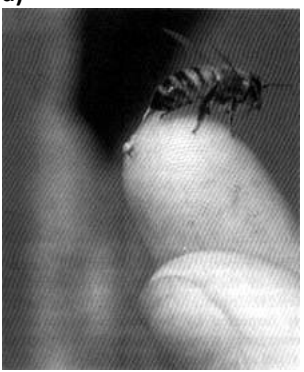
7.1 Introduction

Among the many species of insects, only very few have the capability of defending themselves with a sting and venom injection during stinging. All insects that can sting are members of the order Hymenoptera, which includes ants, wasps and bees. Since the sting is believed to have evolved from the egg-laying apparatus of the ancestral, hymenopteran species, only females can sting. The sting is always at or near the abdominal end, rather than the head. Therefore the pain inflicted by a honeybee, defending its colony, is not caused by a bite, as is frequently said, but by a sting.

There are many other poisonous insects which secrete venom. They usually cover their body with it, spray it, create wounds and secrete it into the wound, or inject it via mouthparts or a sting. In some cases, the venom is used for defense of the

individual or, in the case of social insects, the colony. But venom is also used in killing prey (as with some wasps or spiders) or for immobilizing and preserving prey (for their own or their developing offspring's consumption).

a)



b)

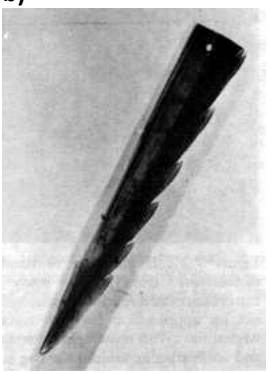


Figure 7.1 : A honeybee worker, stinging the relatively tough human skin, is unable to withdraw its sting lancets because of the

fine barbs (a) unique to the honeybee sting. Once stung by a honeybee, the whole sting apparatus, venom sack and all, almost always remains (b). This occurs only with honeybees and with no other stinging insect.

Honeybee venom is produced by two glands associated with the sting apparatus of worker bees. Its production increases during the first two weeks of the adult worker's life and reaches a maximum when the worker bee becomes involved in hive defence and foraging. It diminishes as the bee gets older. The queen bee's production of venom is highest on emergence, probably because it must be prepared for immediate battles with other queens.

When a bee stings, it does not normally inject all of the 0.15 to 0.3 mg of venom held in a full venom sac (Schumacher et al., 1989 and Crane 1990, respectively). Only when it stings an animal with skin as tough as ours will it lose its sting - and with it the whole sting apparatus, including the venom sac, muscles and the nerve centre (see Figure 7.1 and 7.2). These nerves and muscles however keep injecting venom for a while, or until the venom sac is empty. The loss of such a considerable portion of its body is almost always fatal to the bee.

Figure 7.2 : If disturbed or handled improperly most colonies will defend themselves. Honeybees in many parts of the world are very sensitive to disturbances and react *en masse* to defend their nest, as this innocent dog found out on approaching beehives recently inspected by beekeepers in northern Argentina. With some help from an emergency sting kit (epinephrine injection and antihistamine tablets) the dog survived more than 1000 bee stings.

The median lethal dose (LD_{50}) for an adult human is 2.8 mg of venom per kg of body weight, i.e. a person weighing 60 kg has a *50%* chance of surviving injections totalling 168 mg of bee venom (Schumacher et al., 1989). Assuming each bee injects all its venom and no stings are quickly removed at a maximum of 0.3 mg venom per sting, 600 stings could well be lethal for such a person. For a child weighing 10 kg, as little as 90 stings could be fatal. Therefore, quick removal of the stings is important. However, most human deaths result from one or few bee stings due to allergic

reactions, heart failure or suffocation from swelling around the neck or the mouth.

Used in small doses however, bee venom can be of benefit in trcating a large number of ailments. This therapeutic value was already known to many ancient civilizations. Today, the only uses of bee venom are in human and veterinary medicine.

7.2 Physical characteristics of venom

Honeybee venom is a clear, odourless, watery liquid. When coming into contact with mucous membranes or eyes, it causes considerable burning and irritation. Dried venom takes on a light yellow colour and some commercial preparations are brown, thought to be due to oxidation of some of the venom proteins. Venom contains a number of very volatile compounds which are easily lost during collection.

7.3 The composition of venom

A large number of studies have been carried out on the composition of honeybee venom. Much of the basic identification of compounds, their isolation and the study

of their pharmacological effects was done in the 1950's and 1960's. There are some comprehensive summaries in Piek (1986) which cover the morphology of the venom apparatus, the collection of venom, the pharmacological effects of bee venom and allergies to the Hymenoptera venom of bees, wasps and ants.

88% of venom is water. The glucose, fructose and phospholipid contents of venom are similar to those in bee's blood (Crane, 1990). At least 18 pharmacologically active components have been described, including various enzymes, peptides and amines. Table 7.1 lists the major components as summarized from Dotimas and Hider (1987) and Shipolini (1984). No further discussion of the detailed chemistry and various effects of individual components will be attempted here. Schmidt (1992) presents a comprehensive account of allergies to honeybee and other Hymenoptera venoms. Crane (1990), Dotimas and Hider (1987) and Banks and Shipolini (1986) give a very good overview of its composition, effects, harvesting and use.

Venom from other Apis species is similar, but even the venoms from the various races within each species are slightly different from each other. The toxicity of Apis cerana venom has been reported to be twice as high as that of A. mellifera (Benton and Morse, 1968).

Table 7.1:
Composition of venom from honeybee worker

Class of molecules	Component	% of dry venom[a]	% of dry venom[b]
Enzymes	Phospholipase A_2	10-12	10-12
	Hyaluronidase	1-3	1.5-2.0
	Acid Phosphomonoesterase		1.0
	Lysophospholipase		1.0
	α -glucosidase		0.6
Other proteins and peptides	Melittin	50	40-50
	Pamine	1-3	3

	Mast Cell Degranulating Peptide (MCD)	1-2 0.5-2.0	2 0.5
	Secapin	1-2	1.4
	Procamine		1.0
	Adolapin	0.1	0.8
	Protease inhibitor	13-15	0.1
	Tertiapin[C]		
	Small peptides (with less than 5 amino acids)		
Physiologically active amines	Histamine	0.5-2.0	0.5-1.6
	Dopamine	0.2-1.0	0.13-1.0

	Noradrenaline	0.1-0.5	0.1-0.7
Amino Acids	τ -aminobutyric acid α -amino acids	0.5 1	0.4
Sugars	Glucose & fructose	2	
Phospholipids		5	
Volatile compounds		4-8	

Dotimas and Hider, 1987; [b] Shipolini, 1984
This peptide may not be present in all venom samples

7.4 The physiological effects of venom

7.4.1 Unconfirmed circumstantial evidence

Bee venom has long been used in traditional medicine for the treatment of various

kinds of rheumatism. Although venoms of the different honeybee species differ slightly, there have been reports of successful rheumatism treatment with Apis dorsata venom by Sharma and Singh (1983) and with A. cerana venom by Krell (1992, unpublished).

The list of benefits to human beings as well as to animals is very long. Most of the reports of cures are of individual cases, though several unrelated patients have experienced the improvement or cure of similar ailments. Bee venom treatments are often accompanied by changes in life style, nutrition or other which may account for part, if not most of the benefits from treatments. Reported clinical tests were often conducted in countries with less rigorous methods than the standard Western, double-blind placebo tests. Despite these considerations, many patients did report positive results and many of the successful treatments occurred after established medical or surgical procedures had failed. However, there is a very real resistance in Western medical circles either to accept these results or to test bee venom treatments according to Western medical standards.

The diseases and problems which have been reported by patients or doctors as improved or healed with bee venom therapy are listed below (Table 7.2). This does not constituent an endorsement or recommendation for the treatments. Stinging

should never be tried unless there is immediate access to emergency treatment in case of an allergic reaction.

Table 7.2 List of diseases and health problems improved or healed according to anecdotal reports

Humans		
Arthritis, many typesa	Multiple sclerosisa	Premenstrual syndromea
Epilepsy[a]	Bursitis[a]	Ligament injuries[a]
Mastis[a]	Some types of cancer[a]	Sore throat[a]
Chronic pain[a]	Migraine[b]	General immuno-stimulant
Decreases blood viscosity and	Dilates capillaries and arteries[b]	Decreases blood cholesterol level[b]

coagulability[b]	Rhinosinusitis[c]	Endoarteriosis[d]
Neruoses[b]	Polyneuritis[e]	Radicultitis[ef]
Therosclerosis[d]	Neuralgia[e]	Endoarthritis[e]
Infectious spondylitis[e]	Malaria[e]	Intercostal myalgia[f]
Infect. Polyarthritis[e]	Tropical ulcers[f]	Slowly healing wounds[f]
Myositis[f]	Cancer, temporary[f]	Keratoconjunctivitis[g]
Thrombophiletritis[f]	Iridocytis[g]	Asthma[h]
Iritis[g]		
Animals		

BeeWell, 1993, 1992; [b] Kel'man, 1960; [c] Fotin & Gel'medova, 1981; [d] Poryardin, 1960;
[e] Gaider, 1950; [f] Lavochev, et al., 1958; [g] Naum Iyorish, 1974; [h] Dutta, 1959.

7.4.2 Scientific evidence

During the last seven decades, over 1700 scientific publications on the composition and various effects of bee venom in animals and humans have been published. An overwhelming proportion comes from Eastern Europe and Asia. Most of them concentrate on demonstrating the site specific, physiological effects of individual components such as membrane destruction, toxicity, or the stimulation or blocking of enzyme reactions. This has largely increased our understanding of the processes occurring after a sting, the physiological effects of isolated venom compounds and the substances responsible for most of the allergic reactions. It has contributed little to verifying the increasing claims of different therapeutic values attributed to honeybee venom, however.

A study with whole bee venom on dogs (Vick and Brooks, 1972) and rats (Dunn,

1984) showed that melittin and apamine produce increased plasma cortisol. Together with various other arguments, this suggests that many of the curative effects of bee venom may work through stimulation of the body's enzyme and immune system, in a way similar to the common drug cortisone. Cortisone has been used in the treatment of many ailments, but it is also known to have strong, undesirable side-effects. Melittin also appears to have toxic side effects as do some of the other individual compounds in venom. When whole venom is applied however, no side-effects have been shown, other than in allergic patients (Broadman, 1962 and Weeks, 1992 personal communication).

The anti-inflammatory effects of bee venom are perhaps the best studied and the various mechanisms have been repeatedly described in scientific literature (Rekkaand Kourounakis, 1990; Kim, 1989 and others). The neurotoxic venom compounds have shown a potential benefit for epileptic patients (Ziai, 1990). The protective value of bee venom and melittin against the lethal or damaging effects of x-rays has been investigated (Shipman and Cole, 1967 and Ginsberg et al., 1968). Though these and many other results are encouraging, no clinical studies have been carried out to verify the effectiveness using tests accepted by the Western medical establishment. Nevertheless, more and more physicians and healers are experimenting with this benign treatment after they have tested the patient's

allergic reactions to bee venom. Recently, after long efforts by the American Apitherapy Society and its members, some interest has been shown by national institutions in several Western European countries and the USA for clinical and large scale tests of bee venom therapy.

A good summary of the scientific studies, with further references can be found in Banks and Shipolini (1986) and Schmidt (1992). Summaries of some of the major specific effects of the various venom compounds that are shorter and more easily understood, can be found in Mraz (1983), Dotimas and Hider (1987), Crane (1990) and Schmidt and Buchmann (1992). The American Apitherapy Society keeps records of scientific as well as anecdotal information on the use of bee venom. It is also probably the best source of information on any subject related to apitherapy (see Annex 2).

7.5 The use of venom today

No uses for venom, other than medical ones are known to the author. The only legally accepted medical use of bee venom in Western European and North American countries is for desensitizing people who are hypersensitive (allergic) to bee venom. Since the early 1980's, pure bee venom has been used for

desensitization. The use of whole body extracts has been largely discontinued after a double-blind test proved the higher efficiency of pure venom (Hunt et al., 1978). In Eastern Europe and in many Asian countries bee venom has been used in official medical treatment of a large variety of ailments for a considerable length of time.

The use of pure venom injections and well placed bee stings is increasing in Western countries as an alternative to heavy (and soinetimes ineffective) drug use, which is often associated with numerous side-effects. This is particularly so for arthritis and other rheumatoid inflammations. A list of some other ailments for which successful treatments with bee stings have been reported has been given in section 7.4.1.

Application methods for venom include natural bee stings, subcutaneous injections, electrophoresis, ointments, inhalations and tablets (Sharma and Singh, 1983).

Since bee venom has both a local and a systemic effect, correct placement of injections, or stings and the dosage are very important. Therefore, bee venom therapy must be properly learned. Still, relief of some ailments can be obtained by simply applying a sting or two to the affected area, i.e. to some painful, immobile arthritic joints.

A society for api-acupuncture was formed in 1980 in Japan (see Annex 2). In the following years, many reports of experiences and successes in api-acupuncture appeared (in Japanese) in Honeybee Science (e.g. Ohta, 1983 and Sagawa, 1983). In the Republic of China, bee venom therapy is combined with a knowledge of acupuncture by many hospitals and physicians.

In the West, the American Apitherapy Society (AAS) is collecting case histories and information on bee venom therapy, together with medical uses of other bee products. There may be other national organizations, particularly in Eastern Europe and Asia. IBRA and Apimondia also have a wide collection of reference materials (see Annex 2).

7.6 Venom collection

Early collection methods required surgical removal of the venom gland or squeezing each individual bee until a droplet could be collected from the tip of the sting. Since the early 1960's, extraction by the electro-shock method has been continuously improved and is now standard procedure.

Different extraction or collection methods result in different compositions of the

final products Venom collected under water to avoid evaporation of very volatile compounds seems to yield the most potent venom (Pence, 1981). Venom collected from surgically removed venom sacs showed different protein contents from that collected with the electroshock method (Hsiang and Elliott, 1975). Gunnison (1966) used a cooling system with the standard electro-shock collecting apparatus in order to preserve more of the volatile compounds.

The standard electro-shock method (Morse and Benton, 1964a, b) cannot be recommended for venom collection from Africanized honeybees or the more defensive races in other parts of the world. Colony arousal can become so overwhelming that bees start killing each other and alert other colonies or attack the beekeeper and bystanders. The mass reaction of Africanized honeybees may also result in contamination of the collected venom. Nevertheless, venom is collected by this method in Brazil and Argentina, with only minor modifications.

Even European colonies remain disturbed for up to a week after collection (see Figure 7.5) and it is said by Mitev (1971) that colonies from which venom has been collected every three days produce 14% less honey. Morse and Benton (1964b) found no such evidence for reduced productivity, however. Galuszka (1972) found that when using electro-shock treatment, the most efficient collection cycle was

three 15-minute stimulations at intervals of three days, repeated after 2 - 3 weeks. An Argentinean beekeeper found that by modifying the electric stimulus, his collection efficiency greatly increased and the bees remained disturbed for less time.

The various trap designs stimulate bees by applying a mild electric shock through wires above the collecting tray. The most widely-used designs are modifications of the one first presented by Benton et al., (1963). A review by Mraz (1983) discusses further developments. The trays are placed either between the bottom board and brood chamber at the hive entrance (see Figure 7.3) or in a special box between supers and the hive cover, (Palmer, 1961, USA Patent 3,163,871, 1965, as cited by Crane, (1990).

a) **b)**

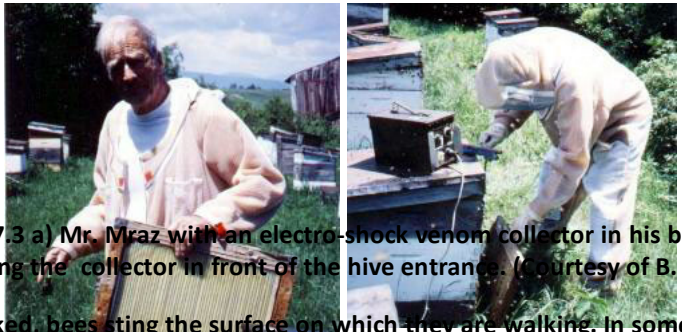


Figure 7.3 a) Mr. Mraz with an electro-shock venom collector in his beeyard. b) Placing the collector in front of the hive entrance. (Courtesy of B. Weeks)

When shocked, bees sting the surface on which they are walking. In some traps, this may be a glass plate or a thin (0.13 mm thick) plastic membrane, nylon taffeta or silicon rubber under which a collecting plate (preferably made of glass) or absorbent tissue receives the venom (see Figure 7.4). Venom dries rapidly on glass plates and can be scraped off with a razor blade or knife. Absorbent tissue is washed in distilled water to extract the venom, which then should be freeze-dried. Collection on glass is generally easier and produces a product which is easier to store, ship and process. During handling of dry bee venom, protective gloves, glasses and dust masks should be worn to avoid any contact with, or inhalation of the highly concentrated venom.

It is unlikely that a bee will eject all the contents of its venom sac, even after repeated stinging. Therefore, typically, only *0.5* to 1.0 jil (0.2 j�l - Crane, 1990) of venom can be collected per bee, with an average of ten stings per bee (Mu~ller, 1939 and O'Connor et al., 1967). This results in less than 0.1 ijg (0.11 jig - Crane, 1990) of dry venom per bee. Consequently, at least 1 million stings are required to make one gram of dry bee venom. Dotimas and Hider (1987) report that 1 g of venom can be collected from twenty hives over a two hour period.

Figure 7.4 : Close-up of collecting device with stings. The steel wires are approximately 6 mm apart and suspended 1 to 3 mm above the

thin silicon rubber film or directly above the glass plate in other models. The wires are alternately grounded and charged to a maximum of 33 volts. A lower voltage is effective, too. Preferably a collecting surface should be used which does not make bees loose their sting. (Courtesy of B. Weeks)

Instead of collecting bee venom, adult bees may be used to sting the patient directly. This is the way to apply the venom in its freshest, most complete and cheapest form. To collect the bees, a small hole is made in the brood chamber, super or inner cover. To avoid colony disturbance, the hole is opened and a collecting jar placed over it until a sufficient number of bees have come out. Small groups (10-100) of workers can be maintained at home for up to 2 weeks. They should be kept in the dark, in a small box (with one side made of fly-screen) and with access to sugar syrup. Care needs to be taken to keep ants away. Alternatively, bees can be collected from frames or the hive entrance by a suction device similar to

the one described in Figure 6.6. However, a screen should be placed over the tube leading to the mouthpiece to prevent any bees from reaching the mouth.

7.7 Venom products

Bee venom may be sold as whole bee extract, pure lidquid venom or an injectable solution, but in either form the market is extremely limited. Most venom is sold in a dry crystalline form.

Figure 7.5: Honeybees outside of the hive shortly after electro-shock treatments. The venom extraction board is still leaning over the hive entrance. (Courtesy of B. Weeks)

Since venom does not need to be processed, it can be prepared wherever bee venom therapy finds sufficient support. Production in small quantities is easy, as long as stringent sanitary controls and aseptic working conditions can be provided. The beekeeper has to work under extremely clean conditions, since most of the venom preparations will later be used for injections into humans or animals.

For injections, the venom can be mixed at the time of injection with injectable fluids, such as distilled (sterile) water, saline solutions and certain oils, or it may be taken from prepared ampoules. Ampoules with set doses of ready-to-inject venom should only be prepared by certified pharmaceutical laboratories, because of the need to maintain stringent aseptic conditions and to measure the dosages very precisely.

There are creams available which include bee venom (e.g. Forapin and Apicosan in Germany, Apivene in France and Immenin in Austria) which are used for external application on arthritic joints (BeeWell, 1993 and Sharma and Singh 1983) but neither the ingredients nor their proportions are known to the author. A general recipe for bee venom ointments is given in section 7.13.

Tablets can be impregnated with quantities of bee venom, but Sharma and Singh (1983) recommended the removal of toxic proteins, such as Melittin and the use of colours to indicate different dosages. The tablets should be placed under the tongue, but no indication is given to the effect or usefulness of such a preparation.

Some specialized laboratories may be able to separate and purify different venom compounds and sell them to scientific and pharmaceutical laboratories. Phospholipase A_2 and highly active peptides are among some of the proteins purified from bee venom for scientific suppliers or laboratories (Schmidt and Buchmann, 1992). Entry to this limited market requires a highly sophisticated laboratory and very well-trained technicians and chemists.

No further use or inclusion of venom in other products is presently known to the author.

Though not directly related, bee sting emergency kits can be sold in some countries, particularly to people who are allergic. They also should be at hand for any beekeeper working with Africanized honeybees and at training centres, police and fire departments, in areas with Africanized honeybees. In the USA, they are now available only against a prescription. Such a kit (e.g. Anakit by Hollister Stier, USA) should contain at least:

1) One syringe with a premeasured content of epinephrine (adrenaline) or atropine, for immediate intramuscular injection - usually 0. 3m1 of a diluted solution of epinephrine (1:1000) in saline solution. There are special, easy-to-use, syringes available from bee supply houses or through pharmacies, which can even be used through clothing (Epipen by Centre Laboratories, USA).

2. anti-histamine tablets.

3. tourniquet.

4. instructions about when, where and how to use the syringe and anti-histamine tablets; when not to use epinephrine, and where to seek medical help.

Epinephrine injections should be given only in extreme emergencies when no other medical help is available. The sting emergency kit has a limited shelf-life and should be kept refrigerated when not in use.

7.8 Buying

The best way to buy bee venom is in the crystallised form, since it is more stable, impurities are easier to detect and adulteration is less likely. The colour of both crystals and powder should be a very light yellow.

Liquid venom as mentioned in section 7.2 should be clear and colourless. Darker venom is slightly oxidized and may have lost some of its effectiveness.

As with all other products where immediate testing is not possible or is very expensive, the producer should be one who is well-known and who can be trusted to produce a high quality product. The producer as well as the buyer should have adequate storage facilities.

7.9 Storage

Even dried bee venom should be stored refrigerated or preferably frozen and it should always be kept in dark bottles in the dark. All producers and buyers should closely observe these conditions. Dried bee venom can be kept frozen for several months, but should not be kept refrigerated for more than a few weeks. Liquid venom and diluted venom can be stored for similar periods if maintained in well sealed, dark glass containers.

7.10 Quality control

There are no official quality standards, since bee venom is not recognized as an official drug or as a food. Purity analysis may be carried out by quantitative analyses of some of its more stable or more easily measured components such as melittin, dopamine, histamine, noradrenaline or those for which contamination is suspected.

A nematode, Panagrellus redivivus was reported to react selectively and specifically to bee venom and a quantitative analysis of the venom in pharmaceutical preparations was developed by Tumanov and Osipova (1966) using this organism.

Pence (1981) describes a method for testing the biological activity of bee venom by measuring electric pulses from muscles of excised honeybee abdomens in response

to the volatile materials from bee venom.

Guralnick et al., (1986) described standardization and quality control methods for purity and effectiveness of Hymenoptera venom, including honeybee venom.

7.11 Caution

Collecting bee venom requires careful work with the highest degree of cleanliness, since the venom will be injected directly without further processing or sterilization. Protection of the collector against the disturbed bees and highly irritative dry venom is very important, too. Since people up to several hundred meters away might get stung by the highly irritated bees, further precautions at the time of collection in the apiary must be considered.

When handling dry venom, laboratory gowns, gloves and face masks should be worn to avoid getting venom dust into the eyes and lungs. All equipment should be carefully washed afterwards. Contact between other people and contaminated material should be avoided People who do not regularly handle bees, who only get stung occasionally or are exposed occasionally to venom dust, run the risk of developing allergies.

Using bee stings for self-treatment of various diseases can be risky, because allcrgies to bee venom can be developed quickly even after long periods of use. An emergency kit (see section 7.7) or quick access to an emergency service should always be available. No other side-effects have been reported, but regular supervision, check-ups and controls should be continued with competent doctors trained in apitherapy.

Since severe allergic reactions are possible, bee venom should not be casually included in any health or medicinal products. Products containing bee venom need labels stating the contents and warning people of possible allergic reactions.

7.12 Market outlook

Bee venom is a highly specialized product with only very few buyers. The market volume is relatively small too, although there are no comprehensive surveys. The main venom producer in the USA has produced only about 3 kg of dry venom during the last 30 years (Mraz, 1982) but there is a large producer in Brazil and more or less significant amounts are produced in many other countries.

Prices in 1990 varied greatly between US$100 and US$200 per gram of dry venom

(Schmidt and Buchmann, 1992). Prepared for injections or sold in smaller quantities, prices can be much higher. However, the beekeeper often does not get this price. The prevailing prices in European and Asian markets are generally slightly lower.

Local manufacturing of the pure venom however, is relatively easy and within the means of many beekeepers; no expensive or high technology processing is required except refrigeration, but its economic feasibility depends on access to the few specialized buyers. In contrast, the venom in less controlled dosages is available almost everywhere, from a beekeeper or one's own hives, free or at very low cost. Often, the only price is the life of the bee.

Though the fractionation of venom goes beyond the means of a small local enterprise, several people working in the field feel that, with further research, there will be a small market niche for specialized laboratories. Since there are several pharmacologically interesting substances in bee venom and since apitherapy may become officially accepted in the future, a better market for the whole product or for special fractions might develop. However, much depends on the official acceptance of bee venom therapy.

7.13 Recipes

Ointments can be prepared by thoroughly homogenizing bee venom with white Vaseline, petrolatum or melted animal fat, and salicylic acid, in the ratio of 1:10:1. The salicylic acid softens the skin, increases its permeability and is a treatment for rheumatism even on its own. The ointment may contain a small amount of silicate crystals to act as an abrasive (Sharma and Singh, 1983).

Other preparations consist of mixing bee venom with sterile, injectable fluids and packaging them in single dosages in glass vials or syringes. In some packages the dry venom is kept separate from the fluid and the two are mixed when the vial is broken.

Techniques for separating the different compounds in venom are far beyond the scope of this book. Such information can be obtained from properly trained chemists.

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CHAPTER 8
ADULT AND LARVAL HONEYBEES

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8.1 Introduction

As adult honeybees are the producers of all the primary products of beekeeping, it is unlikely that a beekeeper will sell these adult bees when he or she is interested in production of primary products. Honeybees or their brood can however, constitute a primary product, and may be sold directly or be processed for other uses. Beekeepers can make a profit from selling their adult bees, often together with combs of larvae. Depending on market conditions, they can sell their bees in the form of package bees, nuclei or small starter hives and whole, full-size colomes

In many countries, bees are considered a nuisance when they nest in or near houses. This is particularly true when they are among the more defensive types. In such cases, beekeepers may be able to charge to remove the bees. If these bees are not used by the beekeeper to strengthen his own operation and were not killed with pesticides, they can be killed and fed to chicken or pigs. Otherwise, they can be composted. The same procedures are even easier with the brood frames of such colonies. Both adults and larvae are a good protein source.

In many African and Asian countries, brood combs are considered a delicacy and consumed immediately when available (see Figure 8.1). They are also particularly rich in protein since they usually contain quantities of beebread, i.e. the slightly fermented pollen stores of the hive. In some Asian countries, worker or drone pupae (in their white stage) are also prepared for human consumption by pickling or boiling. In canned form, they are found in some European or American specialty stores and can be considered a value added product, even if there is not much demand or a broad market perspective in the West.

8.2 The chemical composition of adult and larval honeybees

The chemical composition of mature and immature honeybees has not received as much attention as that of some other primary products. Only data with few details can consequently be presented (Table 8.1). The data for adult bees has been adapted in order to be comparable to the fresh weight data of immature bees. A 1 % glycogen content was estimated rather than the 9.08% sugar content found in the samples in the original analysis, which was probably due to honey in the bees' digestive tracts. On this basis, adults and immatures have very similar protein values. In adults, over 40% of the protein comes from the muscular tissue of the thorax, which is similar in protein to egg-white.

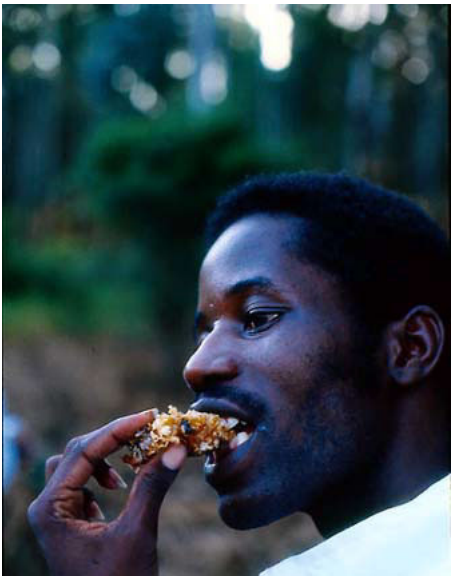

Figure 8.1 : Mr. Lusale, a Zambian beekeeping extension officer, demonstrating an alternative use for bee brood.

8.3 The uses of adult bees and larvae

8.3.1 For beekeeping

The major use of larval and adult bees is undoubtedly that made by the beekeeper for the production of primary bee products. While both can also be considered primary products, the production of complete colonies, starter colonies and packages of bees or queens, are usually not considered as beekeeping !?products[t] (see Figure 8.2). On the other hand, these activities can produce a considerable

amount of additional income, or constitute a whole line of business on their own. A growing beekeeping industry, or growing interest in beekeeping, usually creates a demand for these products.

Their production requires hardly any additional investment if operated on a small scale and profitable sales can be made even if sold one-by-one. However, in many village environments in particular, sales communication between customer and producer often needs to be facilitated by an organization or extension service. A description of how to produce queens, package bees, divide and build-up colonies etc. can be found in all good beekeeping textbooks and manuals. The interested reader is urged to consult these.

Table 8.1:
Composition of mature and immature honeybees compared to beef and soybeans (in % of fresh weight; vitamins in International Units per g fresh weight) modified from Crane, 1990.

	Honeybee			Beef	Soybean[d]

	Mature larvae	Pupae	Adult[a]		
Water	77.0	70.2	72.1	74.1	70.0
Ash	3.0	2.2		1.1	1.5
Protein	15.4	18.2	17.9	17.7b	12.9
Fat	3.7	2.4	2.8	2.8	5.9
Glycogen	0.4	0.8	1	0.1-0.7	2.4c
Vitamin A	107	51.3		0	
Vitamin D	6863	5165			
Chitin/fibre			4.1		1.7

[a] Data corrected for sugar/honey content of analyzed bees, from Ryan et al., 1983;

[b] Data from Krause an Mahan, 1979;

[c] Total sugars;

[d] Soybean data adapted from Smith and Circle, 1972.

8.3.2 For pollination

In the widest sense, one might consider the pollination benefit for agricultural crops provided with honeybee colonies as a value added product. Such benefits increase with more intensive cultivation and more progressive destruction of the natural environment. When planted in monocultures over large areas, crops that require pollination need managed populations of pollinators for any significant production of fruits or seeds (see Figure 8.3). Smaller areas of the same crop may not need the introduction of managed colonies. If they are still surrounded by natural flora, or if alternative floral sources are available to wild pollinators during most of the year. Selection of varieties, and cultural practices such as interplanting can reduce "artificial" pollination requirements for some crops.

Beekeepers in industrialized countries usually charge for pollination services, because they bring the farmer a significant increase in production, are more work for the beekeeper and usually do not produce a honey crop while supplying the

service. A detailed discussion of this subject - the different requirements in infrastructure, environment and agricultural practices - are discussed in another FAO publication (Roubik, 1994).

a)

b)

Figure 8.2 : (a) Packaged bees ready for shipment. (b) Caged, mated queen bee with attendant worker bees and sugar candy, ready for sale, shipment or introduction to a new colony.

Figure 8.3: Honeybee colonies, used for pollination, on the edge of a sunflower field.

8.3.3 As food

Adult and larval honeybees contain reasonable amounts of protein and are non-toxic (Table 8.1). They could therefore serve as a direct food source once the beekeeper has no more need for extra bees or brood, or when undesired colonies have to be removed. Honeybee brood of all ages is eagerly consumed by honey hunters in Africa and Asia and is generally considered a delicious treat. For several cultures, brood is said to form a considerable part of the diet (Hill et al., 1984 and Bailey, 1989; as cited in Schmidt and Buchmann, 1992). In China and Japan, drone larvae are canned for export or, after being covered in chocolate, become a sweet treat. Bee brood is regularly sold alongside honey in markets in many parts of Asia (Schmidt and Buchmann, 1992).

Whether fresh, boiled or fried, larvae have a rich nutty flavour. When fried, they maintain their shape and become nice and crunchy. Eating insects in general is considered normal in many cultures, while others have developed strong inhibitions to this practice.

Development time from egg-laying to the adult larvae is 8 to 9 days. If the larvae are harvested right after the cells are capped, they will have increased in weight approximately 1000-fold. The protein content will have increased only slightly less. This growth rate is not as rapid as that of some fly larvae, but is still much faster

than the growth rate of more traditional protein sources such as cattle or chicken. Many species of insect larvae are easier to grow, but of all the insects to eat, honeybees probably have the highest public appeal and are probably more acceptable than, for example fly larvae or crickets. While it is difficult to imagine that honeybee larvae will become a major source of protein, they are a special delicacy in some countries and may become so in others. Additionally, they can be a useful protein supplement in otherwise poor diets. Human consumption of adult honeybees is uncommon.

If a colony has to be killed, or the death of a colony is detected soon enough and is not due to pesticides, the fresh or dried bees may replace some of the regular feed for small mammals, birds, chickens (Witherell, *1975)* or pigs (Dietz et al., 1976). The author has heard testimonies that indicated both the presence and absence of benefits to poultry. In a similar way, unwanted bees removed from houses or swarm traps may be killed by overheating in a black plastic bag and be composted, or dried and powdered to feed to livestock. However, it is not economically feasible to grow bees for this purpose alone.

Mature drone larvae are in general the preferred choice, probably because of their larger size. In tests with bee larvae as a diet for insect rearing (Coccinellids), frozen

drone larvae appeared to provide a more complete diet than worker larvae (Okada, 1971). Bee larvae have been shown to be an excellent food source for rearing insects, particular various beetles and lacewings (Chrysopidae) used for biological pest control (Okada and Matsuka, 1973; Matsuka et al., 1982 and Hasegawa et al., 1983). All kinds of bee larvae were suitable for rearing songbirds (Gary et al., 1961; Guss, 1967 and Lanyon and Lanyon, 1969). The feeding of dried A. cerana larvae to queens of the same species seems to maintain egg-laying, though no long-term tests have been done (Gondal and Hashmi, 1976). Unfortunately, the data are not sufficient to make any deductions as to whether dried larvae are as nutritive or stimulative as royal jelly.

The greater wax moth (Galleria mellonella), though not a bee product, is a very common pest, little appreciated by any beekeeper. It is very easy to raise, however and its eggs can be readily obtained by any beekeeper. The larvae can be stored alive for over a year at 15 ^{0}C and 60% relative humidity. When deep fried in oil, the larvae burst and look more like popcorn than insects, which may help in marketing. Simple rearing instructions and a "popmoth" recipe are included in the recipe section.

8.3.4 As medicine

Italian psychiatrists observed improvements in respect to the appetite, body weight, hepatic activity, digestion and haemopholetic functions of 15 female psychiatric patients who were suffering from loss of weight and appetite (Monteverdi and Reitano, 1972).

No other references to any medical tests regarding the consumption or the application of whole larvae, adults or their extracts are known to the author. Whole-bee extracts have in the past been used to desensitize people allergic to bee stings, though with unreliable results. This practice has been discontinued since Hunt et al., (1978) reported that whole-body extracts are no more effective for desensitization than no treatment at all. Pure bee venom has now become the standard for immunization therapy. The production of bee venom from adult bees is covered in Chapter 7.

8.3.5 In cosmetics

During the 1950's, when royal jelly was a "fashionable" product, several patents were registered for the use of queen larvae in cosmetics. References on the subject can be found in section 9.5, but no such current use of such applications is known.

8.4 Collection

8.4.1 Adult bees

Adult bees can be collected regularly from colonies during the growing season by shaking bees off the brood frames into packages (see Figure 8.4). This practice is described in all major beekeeping books on Apis mellifera which have a section on package bee production. Whole businesses have been founded on the production of these packages for beekeepers, but they also need to have a queen rearing operation, since bees should not be shipped without a queen. In Canada, a cotton ball wetted with synthetic queen pheromones has recently been tried successfully as a substitute for a queen, but this method has not been tested extensively for commercial applications yet.

Package bee production is suitable for areas that have an early flowering season, i.e. earlier than in the major honey producing areas. Beekeepers have to be willing to pay for bees and queens and transport has to be safe and quick. The same holds true for production and sale of nucleus starter hives and whole colonies, except that the sale of these is not as dependant on early nectar flows. Either are feasible on a large to very small scale.

If a colony has to be removed from a house or other inaccessible place and is intended for consumption by either human beings or animals, the bees should be sprayed with a mist of plain water or sugar water so that they are easier to bag and cannot fly off. Normally, soapy water is used to achieve this effect, but the soap is difficult to rinse out prior to consumption. They should then be either frozen or overheated to kill them. For storage and further processing see section 8.6 and 8.10.

Figure 8.4 : Using a funnel to shake bees into packages in a North American apiary.

8.4.2 Honeybee larvae

The removal of drone larvae will have less affect on colony performance than the removal of worker larvae. Though highly seasonal, drone production can be initiated through feeding and queen selection, and may be promoted further by providing drone size comb or foundation to the colony. In areas where Varroa is controlled by trapping the parasite in drone cells and removing the freshly sealed

drone brood, the use of these otherwise discarded larvae may be considered.

Opened or unsealed cells can be shaken and larvae knocked out, but to avoid breaking the comb, it previously should have been reinforced by wiring. Older, dark-coloured combs should be selected. Ideally, most of the larvae should be of similar age. It is easier to use combs which have been sealed for only a few hours, but larvae should have finished pdupation. The cells are uncapped with a fine, serrated and preferably warmed knife, and the larvae and pupae shaken out onto a sheet of paper, aluminum foil, leaf or other clean surface (see Figure 8.5 to 8.8). If the brood need not be whole, a fork with very long, fine prongs (as also used for honey uncapping) can be used to uncap and retrieve the larvae. Since larvae defecate just before pupation, larvae and pupae should be washed in clean water before further processing. Pupae will have clean, empty intestines.

Another method (Schmidt and Buchmann, 1992) uses a small jet of water from a laboratory wash bottle to remove individual larvae from their cells. The author had reasonable success flooding one side of an uncapped comb. All the cells were filled with clean water, and then the larvae and pupae were shaken out (see Figure 8.8).

If combs are to be discarded after removal from a house or wild nest, the whole

comb may be squeezed or boiled. The latter works best with new combs, but cells should be uncapped prior to boiling. The melted wax will harden at the surface and larvae will sink to the bottom. Some larvae will still have to be removed from older combs and occasionally from cocoons. The flavour is affected by this method.

8.5 Buying

Before purchasing packaged bees, nuclei or full-size colonies, the buyer should first check for diseases, know the producer and/or require a health certificate, if appropriate inspection services are available. It is always risky to bring bees into new areas, no matter where they come from and how well they have been inspected. Importations of bees have spread all major diseases and may drastically change the resistance of local bees to indigenous varieties of disease organisms. Care should be taken that the full strength of the colony, or the number of bees paid for, is obtained.

When buying brood only, the buyer should make certain that live brood is obtained. The time between removal of brood from the colony and processing should be minimal, since unsealed brood away from the colony will soon die and larval protein will decompose very quickly. Brood should be eaten or processed (boiled,

fried or dried) immediately after harvesting. Combs must not be left in the sun under any circumstances.

For larval processing, a comb should contain newly sealed brood of a uniform age. Both larvae and pupae are consumed. Whether there are any preferences and significant nutritional differences, remains unknown. From Table 8.1, it appears that pupae might have a slightly higher protein content. Though no evaluations are known to the author, the highest quantitative nutritive value of larvae is likely to be just before and after metamorphosis into pupae, i.e. a few hours after sealing of the cell.

If processed larvae are bought, it should first be certified that processing was carried out properly under clean conditions, with fresh larvae. Larvae should preferably be dried without exposure to sunlight. Indirect solar drying can be used if the temperature does not exceed 90 ^{0}C. Heat lamps and infrared drying will have the same limitations, but lyophilization will have the least degenerative effect. Particularly if powdered larvae are purchased, adulteration needs to be checked.

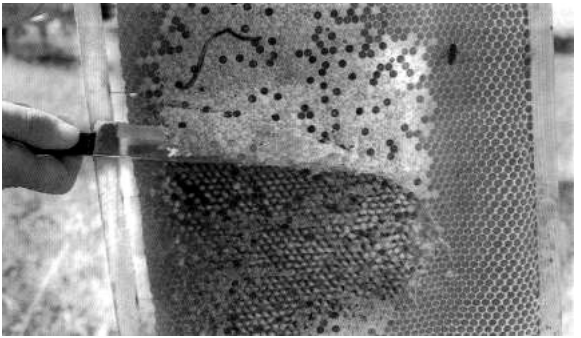

Figure 8.5 : Uncapping of recently sealed brood with a serrated knife. The comb is reinforced with wire but should be darker, i.e. older, to prevent breaking during shaking.

Figure 8.6: Uncapped comb of similarly aged larvae just prior to pupation. Larvae in slightly deformed cells are difficult to remove.

Figure 8.7 : Shaking out larvae on to a clean surface works best with a darkcoloured, wire reinforced comb.

8.6 Storage

Packages of live bees with a queen can be stored for several days more - and up to several weeks if stored with sufficient ventilation and food. In hot climates, bees need water and ventilation to stay cool. Overheating is a serious problem than exposure to cold temperatures. Bees should always have access to sugar syrup or honey. During transport, packages or colonies should not be left sitting in the sun for any amount of time. Transport at night is preferable where no other hazards exist.

Live brood should only be stored inside a hive. Sealed brood can also be maintained (kept alive) in a well regulated incubator, at a temperature of 32 to 35 ^{0}C (90 to

95 ^{0}F). Dead brood and bees need to be refrigerated immediately. All processing should be completed within 24 hours of killing and in hot and humid climates in less than 6 hours.

Larvae dried at 70 - *75* ^{0}C store well in sealed plastic bags at room temperature. Caramelization starts at higher drying temperatures. Drying under vacuum or reduced pressure may be advantageous. Deterioration is significant after 7 months of storage at room temperature, but storage deterioration over shorter periods has not been reported. Diets of dried, pulverized drone larvae performed well after storage for 7 months at either - 15~ or *5* ^{0}C and satisfactory after 7 years at *5* ^{0}C. Exposure to sunlight increased the rate of deterioration, as did heating to 120^{0}C (Sakai et al., 1978). Heating to 90^{0}C for 20 minutes had no noticeable deleterious effect, nor did y-radiation at a level of 2.5-3.5 x 106 rad (Sakai et al., 1978). This exposure kills many pathogens, including those of AFB. Fried or boiled larvae should be treated like other protein foods and should be consumed quickly, since even refrigerated they will keep only for a few days.

Figure 8.8: If brood cells are filled with water, most of the larvae can be dislodged much easier. This works even better with younger unsealed

Preservation methods other than freezing and drying include smoking, pickling and canning. Smoked larvae were found to spoil after a few days unless the larvae were smoked for at least 12 hours at 60 - 90 ^{0}C and 30% relative humidity (Hocking and Matsumura, 1960). Pickling in 15 % and 20% salt solutions was unsatisfactory, mainly because the brood floated in a compact mass on the surface where decomposition was quiteadvanced after three weeks. Freshly killed larvae were pickled satisfactorily in a mixture of malt vinegar, whole mixed spices and 1 % salt. Brandy (alcohol) pickling was very effective with a 1:1 mixture of brandy and brood, changing the brandy after a few days. According to Hocking and Matsumura (1960) neither of the pickling methods produced a product of acceptable flavour.

Some of the above preservation methods and recipes described below lend themselves to canning. Standard canning methods and precautions should be observed.

8.7 Quality control

Quality control of purchased live bees and colonies should follow the guidelines given in the buying section. Beekeepers should ensure bees are healthy with young fertile queens.

Since there are no specific quality standards for honeybee larvae, national or international standards for similar foods should be applied, such as those for canned, dried or pickled meats. Even chocolate covered larvae are probably better treated as meats than sweets, because of their high protein content. Local laws and food standards have to be observed or exceeded. Because of the high protein content and perishability of the larvae and bees, good hygiene and attention to proper processing and handling conditions are essential more so than for most other bee products.

8.8 Caution

The greatest threat to live bees and colonies are diseases and overheating, both of which have to be carefully avoided.

For direct consumption of brood or larvae, care should be taken that no whole bees (alive or dead) are accidentally eaten, since the sting of even a dead bee can release venom when chewed. For the same reason, particular care should be taken when handling freshly frozen bees. Dried adult bees may be pounded or ground to avoid similar problems with livestock. Once the adults have been boiled or fried, the venom is no longer active.

8.9 Market outlook

As mentioned earlier, packaged bee production can be a considerable income source for beekeepers, as can the sale of queens, nuclei/starter colonies and full size colonies. Which of the forms of adult bees are most marketable in a country depends very much on the type of bees and the kind of beekeeping practised.

Nuclei colonies require frame hive beekeeping in standard sized bee hives. Whole colonies instead, can be sold in all sorts of traditional bee hives but buying or selling packaged bees only makes good sense in more intensive, frame hive beekeeping. These conditions, in addition to beekeepers' attitudes and the profitability of beekeeping vary too much from country to country to allow any valid generalizations. Markets, however can be tested easily since small scale sales and production do not require any additional investments.

For the consumption of larval and adult honeybees as food, specialized markets may be accessible where, for example, ethnic communities might consume such foods. Good tasting snacks can be prepared, packaged and sold where no prejudice exists against the consumption of insect larvae. For example, deep fried, salted or sweetened larvae can be packaged as special snacks and larvae flour can be used to

enrich wheat flours, but local marketing will be very limited in size and external markets extremely difficult to reach and develop. The People's Republic of China, Taiwan and Japan have small local markets and there may be some trade between these countries (Crane, 1990). Cans of chocolate-covered honeybee drone larvae may be seen in some specialty Asian food stores in Europe and the USA, but according to recent enquiries they are rather difficult to find.

The sale of fresh combs with brood for consumption may be possible in some areas. Broken combs with brood and some pollen bathed in honey could be sold as a very nutritious snack in some local markets. The problem is that the removal of brood combs during honey harvest is destructive and can therefore adversely affect other aspects of beekeeping.

8.10 Recipes

Honeybee larvae or many other insect larvae can be grown cleanly and easily to enrich staple foods with protein. Many types of insect larvae are eaten in the world and most of them can substitute for honeybee larvae in the following recipes.

8.10.1 Preparation of mature and immature bees for human consumption

One way to kill adults or larvae is by freezing them, but if a large quantity of adult bees are placed in a freezer, many of them may still be alive after several days. Bees are much more sensitive to overheating than to cooling and when placed in the sun inside a plastic bag, will die within a few minutes. However, they must be removed from the sun as soon as they are dead since decay will quickly occur. Larvae should be kept alive as long as possible. Once dead, both larvae and adults need to be processed or eaten immediately (see also section 8.6).

After killing, and particularly if they have been killed by overheating, bees should be rinsed in cool, clean water. Once rinsed, they need to be patted dry and either be frozen, cooked or dried. Even when dead, adult bees can still sting and their venom remains active so that during washing and subsequent operations, the sting may penetrate the skin and inject venom. Dried adults should be ground to avoid any dangers of injury from stinging. The venom remains active after drying or freezing, but is deactivated by cooking or frying.

Once removed from the combs, the larvae are ready for processing and preservation, after a short rinse in fresh, clean water (see Figure 8.9).

If larvae are refrigerated immediately, freezing, drying, boiling or frying should be

completed less than 24 hours after collection of larvae to avoid any spoilage since insect proteins decay much faster than those of beef, chicken, lamb or pork. Where no refrigeration is available, processing will have to be started immediately after collection. Cooked larvae or pupae can be preserved by freezing. If there is no freezer or refrigerator, the boiled larvae should be consumed within a day. Fried larvae will keep a little longer.

8.10.2 Bakutig traditional recipe from Nepal (Bur2ettg 1990)

Brood combs from traditional honey hunts in Nepal are placed into coarse woven fabric or bags and squeezed. The resulting juice is collected and heated over a fire while stirring. The result is described as having a texture similar to that of scrambled eggs but the flavour should be richer.

8.10.3 Frozen larvaeg pupae or adults

Figure 8.9: Bee larvae in a strainer for rinsing.

Fresh and clean larvae, pupae or adults are frozen in small batches or spread on metal sheets for faster freezing. If plastic bags are used, these should be half filled and flattened on the freezing trays. In larger scale bulk freezing, and especially with pupae or larvae that are already dead, the centre of a large volume freezes more slowly, leaving enough time for larvae or pupae to darken due to oxidation.

8.10.4 Rawg fried and boiled larvae

Honeybee larvae can be consumed like other insect larvae - raw, fried or boiled. The raw larvae can be chewed while still inside the comb or after removal. Chewing comb which also contains pollen further increases the nutritional value. The age of the larvae is not very important, but whiter or newer combs are preferred for chewing.

If skins of larvae are intact after collection, they may be rinsed briefly. Then, larvae can be boiled for 10 minutes (some people prefer 30 minutes) in salty or spiced water just like sea food. Once boiled, they can be added to other recipes or eaten as they are.

Like sea food, larvae may be deep-fried either plain (see Figure 8.10) or after being rolled in flour or dipped in batter. Deep-fat frying at 150^0C for only 1 minute is sufficient (Hocking and Matsumura, 1960). After one minute, the larvae should be removed and briskly shaken and drained on a slope, and/or covered with absorbent material to eliminate some of the excess fat. Frying in butter results in uneven browning and more broken larvae.

Figure 8.10: Frying bee larvae in oil.

8.10.5 Dried larvae and adults

Larvae and adults may be sun-dried in a solar drier. They should be kept out of direct sunlight and protected from dust and insects. If the weather is not favourable for quick drying, the insects may be roasted carefully to avoid deterioration. After drying, they may be chopped or ground to a powder. The powder may be used to enrich other meals or flours. If used as an additive to animal feed, they can be added whole. The flavour of these meals is not affected if the insects are used in moderate quantities.

8.10.6 Basic general recipes

The basic recipes and many of the following ones are adapted from Taylor and Carter's "Entertaining with Insects " (1976). Some modifications have been included to adjust the recipes for more general use and for readily-available ingredients. Once frozen, smoked, dry-roasted, solar-dried, or made into a flour, insects can be incorporated into basically any other food dish. In any of the dried forms, including the flour, they can also be readily marketed.

Dry roasted larvae or adults

Spread the cleaned, fresh or frozen insects on paper towels (not newspapers) on a cookie sheet. Bake at 70–90 - 94^{0}C for 1-2 hours until the desired state of dryness is obtained. Check the dryness by attempting to crush the insects with a spoon.

Alternatively, the insects can be roasted in a large frying-pan, pot or metal sheet over medium heat. If their temperature exceeds 100^{0}C they will caramelize. They should be stirred frequently to prevent them from burning. A coffee roaster could probably be used. Drying larvae by smoking did not produce a good, smoky flavour.

Bee flour

Bees should be dry-roasted or sun-dried as above and reduced (in an electric blender) to a fine powder. For those relying on manual skills, grind or pound until all insects are reduced to a fine powder. This powder can be further enriched with equally fine ground dry pollen pellets or can be mixed directly with any other flour, dough, bread, vegetable dish or soup. It thus remains unnoticeable by taste and texture, but enriches the diet. If kept dry and packed immediately in plastic bags, it should keep fresh long enough for local marketing and consumption. Cold storage is

recommended and customers should be alerted to this and its short shelf-life. Do not process or package bee flour during the rainy season since the flour cannot be kept dry enough.

Basic cooked insects

1 cup	*Cleaned bees (adults or larvae)*
2 cups	*Water*
1 teaspoon	*Salt*
2 dashes	*Pepper*
1 tablespoon	*Butter*
½ teaspoon	*Sage*
2 table spoons	*Onions, finely chopped*

Quickly brown the onions in the butter or other available fat or oil. Then add all the other ingredients. Bring to a boil and simmer for 30 minutes or until tender. The sage can be replaced with other spices such as red peppers (chili peppers),

laurel, thyme, rosemary or curry, according to local taste. For immediate consumption, boiling for 5 to 10 minutes is sufficient.

Bee stew

Prepare your favourite soup or stew with vegetables and, instead of meat, add a similar or slightly smaller quantity of whole or crushed insects. The cooking time does not need to be as long as with meat. Only boil until the vegetables have cooked, because the insects will be boiled sufficiently after 10 minutes. If you miss the familiar flavour of meat, add some animal fat or marrow bones - they do not require extra cooking time.

Garlic butter fried bees

*cup	*Butter or cooking oil*
6 cloves	*Garlic*
1 cup	*Cleaned bees (larvae)*

Heat the oil or butter over low heat in a frying-pan or pot. Slowly fry the garlic so

that in about 5 minutes it is slightly brown. Add the insects and continue frying at the same temperature for another 10 to 15 minutes, stirring occasionally. Do not overheat or the garlic will burn.

The insects can then be included in rotis and tacos, used as condiments with rice and tortillas or be offered as appetizers (see Figure 8.11). If drained well, they can be served as snacks at any time or be packaged like nuts.

Figure 8.11 : Honeybee larvae prepared as appetizer in three different ways (from left to right): fried with garlic, boiled and fried in oil after covering with flour.

Insect marinade

A marinade can be prepared from a variety of ingredients to give the insects a stronger and spicier flavour and/or to preserve them for longer.

A very simple but tasty marinade is made of:

1	*Large clove of garlic, crushed or minced*
1	*Dried red pepper (chili pepper) crushed or minced*
2 tablesp	*Fresh ginger, minced or grated*
1 to 1.5 cup liquid	*The liquid may be soy sauce with a little sake (rice wine) or grape wine, salt and lemon juice, or other*

strongly flavoured juices or extracts with salt.

2 table spoons Onions, finely chopped

Once all the ingredients are combined, cover 1 cup of insects with the marinade and leave it for several hours. The process can be accelerated by simmering the mix for 20 to 30 minutes over low heat.

To pickle or preserve the insects, use a very thick soy sauce or, prepare a spicy and/or flavoured vinegar mixture with herbs and spices. Add the raw or cooked insects. Pickling arvae in vinegar or brandly alone does not produce a pleasant flavour. For long-term storage, some recipes recommend boiling after marination, others only use marination. Each region has its own way of pickling vegetables or meats, which can also be applied to insects. When adding large quantities of insects ensure the vinegar is concentrated enough and is not excessively diluted by water from the insects blood. Drain the vinegar after two days and replace it with fresh marinade. Chutney is a form of pickling where insects can be added, or used to replace one of the other ingredients.

8.10.7 Bee mango chutney

Principal ingredients:

15	*Medium size, peeled chopped mangoes*
8	*Medium size, chopped papayas*
1-2 cups	*Boiled bee larvae, chopped*
To be mixed with:	
3 tablespoons	*Chopped ginger candied if possible*
½ cup	*Chopped citron or other candied fruit*
½ cup	*Chopped candied lemon peel or ½ cup chopped, preserved kumquats*
Spice bag:	
2	*Cinnamon sticks*
30	*Whole cloves*
½ teaspoon	*Coriander seeds*
Sweet vinegar:	

6 cups	*Sugar*
4 cups	*Cider vinegar*

Heat the sweet vinegar to boiling, add the other ingredients including the spice bag and simmer for 5 minutes. Remove the spice bag and pour the boiling mixture into clean, sterilized jars, seal and continue heating for another 15 minutes in a water bath. when filling the jars leave a few centimetres of empty space between the chutney and the lid.

Use vinegar of at least 5-6% acetic acid. Other spices such as red peppers, turmeric or curry may be added. When using other vegetables like tomatoes, apples or onions, simmer them first for ½ hour in an equal volume of sweet vinegar.

8.10.8 Bee chapattis

1 ½ cups	*Flour (all-purpose, white or whole grain from wheat or other grains)*
½ cup	*Bee flour (see recipes in 8.10.6)*
1 ½ cups	*Water*

q.s.	*Salt, to taste*
q.s.	*Melted butter, lard or oil*

Mix water and flours until a stiff dough is obtained. Add the salt. Knead the dough until it is smooth. Pinch off pieces of dough and mould into balls of about 4-5 cm in diameter. Roll each ball in flour and place it on a flour-covered board. Flatten the balls to approximately 5-6 mm thickness. Heat a large non-greased frying-pan. Place a flattened ball in the pan and fry for 2 minutes on each side. Remove the chapatti and apply a little melted butter or oil on each side and fry until dark brown spots begin to appear on the heated faces.

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