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.

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

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 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, 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		Olariu et al, 1989
Bacteroides nodosus	reduction of foot-rot in rams	Muñoz, 1989
Klebsiella pneumoniae		Dimov et al., 1991
reduced or no bactericidal activity		Brumfitt et al., 1990
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	Sosnowski, 1984
	anti-herpes ointment patent	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

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	Grumberger 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	Gabcys 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	Tóthné 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	Gubieza 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 37^0C (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.

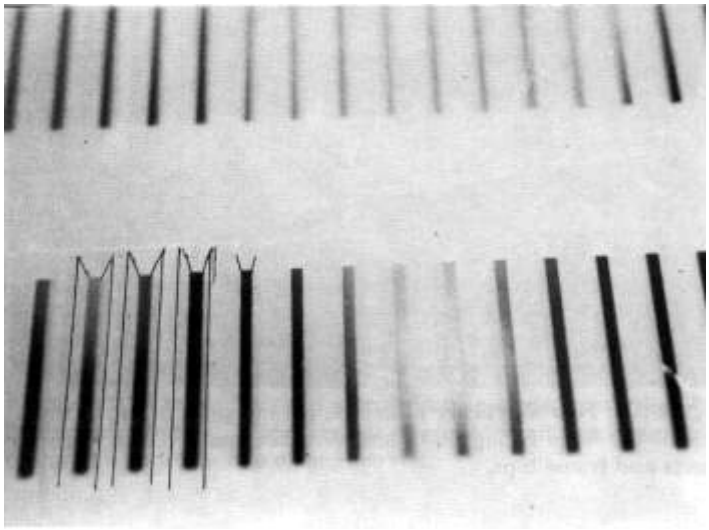

Figure 5.2*:* Flexible, 3 mm plastic sheets with rows of slots, 2 mm side on one side and 4 mm on the other

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.

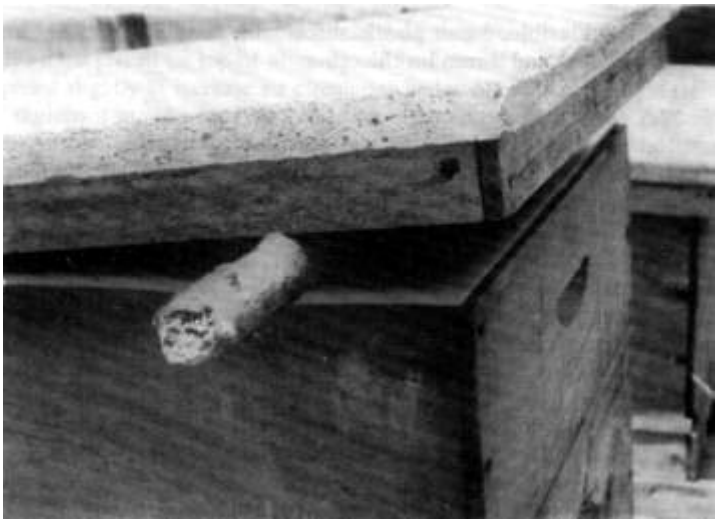

Figure 5.4: The cover is left open a little to increase ventilation and let light in. This stimulates the bees to seal the slots with propolis.

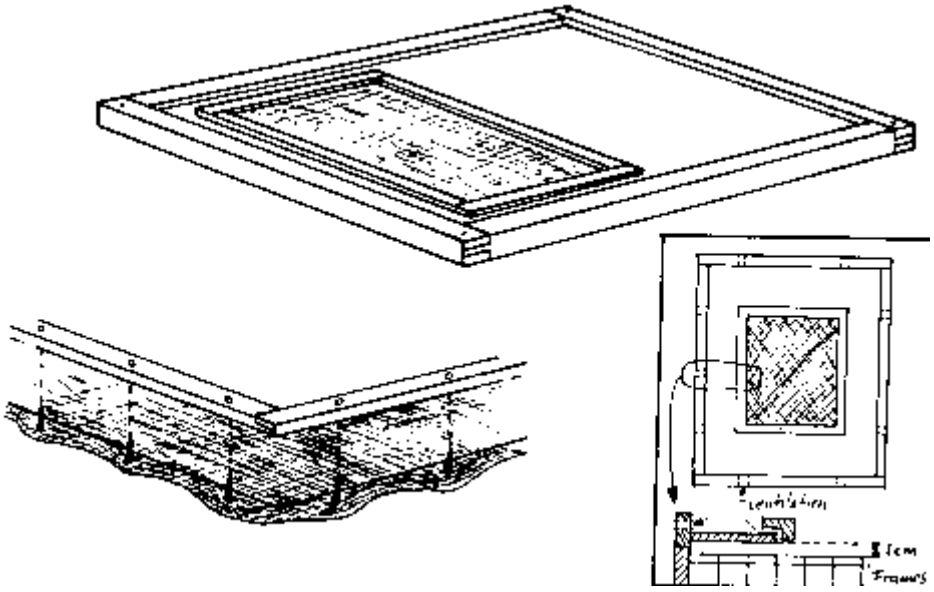

Figure 5.5: A simple design of a propolis trap made from nylon, fly or mosquito screen. The screen is removable and can be quickly replaced with a new one during harvest.

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).