The Bee Pollen Book

Wild Thyme and Sav'ry set around their cell
Sweet to the Taste and fragrant to the smell
Set rows of Rosemary with flow'ring Stem,
and let the purple Vi'lets drink the Stream

Virgil, "Georgica", 20 BC

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Pollen: Collection, Harvest, Composition, Quality

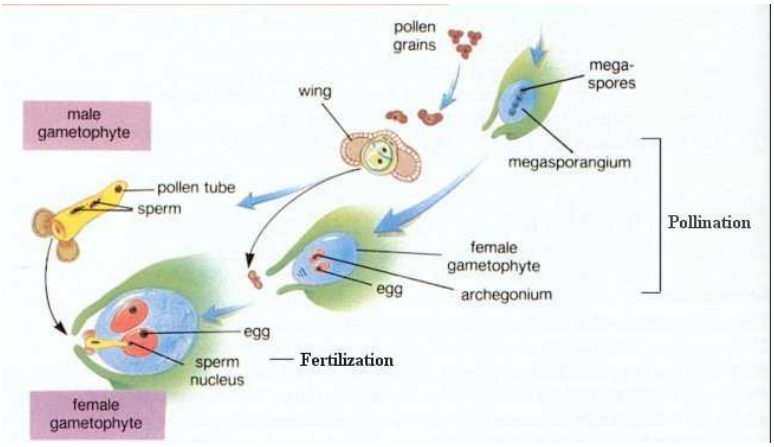
Stefan Bogdanov

The old Egyptians describe it as "a life-giving dust." In ancient Greece the pollen pellets, carried on the bee's legs were considered to be made of wax. Aristotle in his Historia animalism observes, that they resemble wax in hardness but are in reality sandarace or bee-bread. Later it was called farina.The name bee bread persisted until many centuries. Pollen (a Latin word for fine flour or dust) was used for the first time by John Ray in Historia plantarum (1686). The first works on the mechanism of pollen foraging were carried out by Meehan in 1873.

By determining pollen in soil sediments information on the vegetation history of the earth is gathered. The importance of pollen for the determination of honey origin was realized at the beginning of the 20th century.

POLLEN AND POLLINATION

The pollen contains the plant's male reproductive organs or gametophytes. They are situated in he anthers of the higher flowering plants. The pollen is transferred onto the stigma of a flower, a process called pollination. This transfer is carried either by the wind, by or by insects Each pollen grain carries a variety of nutrients and upon arrival at the stigma it divides into several cells and grows a tube through the often very long stigma of the flower. Growth continues to the embryo sac in the ovary of the flower, inside which one egg cell will fuse with a sperm cell from the pollen and complete the fertilization. There are two types of flowers: naked and covered. The open flowers are pollinated by wind, the covered ones, mostly by insects. The pollination by insects came into being some 100 millions years ago. Pollen are well preserved in the earth. The science, by which pollen are studied in order to gain knowledge about the vegetation on earth in called paleopalynology.

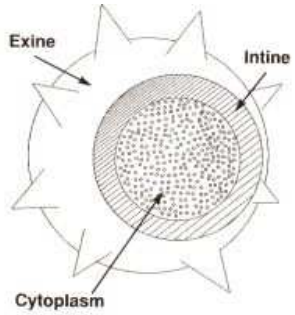


Bees pollinate around 40 000 plant species. The importance of bee pollination for ecology and agriculture is immense. The economic importance for the USA in 1973 was estimated to be the 143 fold of the honey harvest, that is 18.9 billions $ [32]

In the USA in other countries beekeepers are paid by the peasants for pollination services. The importance of bees for the pollination and multiplication of wild plants is also immense, but cannot be quantified in a money value.

ANATOMY AND POLLINATON

Pollen itself is not the male gamete, but each pollen grain contains vegetative (non-reproductive) cells (only a single cell in most flowering plants but several in other seed plants) and a generative (reproductive) cell containing two nuclei: a tube nucleus (that produces the pollen tube) and a generative nucleus (that divides to form the two sperm cells). The group of cells is surrounded by a cellulose-rich cell wall called the intine, and a resistant outer wall composed largely of sporopollenin called the exine.



The transfer of pollen grains to the female reproductive structure (pistil in angiosperms) is called pollination. This transfer can be mediated by the wind, in which case the plant is described as anemophilous (literally wind-loving). Entomophilous (literally insect-loving) plants produce pollen that is relatively heavy, sticky and protein-rich, for dispersal by insect pollinators attracted to their flowers. Bees and many insects and some mites are specialized to feed on pollen, and are called palynivores.

HOW AND WHICH POLLEN ARE GATHERED BY THE BEES?

How bees collect pollen

When visiting flower's blossom bees touch the stamen and its body is covered with pollen dust. The honey bee uses hind legs this plate to compress the pollen into the pollen basket. The bee moistens the pollen with secretion from it's mouth which helps the pollen cling together and to the basket hairs. This secretion contains different enzymes, e.g. amylase and catalase. A pollen load contains up to 10% nectar, which is necessary for packing. To collect a load of pollen, on the average about 8 mg, a bee has to visit about 200 different flowers. Mostly, a load contains pollen from the same flower. Approximately 10 trips a day are made for pollen by a worker. In good weather 50,000 to pollen loads were brought into the hive daily. In the hive, pollen is removed from the rear legs by a spike on the mid legs and is placed in cells. Often the head is used to pack the pollen in cells. Honey is added to maintain pollen quality, which is called beebread.

While honey is the energy source of the bee colony, pollen, is the bees' main source of the other important nutrients: proteins, minerals, fats and other substances. Consequently, an adequate pollen supply will be essential to ensure the long-term survival of a colony and to maintain its productivity**.**

Honey bee foragers mix freshly collected pollen with some nectar before packing it into their corbiculae. In the hive, the workers add more nectar and glandular secretions to the pollen, which then undergoes lactic acid fermentation.

Pollen is a very important factor for the development of the colony, in the first place for producing brood. It supplies the necessary food: proteins, lipids and minerals. It seems that under normal conditions bees will gather enough pollen for a sufficient colony development. Thus there is no need for a pollen supplement.

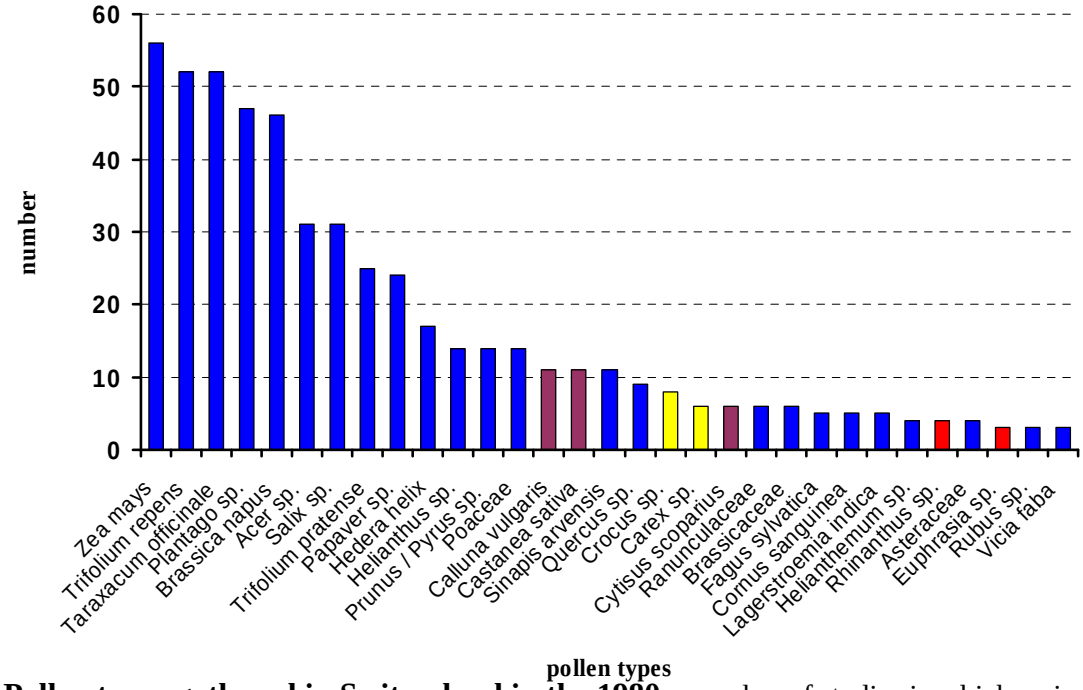
See further information at [22, 23]

Which pollen types are gathered by the bees?

Basic studies on pollen collection by bees were carried out in the Swiss Bee Research Centre by Wille and collaborators during the 1980's. These studies have been recently reviewed by Keller et al. [22, 23] and are summarised here: *Different investigations show that the bulk of the pollen generally came from few plant species.. The five most common pollen sources yielded on average more than 60% of the total collected pollen. Following conclusions are based on this study, carried out in different locations of Switzerland ,in the most important pollen sources were plants occurring at high densities either naturally or due to cultivation: white and red clover (Trifolium repens and pratense), corn (Zea mays), rape (Brassica napus) and sunflower (Helianthus sp.). Other plants such as plantain (Plantago sp.), dandelion (Taraxacum officinale) and mustard (Sinapis arvensis) are generally abundant in meadows and pastures [7]. A third group of important pollen sources included different tree species such as maple (Acer sp.), willow (Salix sp.), stone fruit (Prunus sp.) and pome (Pyrus sp.). Of course, this list is strongly influenced by the overrepresentation of study sites from the Swiss midland, and in other sites many other plant species may be important. Thus, pollen from heather (Calluna vulgaris), sweet chestnut (Castanea sativa) and scotch broom (Cytisus scoparius) was quite dominant in samples from Intragna in southern Switzerland (black bars in fig. 1), but was not found at other localities. In two other sites lying the subalpine region of*

Switzerland at an elevation of 1250 and 1560 m above sea level respectively dominant plants were: crocus (Crocus sp.) and sedges (Carex sp.), besides Rhinanthus sp. and Euphrasia sp., found exclusively in one of the locations In another study the composition of bee-collected pollen was compared with the composition of the surrounding flora and was found that the bulk of the pollen indeed came from common plants. However, it is likely that the pollen composition does not simply reflect the proportions of different flowers in the surroundings but is, at least to some extent, determined by the preferences of the bees.

At the beginning of the vegetation period, a uniform pattern was observed across most available studies with a very pronounced dominance of different tree species as the most popular pollen sources. These included maple (Acer sp.), ash (Fraxinus sp.) different fruit trees (Prunus sp. and Pyrus sp.), poplar (Populus sp.), oak (Quercus sp.), willow (Salix sp.) and elm (Ulmus sp.). At some Swiss locations, dandelion (Taraxacum officinale) was also an important pollen source in spring. In May and June, the spectrum of pollen types became much more diverse and generalisations across all study sites were hardly possible. In Ireland and England, some shrub species such as hawthorn (Crataegus monogyna) and elder (Sambucus sp.) were important pollen sources whereas rape (Brassica napus) was frequently collected at several of the Swiss locations. In midsummer and early fall, pollen from red and white clover (Trifolium pratense and repens), corn (Zea mays) and plantain (Plantago sp.) dominated the samples from all locations from the Swiss midland. In southern Switzerland, European chestnut (Castanea sativa) and heather (Calluna vulgaris) were the dominant pollen sources at this time of the year. In Ireland, on the other hand, large amounts of pollen were collected from blackberry (Rubus sp.) and meadowsweet (Filipendula ulmaria). Towards the end of September, ivy (Hedera helix) became the dominant pollen source at several locations.



Pollen types gathered in Switzerland in the 1980s: number of studies in which a given plant taxon ranked among the five most common pollen sources after [22, 23]

How much pollen do bees collect?

There are two types of pollen: hand collected and bee collected. Only in the cases where one wishes to collect the pollen of a certain plant it can be collected by hand. There is only bee collected pollen on the market. Beekeepers collect pollen by means of pollen traps, which also provide quantitative estimates of the pollen harvest of a colony. The information here is taken from the review of Keller at al.[22, 23]: *There is a large variety of different trap designs, but all consist of some type of grid, which removes the pollen pellets from some of the returning foragers as they enter the hive. The pollen is collected in a tray and can be easily removed by the researcher. The grid is installed either in front of the hive entrance or horizontally underneath the entrance to the brood nest (O.A.C. trap design) The percentage of pollen actually retained in a trap may be quite variable, but will always be considerably less than 100%. Extensive observations by*

Imdorf showed that the efficiency of a trap at one colony could vary between 3 and 25% during the course of the vegetation period. Still larger variation (15 – 43%) was observed between different colonies, even if the same trap type was used. Such discrepancies may stem from small differences in the material used for the individual traps. Alternatively, honey bee colonies may vary in the average size of the workers or may collect a different spectrum of pollen types. The species composition of the collected pollen appears to be of particular importance. Thus, it was found that the average efficiency of their traps increased from 33% to 60% when they were moved to a different location where different flowers were available, and the foragers collected significantly larger pollen pellets.
From the above discussion it becomes clear that accurate estimates of the actual quantity of pollen collected by a colony are virtually impossible. It is also not well understood to what extent honey bee colonies might be harmed by the permanent use of pollen traps.
In different studies the amount of pollen, gathered in different locations in Europe and the USA was determined The available estimates of the amount of pollen collected per colony and year in different European and one American location range between 5.6 kg and 222 kg. Assuming an average trap efficacy of 20 % the amount gathered by the pollen trap varies from 1.1 to 40. 4 kg. The maximum of 40.4 kg, found in the pollen traps in California, was considerably higher than the amounts gathered in Europe, which varied between 1.4 and 9.2 kg. This difference is probably the result of a longer collection period. In the study by Eckert more than 50 kg of pollen were actually retained in the traps. Factors for pollen gathering are abundance of pollen, weather conditions and the nutritional need of the colony may influence the foraging behaviour of the bees.
The amount of pollen available for consumption at any given point in time is determined not only by the intensity of pollen collection but also by the pollen stores of a colony. In experimental colonies, the intensity of pollen foraging could be decreased by adding and increased by removing pollen stores. In apiaries specialized on the production of bee pollen in countries with a longer vegetative period up to 10 to 20 kg per colony can be harvested, the normal however is lower, about 5-15 kg per hive.

Pollen is collected with a pollen trap, made out of a grid, placed on the entrance of the hive. These traps vary greatly in size, appearance, and method of installation on the hive. Each has some feature that makes it particularly adaptable for a specific purpose. All traps, however, have two basic elements: 1. a grid through which pollen-carrying bees must crawl to separate the pollen pellets from the bees' legs, and 2. a container to store these pellets. Upon entering the hive the pollen loads of the bees are stripped away and fall in a drawer beneath.

HARVESTING BY THE BEEKEEPER

Fresh, bee collected pollen contains about 20-30 g water per 100 g. This high humidity is an ideal culture medium for micro-organisms like bacteria and yeast. For prevention of spoilage and for preservation of a maximum quality the pollen has to be harvested daily and immediately placed in a freezer. After two days of storage in the freezer, the pest insects will be killed[29] After thawing pollen can be kept only for a few hours and should be further processed as soon as possible.

Drying

The pollen is best dried in an electric oven, where humidity can continuously escape. Then it is purified by a special machine, similar to the seed cleaning machine. The maximum temperature is 30°C and the drying time should be as short as possible in order to avoid vitamin losses.
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Today pollen is dried generally in electric ovens, where humidity can continuously escape. The prescribed maximum temperature was 40°C. However this temperature seems to be high. The effect of different methods of preservation (freezing, drying at about 40ºC and lyophilisation) on selected parameters attributed to the biological

quality of bee pollen were tested in Poland. Freezing caused no substantial changes in the chemical composition of the pollen loads, so this technique should be recommended when the preservation of the pollen load for nutrition or therapeutic purposes is important. Lyophilisation markedly decreased vitamin C and provitamin A content, but drying at 40ºC revealed the most disadvantageous effect [46].

A Brazilian study found that pollen drying for 6 hours at 45 °C led to significant losses of vitamin E and β-carotene, as well as pro-vitamin A by 15 to 25 % [9]

A Spanish study showed that freeze drying is better for the preservation of the chemical and the biological properties of pollen than oven-dried one[12], the result being confirmed by a Portuguese study[11].

A Portuguese study revealed that quick drying of bee pollen (3 times for 45 seconds) at 50^{o} C in an infra-red oven did not lead to losses of anti-oxidant activity

Concluding the above results, pollen should be dried at possible low temperatures, a maximum of 30 °C. The better alternative is to use freeze drying. A pollen freeze drying machine is described in the literature [14] , but its effect on pollen quality has not been tested.

Drying changes the aroma profile of bee collected pollen[13]

Storage

Experience in Switzerland showed that from a microbiological and sensory point of view pollen remains stable until 1.5 years of storage at room temperature. Under these conditions pollen keeps its sensory and microbiological quality for a storage period of 2 years, if stored in a cool, dry and dark place [3].

As a functional food one of the main health enhancing properties is the strong antioxidant activity of pollen.

Pollen loses a considerable amount of its antioxidant activity (about 59%) after one year[4]. This loss might be due to the decrease of phenolic compounds, observed in another study[37]

The amounts of four out of nine constituents examined (reducing sugars, total proteins, vitamin C, and provitamin A) markedly decreased upon storage. Taking into account the methods of production practical recommendations for the means of preservation and optimum conditions for the storage of pollen loads are suggested. Freezing followed by storage at -20ºC in pure nitrogen guarantees high biological qualities of bee pollen kept for up to 6 months. Pollen stored for a longer periods should, however, be dried by lyophilisation and stored at -20ºC in pure nitrogen to preserve its highest biological activities. Storage of pollen at 0 to 10 degrees in vacuum has been proposed in order to prevent antioxidant spoilage [42].

A Brazilian study found no loss of vitamin C and losses of vitamine E and beta-carotenes by 15 to 20 % upon storage of dry pollen for one year at room temperature [10]

Fresh, frozen purified pollen should be stored under nitrogen until consumption for preservation of optimal biological and nutritive properties [31].

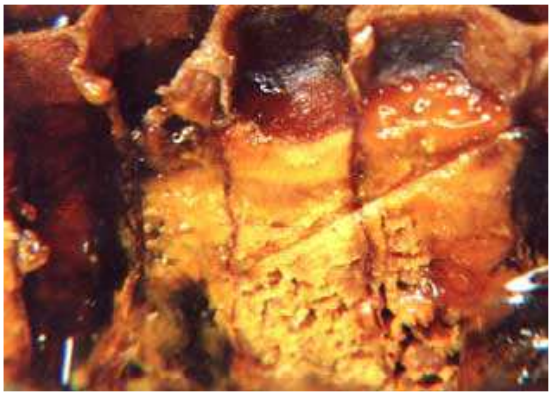
Harvesting of unifloral pollen and of specific pollen types

Normally beekeepers collect mixed pollen. Harvesting of unifloral pollen is important because only this type of pollen has constant composition and thus can be successfully used in nutrition and medicine. A machine was constructed in Austria, by the help of which bee pollen can be sorted into different types, the purity of the sorted pollen being about 90 %[34].
The different pollen types differ in colour. This properties was used for the development of a computer based differentiation of pollen loads[6], however, the hardware has not been developed.

Fresh freeze-dried pollen

Patrice Percie du Sert from France invented and patented a technique in 1994 that allows all the nutrients in fresh bee pollen to be preserved. The pollen is frozen at collection and packed in a nitrogen filled package; oxygen is excluded, eliminating decay. This process allows the pollen to be presented as close to its pure state as possible. Fresh, purified pollen can be frozen and stored under nitrogen until consumption for preservation of optimal biological and nutritive properties[31]

Bee bread



Bees store pollen in the hive as beebread. Pollen is mixed with honey and bee secretions and stored in the combs. Bee bread undergoes a lactic acid fermentation and can be thus preserved. Beebread combs will often be sold as a whole. For that purpose a bee queen separator is placed between brood and honey combs during a period of a maximum pollen gathering activity. When the combs are full, the pollen is harvested by means of a scraper and filled into a jar.

Production of "home bee bread" after Dany 1988, as described by Krell[25]

The term "bee bread" is reserved for the original bee pollen stored in the combs. Thus, the product described below cannot be called "home bee bread" or fermented pollen.

Normally, the term beebread refers to the pollen stored by the bees in their combs. The beebread has already been processed by the bees for storage with the addition of various enzymes and honey, which subsequently ferments. This type of lactic acid fermentation is similar to that in yoghurts (and other fermented milk products) and renders the end product more digestible and enriched with new nutrients. One advantage is almost unlimited storability of beebread in comparison with dried or frozen pollen in which nutritional values are rapidly lost. The natural process carried out by the bees can more or less be repeated artificially with dry or fresh bee-collected pollen. It is important however, to provide the correct conditions during the fermentation process.

The container

Wide-mouthed bottles or jars with airtight lids are absolutely essential. Airtight stainless steel or glazed clay pots can also be used. Containers should always be large enough to leave enough airspace (20 to 25 % of the total volume) above the culture.

The temperature

The temperature for the first two to three days should be between 28 and 32^{0}C; the bees maintain a temperature of approximately 34°C. After the first two or three days the temperature should be lowered to 20°C.

The high initial temperature is important to stop the growth of undesirable bacteria as quickly as possible. At this ideal temperature all bacteria grow fast so that an excess of gas and acid accumulates. Only lactic acid producing bacteria (lactobacilli) and some yeasts continue to grow. The former soon dominate the whole culture. This final growth of lactobacilli should proceed slowly, hence the reduction in temperature after 2-3 days.

The starter culture

It is best to start the culture with an inoculation of the right bacteria such as Lactobacillus xylosus or lactobacilli contained in whey. Freeze-dried bacteria are best if they can be purchased, but otherwise, the best cultures are those that can be obtained from dairies. Whey itself can be used. If the whey is derived from unprocessed fresh milk it should be boiled before use. A culture can also be started with natural beebread.

Preservation

Fermentation produces a pleasant degree of acidity (ideally pH 3.6-3.8). Some pollen species may promote excessive yeast growth but this does not spoil the beebread. If the flavour is strange or some other mildew-like or unpleasant odours arise from the beebread, discard it and try again. The final product, can be stored for years, once unsealed, it can be dried and thus is storable for many more months.

General conditions

For successful fermentation, exact quantities are less important than the correct conditions:

- the pollen to be fermented needs to be maintained under pressure
- the air space above the food needs to be sufficient (20-25 % of total volume)
- the container needs to be airtight
- the temperature should not drop below 18°C

Ingredients (in parts by weight):

10 Pollen; 1.5 Honey; 2.5 Clean water 0.02 Whey or very small quantity of dried lactic acid bacteria

Clean and slightly dry the fresh pollen. If dried pollen is used, an extra 0.5 parts of water is added and the final mix soaked for a couple of hours before placing it in the fermentation vessels. If the mixture is too dry, a little more honey-water solution can be added.

Heat the water, stir in the honey and boil for at least 5 minutes. Do not allow the mix to boil over. Let the mix cool. When the temperature is approximately 30-32 ^{0}C, stir in the whey or starter culture and add the pollen. Press into the fermentation container.
When preparing large quantities in large containers, the pollen mass should be weighted down with a couple of weights (clean stones) on a very clean board.
Close the container well and place in a warm place (30-32 ^{0}C).
After 2-3 days, remove to a cool area (preferably at 20^{0}C). 8 to 12 days later the fermentation will have passed its peak and the beebread should be ready. The lower the temperature, the slower is the progress of fermentation.
Leave the jars sealed for storage.

COMPOSITION

The pollen composition varies greatly according to its botanical origin:

Pollen composition after[5]

Main Components	**Content Minimum – Maximum g/100g dry weight**
Proteins	10-40
Lipids	1-13
total Carbohydrates*	13-55
Dietary fibre, Pectin	0,3-20
Ash	2-6
undetermined	2-5
Minerals, trace elements	**mg/kg**
Potassium	4000-20000
Magnesium	200-3000
Calcium	200-3000
Phosphorus	800-6000
Iron	11-170
Zink	30-250
Copper	2-16
Manganese	20-110
Vitamins	**mg/kg**
β-Carotene	10-200
B1; Thiamin	6-13
B2; Riboflavin	6-20
B3; Niacin	40-110
B5; Pantothenic acid	5-20
B6; Pyridoxin	2-7
C; Ascorbic acid	70-560
H; Biotin	0.5-0.7
Folic acid	3-10
E: Tocopherol	40-320

Carbohydrates

They are mainly polysaccharides like starch and cell wall material[43]

The calculated carbohydrate content is higher than the one, determined by analytical methods. The reason is that a part of the carbohydrates is composed by crude fibre and cell wall material, which are generally not determined by chemical methods, while their part can be calculated: 100 less the sum of water, fat, protein and ash content.

The sugars fructose, glucose and sucrose comprise about 90 % of all low molecular sugars[39]

Crude fibre

The crude fibre is composed of starch and insoluble polysaccharides like callose, pectin, cellusose and sporopollenin[43]. There is quite a large variation between the minimum and the maximum values, due probably to the different methods and to the different plants measured[1, 17, 39, 41]

Protein and amino acids

The protein concentrations in hand-collected pollen from 377 plant species from 93 families . Pollen from different species may vary considerably in protein content, with values ranging between 2.5% in the cypress *Cupressus arizonica* and 61.7% in *Dodecatheon clevelandii* (Primulaceae). Within plant families, however, protein concentration appears to be highly conserved, except in the species-rich Cactaceae and Fabaceae. On average, animal-pollinated plants do not appear to be richer in pollen protein than wind-pollinated plants [36].

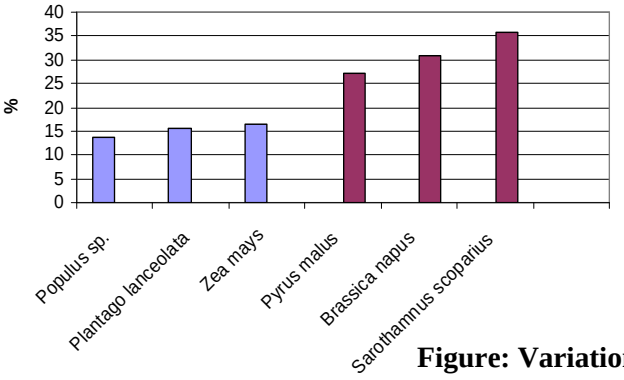


Figure: Variation of protein in pollen gathered in Switzerland, after [26]

Only about 1/10 of the total protein comes from free amino acids. Generally, there appear to be few qualitative differences in the amino acid composition of different pollen types and most of them contain all essential amino acids[36] Wille et al. detected also very similar proportions of the different amino acids in bee-collected pollen samples from 99 plant species[22]. Pollen proteins play a key role as an allergens[35].

Lipids

There are considerable differences of the fat composition, depending on the botanical origin. There are mainly polar and neutral fats (mono-, di and triglycerides), as well as small amounts of fatty acids, sterines and hydrocarbons.

In one study 3 % of the total lipids are free fatty acid are reported. about half of them are the unsaturated acids oleic, linoleic (omega-6) and linolenic (omega-3) [43], while in a study of pollen of different geographic origin it is reported that 50 to 60 % of the fatty acids are unsaturated (oleic, linoleic and alpha-linoleic) while the rest being saturated, mainly palmitic acid [44]

Other physiologically important compounds are the sterols.

STANDARD AND QUALITY

From hygienic point of view the microbiological safety is the main quality criterion. It is important to control the microbiological quality of pollen, especially the absence of pathogenic germs and fungi. Destruction of bacteria by irradiation, ozone treatments[47] or chemical fumigants[38] is not necessary and leads to toxic residues..

For specific use the composition of biological active components e.g. flavonoids (Campos et al. 1997, Serra-Bonvehi et al., 2001) or vitamin content should be evaluated.

Pollen is the bee product, least influenced by contaminants from beekeeping[2]. However, it can be polluted by air contaminants, e.g. by heavy metals and pesticides. Thus, for optimum quality pollen should be gathered in areas which are at least 3 km distant from contamination sources such as heavy traffic and pesticide-treated agricultural areas.

In the last few years there are genetically manipulated plants and also pollen. No studies on the negative effect of such pollen on human nutrition have been published. The consumer should be aware of that. In the EU there is a compulsory indication of the content of genetically manipulated organisms (GMO) in food (and also of pollen, if there the GMO content exceeds 1 %.

Analysis	Quality criteria
Sensory examination	Typical odour and taste, no visible contaminants
Microscopic examination	Origin test (botanical, geographical)
Microbiological testing	Bacterial load should be within legal hygienic limits
Chemical Examination	Water content: maximum 6 g/100 g pollen
	Content of main ingredients, carbohydrates, fat and protein, if labelled accordingly:
Contamination	Pesticides, heavy metals

Sensory Analysis

Colour, appearance, odour and taste vary according to the botanical origin.
Colour: mostly yellow or yellow-brown, but many different colours are possible[18, 24]
Appearance: as so called „pollen loads“
Odour: hay-like
Taste: sweet, sour, bitter, spicy,
Defects: off-odour and taste, “molds”, fermented, rancid, visual impurities

Microscopical examination

The pollen should not contain impurities like bee parts, wax, plant particles or other extraneous matter.

Pollen analysis can be used for the determination of the botanical origin. The same methodology, as used for pollen analysis of honey can be used [28]

There is no international standard. Some countries as Brazil, Bulgaria, Poland and Switzerland have national standards [5]. A proposal has been recently made [5]:

Proposal for a chemical standard

Component	Requirement	Content
Water content	not more than	8 g/100 g
Total protein content (N x 6.25)	not less than	15 g/100 g
Sugar content (total)	not less than	40 g/100 g
Fat	not less than	1,5 g/100 g

Water content

The maximum allowed humidity varies from country to country: Brazil, 4 %, Switzerland, 6 %, in Russia: 8-10 %, Bulgaria: 10 %. More than 10 % makes the pollen susceptible to fermentation. The examination of the sensory quality in Switzerland concluded that humidity of less than 6 % makes the pollen too dry and less acceptable from sensory point of view.

The determination of pollen water content is carried out after drying to a constant weight in a cabinet dryer or infra-red oven drier [16, 30] or by Karl-Fischer method [15, 40].

Carbohydrates

Generally the carbohydrate content in g/ per 100 g will be determined by calculation, as the total carbohydrate content cannot be determined easily: 100 less the sum of water, fat, protein and ash content.

Proteins and amino acids

Protein content is a standard determination after Kjedahl, using a factor of 6.25 or 5.6 [33] (Rabie *et al*., 1983). According methods for protein content in pollen loads we recommend to use for calculation (Kjeldahl method) N x 5.6 rather than N x 6.25 This factor is used by other authors too.

Lipids

Lipids are determined by extraction with petrol ether [45]

Contaminants

Pollen is the bee product that is most susceptible to pesticide contamination [2], and a 2016 study showed that indeed the pollen from rape and near arable crops were heavily contaminated[8]. Thus for secure consumption pollen from areas without intensive agriculture or from organic apiculture should be taken. Pesticides should be tested whether they conform to the requirements. Also pollen should be tested for microbial purity: pollen can be contaminated by funghi, which can produce mycotoxins[21]

LABELLING

Composition

The composition of pollen varies greatly depending on the botanical composition of the pollen. There are two possibilities.

1. Determine the composition of each lot and state the composition:
2. Indicate an average composition, example for Swiss pollen:
100 g pollen contain on the average 20 g protein, 60 g carbohydrates 8 g fat and approx. 300 calories.
Also the fiber content could be indicated,
Serving: 2 tea spoons daily (approx. 10 g); children: half dose.
Warning: It is recommended that people who are susceptible to allergies or asthma should avoid intake of bee pollen.
Storage: store in the dark in a cool dry place
Best before (valid after packaging of product)

Dried pollen stored at room temperature:	12 months
Dried pollen packed in vacuum:	24 months
Frozen fresh pollen stored in the freezer:	12 months

TRADE

There are no official figures on pollen trading. Mainly bee gathered pollen is traded, with the exception of maize pollen, which is also gathered by special machines. There are no official figures about the trade of pollen, but according to Crane the production of pollen is the greatest among the secondary bee products (all besides honey). According to the same sources 1986 60-130 tons were produced in in West Australia[7]

In Europe production is greatest in Spain, Portugal, France, Germany and Italy, as well as Eastern Europe[19, 20] Spain is the biggest producer in Europe, in 1986 about 1200 tons were produced, 943 tons of which being exported[39].

Other countries like like Canada, USA as well as the Latin American countries and China are also good pollen producers and export some pollen. Especially China is becoming a leading producer and exporter in the world, it produces at present about 2500 tons per year[27]

Storage in glass for one or more years results in decrease of antioxidant activity. Packing in vacuum or under N_2 is better.

Pollen packed in vacuum packed air-tight plastic bags prevents oxidation and decrease of antioxidant activity due to contact with oxygen.

Harvesting of unifloral pollen ensures constant and reproducible concentration of biologically active ingredients.

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