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BIOCHEMISTRY AND MICROBIOLOGY OF BEE-COLLECTED ALMOND (*PRUNUS DULCIS*) POLLEN AND BEE BREAD

I. — Fatty Acids, Sterols, Vitamins and Minerals

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SUMMARY

Almond (*Prunus dulcis*) pollen was hand- and honey bee-collected (corbicular) for chemical analysis. Corbicular pollen was packed by bees into wax combs for conversion (via natural fermentation) into bee bread. Chemical analyses of 12 fatty acids, 3 sterols, 3 vitamins, inositol, titratable acidity, and 5 minerals were made on the hand-collected and corbicular pollen. The bee bread was analyzed after 7, 21, and 42 days for all of the above except the fatty acids and sterols. Lipoidal and acidic additions by the bees constituted the greatest differences between hand-collected and corbicular pollen. Sitosterol content decreased while titratable acidity increased during conversion to bee bread. The only vitamins which decreased as the pollen was converted to bee bread were ascorbic acid and pyridoxine.

INTRODUCTION

Honey bee (*Apis mellifera* L.) use pollen as their nutritional source of protein, fatty acids, lipids, sterols, vitamins, minerals and certain carbohydrates (DIETZ, 1975). Pollen stored in wax combs in a honey bee colony is referred to as bee bread. Extensive analyses have been conducted on various hand-collected and bee-collected pollens but little information is available on the chemical changes occuring in pollen as it is converted to bee bread. HERBERT and SHIMANUKI (1978) have recently published chemical and honey bee brood rearing nutritional bioassays of mixtures of pollens and bee bread from seven locations in the United States. PAIN and MAUGENET (1966) used ovarian development of queenless nurse bees as a bioassay of

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bee bread and hand- or bee-collected pollen. HERBERT and SHIMANUKI and PAIN and MAUGENET did not find any increase in nutritional value due to the fermentation process although HERBERT and SHIMANUKI reported earlier consumption and brood rearing with bee bread.

Analyses of pollens are found in the papers by TODD and BRETHERICK (1942), VIVINO and PALMER (1944), LUNDEN (1954), NIELSEN *et al.* (1955), and MCLELLAN (1977). These papers indicate a wide range in levels of protein, minerals, sugars, lipids, and vitamins present in pollen of diverse origin. The sterol content has been examined by BARBIER *et al.* (1960), HÜGEL (1965), STANDIFER (1966), STANDIFER *et al.* (1968), and THOMPSON *et al.* (1978). NILSSON *et al.* (1957) identified long-chain alcohols and long-chain hydrocarbons in Swedish pollens and found that the exine of gymnosperm pollen (*Pinus mugo* Turra) was composed of large amounts of alcohols, whereas the exine of angiosperm plants (*Zea mays* L., *Alnus glutinosa* (L.) Gaertn.) was composed of only small amounts of the hydrocarbons. FARAG *et al.* (1978) reported the fatty acid composition of six species of bee-collected pollens collected in Egypt, and suggested that some fatty acids may play a role as plant growth substances. Other long-chain fatty acids and pigments (lutein esters) found in pollen have been implicated as phagostimulants by HOPKINS *et al.* (1969) although tests conducted by Loper (unpublished) did not confirm this activity.

Pollen is a rich source of vitamins, especially the water-soluble B vitamins that have a vital role in honeybee larval nutrition (SERIAN-BACK, 1961; HAYDAK and DIETZ, 1965, 1972; ANDERSON and DIETZ, 1976). The following vitamins have been found in pollens : pro-vitamin A, thiamine, ascorbic acid, cyanocobalamin, pyridoxine, biotin, niacin, riboflavin, folic acid, and pantothenic acid. Pantothenic acid is of particular interest since it occurs in royal jelly in concentrations about 17 times the average value found in pollen (PEARSON, 1942). HAGEDORN and BURGER (1968) found a positive correlation between the loss of ascorbic acid with age of the pollen and lowered nutritional value. They concluded that the autooxidation of ascorbic acid may cause it to be one of the most limiting vitamins in honeybee nutrition. In contrast, STANDIFER and MILLS (1977) found that ascorbic acid did not decrease in concentration in the larval food produced by nurse bees of differing ages fed different diets.

Analysis of the mineral contents of pollen has been fairly complete (TODD and BRETHERICK, 1942; VIVINO and PALMER, 1944; NIELSEN *et al.* 1955; NATION and ROBINSON, 1971; DIETZ, 1971 a and 1971 b; and MCLELLAN, 1977).

With mastication and consequent addition of mandibular gland secretions, including 10-hydroxy-2-decenoic acid (LUKOSCHUS and KEULARTS, 1968), honeybees pack most of the collected pollen into cells of the honey comb. They also add small amounts of nectar or honey. This mixture undergoes fermentation that is essentially complete after 2 weeks in the warm (34-36 °C), moist environment of the

hive (CHEVTCHIK, 1950; PAIN and MAUGENET, 1966). See also AVETISIAN (1935), FOOTE (1957), and HAYDAK (1958) for insight into certain aspects of this process.

GILLIAM (1979 a, 1979 b) isolated 110 yeasts belonging to seven genera and 41 bacteria (genus *Bacillus*) from the same almond pollen (hand-collected, trapped, and bee bread) used as the source material for this paper. CHEVTCHIK (1950) and PAIN and MAUGENET (1966) have described a sequence of organisms and chemical changes occuring during fermentation. It appears that aerobic bacterial action, aided by a release of the pollen cell contents, proceeds for about 12 hours. This period is followed by a buildup of lactic acid-producing organisms, leading to increased acidity. Then *Lactobacilli* become active, increasing the lactic acid content until a *p*H of about 4 is reached. At this point, the pollen is essentially « preserved » or « pickled », a process analagous to the ensiling of forage vegetation. After this, various molds develop and utilize much of the lactic acid so that the resulting bee bread is somewhat « predigested » and possibly rendered more palatable to the honey-bee. Part of this pre-digestion involves the hydrolysis of sucrose to glucose and fructose (CASTEEL, 1912). An increase in soluble proteins as a result of the fermentation process has also been reported (LANGER, 1931). HITCHCOCK (1956) demonstrated the presence of a « milk-digesting enzyme » in bee bread which was not present in the pollen collected from foragers. HAYDAK and VIVINO (1950) reported that vitamin K activity was present in bee bread, but was absent in the fresh pollen (see also VIVINO and PALMER, 1944); HAYDAK and PALMER (1938, 1941) found similar results regarding vitamin E activity.

We have found no literature on changes in individual lipids, fatty acids, sterols, or minerals during formation of bee bread from pollen. Thus, previous chemical and biological assays have not investigated the influence of microbiological changes of a specific pollen of known chemistry before, during and after fermentation leading to the end-product that bees actually consume. The objective of this work was to study fermentation of a specific pollen to determine the chemical and microbiological changes with occur. This paper presents data on the content of fatty acids, sterols, vitamins and minerals in almond pollen samples and the content of vitamins and minerals in bee bread.

MATERIALS AND METHODS

We analyzed the following samples of almond pollen : fresh pollen collected by hand from the flower (obtained by sifting shredded anthers through 60-mesh copper screen) in March 1977; pollen pellets removed from the bees' legs (corbicular pollen) by traps placed on 15 colonies in an almond orchard near Davis, California in February 1976 (pollen was collected hourly from the pollen traps and frozen in liquid nitrogen and stored in a freezer until used); and bee bread (from the 1976 pollen) stored in comb cells for 7, 21, and 42 days. On the basis of uniform color and size and microscopic examination of the pellets, the corbicular pollen was at least 98.9 % almond pollen.

To obtain the bee bread, four colonies of honey bees were established in September 1976 and maintained in a polyethylene greenhouse. Each colony contained nine frames, one frame with sealed and

unsealed brood, and the others containing only drawn combs. Queens did not have access to the combs. At 4-hour intervals, two almond pollen pellets were placed in each cell on both sides of three drawn combs from each colony. During the 4-hour intervals, the bees packed the pollen into the cells. After 2 or 3 days, most of the comb cells were 1/2-3/4 filled with packed pollen. The bees had access only to water and the almond pollen. Then, three combs from each colony containing the packed pollen were placed in a clean room maintained at 34 °C and 55-60 % RH to simulate storage in the colony and to avoid possible overgrowth of the pollen by molds. On the sampling dates, the bee-bread was extracted by freezing the comb in liquid N_2 and « popping » the contents out. Each bee bread sample was a composite collection from all the combs that contained pollen pellets to be sampled on the appropriate dates.

Fatty Acid Analyses

The samples were brought to an equal moisture content, and 1-gram subsamples were placed in a Soxhlet extractor (50 μl of C_{15} acid was added as an internal standard). The pollen was extracted for 16 hours in $CHCl_3$-MeOH (2 : 1). The extract was then brought to dryness on a rotary evaporator at 40 °C. The extract was acidified to *p*H 2-3 with 0.1 N HCl and extracted three times with hexane. The hexane extracts were taken to dryness on a rotary evaporator and weighed. The lipoidal residues were saponified in 4 ml of ethanolic KOH. After saponification and removal of the non-saponifiables, the fatty acids were extracted in hexane.

The fatty acids were methylated in 5 ml of boron trifluoride (12 %) in MeOH by heating at 100 °C for 5 minutes. The methylated fatty acids were separated by gas liquid chromatography (GLC) using a 3.05 m glass column packed with 12 % diethyleneglycolsuccinate using N_2 as the carrier gas. The detector and injector temperatures were held at 225 °C; the column was at 160 °C for 10 min, and then programmed to 220 °C at 2°/min. The areas under the peaks were electronically integrated. Identification of the fatty acids was accomplished by comparison of retention times with standards chromatographed the same day. The data were expressed as percentages of each acid. Each sample was chromatographed twice.

Sterol Analyses

Almond pollen extraction and separation of esterified and unesterifield sterols. The pollen was mechanically stirred and extracted twice with a solvent mixture of $CHCl_3$-MeOH-H_2O [3 : 3 : 2) 7 ml/g of pollen]. The extract was filtered and combined filtrates were concentrated under vacuum to near dryness and diluted with a solution of 15 % NaCl and the residue was reextracted with dichloromethane. The dichloromethane was washed with water, dried over Na_2SO_4, and removed under vacuum. Chromatography of the residue over 15 g of Woelm ® [6] neutral alumina activity grade II (1 g of residue/30 g of alumina) using 50 ml each of the following solvents : hexane, hexane-C_6H_6 (1 : 1), C_6H_6, C_6H_6-Et_2O (3 : 1) and Et_2O gave hydrocarbons, sterol esters and waxes, triglycerides, alcohols, and sterols, respectively. The sterol ester and wax fractions were saponified and the esterified sterols were similarly isolated. The esterified and unesterified sterols were separately acetylated with Ac_2O in pyridine.

Separation and Identification as Sterol Acetates of Unesterified and Esterified Sterols. The unesterified sterol acetates, when analyzed by GLC on an SE-30 column, showed a major peak and a minor peak for sterol acetates, whereas thin layer chromatographic (TLC) analyses ($AgNO_3$-Silica Gel) showed three spots for sterol acetates. Thus, the sterol acetates were chromatographed over 18 g of 20 % $AgNO_3$-Unisil column (1,4 cm × 28 cm) and eluted with 50-ml vol. containing 1, 2, 3, 4, 5, 6, and 10 % ether in hexane. The fractions monitored by GLC and TLC ($AgNO_3$-Silica Gel) analyses showed the following distribution of sterol acetates : hexane-ether (98 : 2) sitosterol, hexane-ether (96 : 4) isofucosterol, and all other fractions thereafter, 24-methylenecholesterol.

The esterified sterol acetates were similary separated and purified. The sterol acetates of both the unesterified and esterified sterols were quantitatively analyzed by GLC on an SE-30 column.

(6) Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

Analyses of Vitamin Content

Pooled pollen samples from each treatment and date were analyzed for ascorbic acid, pantothenic acid, niacin, inositol, and pyridoxine (WARF Institute Reports, 1977).

Mineral Analyses

Mg, Ca, K, Zn, and Pb were determined by flame absorption spectrometry on a Perkin-Elmer atomic absorption spectrophotometer. One-gram samples were placed in digestion containers and alternate aliquots of concentrated HNO_3 and H_2O_2 were added. Samples were boiled and dried at 130 °C until the residual ash was white or light gray. Usually three cycles were sufficient. Residues were taken up in dilute HCl and determined directly for Zn and Pb. For Mg, Ca, and K analyses, the samples were diluted 50-100 times to provide concentrations in the proper absorption range.

RESULTS

Fatty Acids and Sterols

Table 1 reports the fatty acid and sterol composition of the hand-collected and corbicular almond pollen. The lipid extraction and fatty acid analysis of the bee bread samples revealed that the method of extracting the bee bread resulted in beeswax and larval cocoon contamination. Therefore, those data are not presented. Although there was an increase in crude lipids, the corbicular pollen had a fatty acid composition very similar to that of the hand-collected pollen. There did appear to be a small shift toward more saturation among the 18-carbon acids in the corbicular pollen.

In all samples, the principal sterol was identified as 24-methylenecholesterol; two minor sterols were sitosterol and isofucosterol (Table 1). The identities of the sterols were confirmed by GLC, TLC, infrared, and mass spectral analyses. The concentration of sitosterol in hand-collected pollen was considerably greater than that found in the corbicular pollen (Table 1). Conversely, the content of the unesterified 24-methylenecholesterol was considerably lower in the hand-collected pollen than in the corbicular pollen.

Vitamins

The results of the vitamin assays are given in Table 2.

Ascorbic acid. There was insufficient hand-collected pollen for this analysis. Two analyses of the corbicular pollen revealed 20,6 mg of ascorbic acid/100 g; the concentration of ascorbic acid in bee bread decreased to 5.9 mg/100 g after only 7 days of storage. Ascorbic acid concentration slowly decreased to 4.7 mg/100 g after 6 weeks of storage.

Pantothenic acid. Hand-collected pollen contained 3.38 mg/100 g, but the corbicular pollen contained only 0.76 mg/100 g. The concentrations in the bee bread were between 1.87 and 2.40 mg/100 g – about 2/3 of the pantothenic acid content of hand-collected pollen.

TABL. 1. — *Fatty acid and sterol composition of hand-collected and corbicular almond pollen.*

Fatty Acids				Sterols[a]		
% in $CHCl_3$: MeOH (2 : 1) Extract				Mg/100 g of Sample		
Name, carbon atoms and double bonds	Average[b] or Range	Hand-collected	Corbicular	Name and Esterification State	Hand-collected	Corbicular
Caprylic (8)	Av.	0.22	0.06	*Sitosterol*		
	Range	0.19-0.26	Tr[c]-0.06	unesterified	79	7
Capric (10)	Av.	N.D.[d]	0.56	esterified	75	31
	Range	—	0.41-0.71	*Isofucosterol*		
Lauric (12)	Av.	0.43	0.90	unesterified	4	7
	Range	0.41-0.45	0.82-0.99	esterified	9	8
Myristic (14)	Ave.	0.54	0.22	*24-methylene-cholesterol*		
	Range	0.52-0.57	0.22-0.23	unesterified	164	247
Palmitic (16)	Av.	23.34	26.46	esterified	264	297
	Range	22.46-24.23	26.20-26.73			
Stearic (18)	Av.	3.89	4.09			
	Range	3.72-4.06	3.99-4.19			
Oleic (18 : 1)	Av.	10.41	15.78			
	Range	10.26-10.56	15.48-16.08			
Linoleic (18 : 2)	Av.	33.46	26.20			
	Range	33.23-33.70	25.61-26.79			
Linolenic (18 : 3)	Av.	22.61	18.71			
	Range	22.36-22.86	18.24-19.22			
Arachidic (20)	Av.	1.71	3.20			
	Range	1.39-2.01	2.96-3.43			
Behenic (22)	Av.	2.27	1.78			
	Range	2.17-2.37	1.29-2.26			
Lignoceric (24)	Av.	0.59	0.32			
	Range	0.18-1.00	Tr-0.32			
% Extractable lipids		0.15	3.96			

[a] Quantitated and reported as sterol acetate.
[b] Average of 2 gas chromatographic analyses of one extract.
[c] Tr = Trace.
[d] N.D. = Not Detectable.
[a] Quantitated and reported as sterol acetate.
[b] Average of 2 gas chromatographic analyses of one extract.

Niacin. The highest concentration was in the hand-collected pollen (8.05 mg/100 g); a gradual loss occurred during the 42 days to a low of 6.20 mg/100 g.

Inositol. There was a decrease with time from a high of 228 mg/100 g in the hand-collected pollen to 137 mg/100 g in the 42-day bee bread sample.

TABL. 2. — *Vitamin content and titratable acidity of hand- and honey bee-collected (corbicular) almond pollen before and 7, 21, and 42 days after storage in comb cells.*

Vitamins	Concentration (mg/100 g)				
	Hand-collected Pollen	Corbicular Pollen	Stored Pollen *Days in Comb Cells*		
			7	21	42
Ascorbic acid	N.A.[a]	20.6	5.9	5.5	4.7
Panthothenic acid	3.38	0.76	2.40	1.87	2.19
Niacin	8.05	7.10	6.17	6.63	6.20
Pyridoxine	0.91	0.61	0.45	0.42	0.42
Inositol	228	188	156	191	137
Titratable acidity (ml)	0.8[b]	9.2[c]	5.8[c]	6.1[c]	6.2[c]

[a] N.A. - Not analyzed, insufficient sample.

[b] 1 g pollen dispersed in 100 ml boiled water required 0.8 ml of 0.05 *N* base to reach neutrality with phenolphthalein indicator.

[c] ml of 0.5 *N* base required to bring 1 g sample in 100 ml water to *p*H 8.2 (too highly colored to give phenolphthalein end point).

Pyridoxine. The hand-collected pollen (0.91 mg/100 g) had 1/3 more pyridoxine than the corbicular pollen (0.61 mg/100 g). The bee bread had a nearly constant level (0.45-0.42 mg/100 g) that was a little less than 1/2 of that found in the original pollen.

PH and Titratable Acidity

The *p*H was not determined in the hand-collected pollen. The corbicular pollen had a relatively low *p*H (4.2), and the fermented bee bread was in the range expected for lactic-acid anaerobic processes (4.5). Titratable acidity (Table 3) was very low in the

TABL. 3. — *Minerals (ppm, dry weight basis) analyzed in honey bee-collected (corbicular) almond pollen before and 7, 21, and 42 days after storage in comb cells.*

Mineral	PPM[a]			
	Corbicular pollen	Stored Pollen Days in comb cells		
		7	21	42
Mg	775	675	475	649
Ca	1 150	920	780	910
K	5 670	5 850	3 456	5 050
Zn	103	93	54	81
Pb	6	15	6	6

[a] Results are from 1 sample digest run on a Perkin-Elmer atomic absorption spectrometer.

hand-collected pollen (0.8 ml of 0.05 N NaOH), and there was a large (115-fold) increase in the acidity of corbicular pollen (9.19 ml of 0.5 N NaOH). The acidity in bee bread dropped to a relatively uniform level (5.8 - 6.2 ml of 0.5 N NaOH).

Minerals

The contents of five minerals (Ca, Mg, K, Zn, and Pb) determined by atomic absorption spectrometry in one sample from each date are given in Table 3.

DISCUSSION

Lipoidal and acidic additions by the bee constituted the greatest differences between hand-collected and corbicular pollen noted in this study. The large change in titratable acidity is probably due to the addition of mandibular and hypopharyngeal gland secretions (LUKOSCHUS *et al.*, 1968). It is presumed that varying amounts of sugars were also added by the bees at this time. There was also a remarkable drop in sitosterol content, although changes in the other sterols were minor. Semi-quantitative analyses for sterols in the bee bread samples indicated that there were no further large changes in sterol content, i.e. 24-methylenecholesterol remained the dominant sterol. This sterol has been found in the tissues of queen and worker bees (BARBIER and SCHINDLER, 1959) and in royal jelly (BROWN *et al.*, 1961).

Although absolute vitamin requirements have not been established for honey bees, DIETZ (1975) recommends diets with large quantities of ascorbic acid. The loss of ascorbic acid (and to a lesser extent, all the vitamins) during fermentation of the bee bread, undoubtedly influenced by the large changes in microbial flora reported by GILLIAM (1979 a, 1979 b), should be investigated further to see if nutritional values were affected by this apparent loss.

The mineral element analysis was not extensive, but the results are similar to those reported by NATION and ROBINSON (1971) and indicate that almond pollen is not unique in its mineral content. The decreases in mineral concentration in the bee bread samples probably reflects dilution due to the addition of lipid material. The relatively high lead content may reflect the fact that the orchard was near a major highway.

In brood rearing bioassays of the relative nutritional value of several different species of pollens, we have found almond pollen (not bee bread) to be one the most adequate nutritionally although the coefficient of variation of test means (45 %) was almost twice that found with saguaro (*Cereus giganteus* Engelm.) (LOPER and BERDEL, unpublished (3)).

(3) LOPER, G. M., BERDEL, R. L., 1980. — A nutritional bioassay of honeybee brood-rearing potential. (Unpublished, to be submitted to Apidologie.)

This paper, along with those of GILLIAM (1979, 1980) and STANDIFER *et al.* (1980), provides microbiological and chemical information concerning what happens to almond pollen during storage; further work is necessary to determine the effect of storage on the nutritional value of bee bread derived from almond pollen.

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RÉSUMÉ

BIOCHIMIE ET MICROBIOLOGIE DU POLLEN D'AMANDIER *(PRUNUS DULCIS)* RÉCOLTE PAR L'ABEILLE ET DU PAIN D'ABEILLES.
I. ACIDES GRAS, STÉROLS, VITAMINES ET MINÉRAUX

Le pollen d'amandier *(Prumus dulcis)* a été récolté à la main et par des abeilles (pollen de corbicule) pour être analysé chimiquement. Le pollen de corbicule a été tassé par les abeilles dans les rayons pour sa transformation (par fermentation naturelle) en pain d'abeilles. On a effectué les analyses chimiques de 12 acides gras, 3 stérols, 3 vitamines, de l'inositol, de l'acidité titrable et de 5 minéraux sur le pollen récolté à la main et le pollen de corbicule. Au bout de 7, 21 et 42 jours de conservation on a analysé dans le pain d'abeilles les mêmes substances, sauf les acides gras et les stérols. Les variations dans les pourcentages d'acides gras ont été faibles; il y a eu une augmentation des acides palmitique et oléique et une diminution des acides linoléique et linolénique des échantillons de pollen récolté à la main aux échantillons de pollen de corbicule. On a isolé dans les échantillons de pollen le sitostérol, l'isofucostérol, le 24-méthylénécholestérol. Le sitostérol est particulièrement instable puisqu'il s'en perd environ 75 % entre le pollen récolté à la main et le pollen de corbicule. On a déterminé les teneurs en 4 vitamines et en inositol; l'acide ascorbique est particulièrement instable dans les cellules des rayons. A la fin du test, le pain d'abeille ne contenait plus qu'un quart de la teneur en acide ascorbique du pollen de corbicule. Les teneurs en pyridoxine ont également diminué de moitié. Puisque l'acidité titrale du pollen de corbicule a augmenté 115 fois par rapport au pollen récolté à la main, on suppose que les abeilles ont ajouté au pollen des sécrétions acides des glandes mandibulaires et hypopharyngiennes. Le stockage du pollen dans les rayons a réduit l'acidité titrable d'un tiers par rapport à celle du pollen de corbicule. On a déterminé les quantités de Mg, Ca, K, Zn et Pb dans des échantillons de pollen de corbicule et de pollen stocké. En conclusion, nous avons montré divers changements dans la composition chimique du pollen et du pain d'abeilles qui pourraient influencer la valeur nutritive du pain d'abeilles résultant.

ZUSAMMENFASSUNG

BIOCHEMIE UND MIKROBIOLOGIE VON MANDELPOLLEN (*PRUNUS DULCIS*) AUS DEN POLLENKÖRBCHEN DER BIENEN UND AUS BIENENBROT
I. FETTSÄUREN, STEROLEN, VITAMINE UND MINERALE

Pollen der Mandel *(Prunus dulcis)* wurde für die chemische Analyse sowohl von Hand wie von Bienen (als Pollenhöschen) gesammelt. Der Höschenpollen war von den Bienen in Waben zur Umwandlung in Bienenbrot (durch natürliche Fermente) gelagert worden. Sowohl von handgesammelten wie von Höschenpollen wurden chemische Analysen zur Bestimmung von 12 Fettsäuren, 3 Sterolen, 3 Vitaminen, Inositol, tritrierbarer Azidität und 5 anorganischen Elementen durchgeführt. Die Ergebnisse sind in den Tabellen 1 - 3 zusammengestellt.

Die Veränderungen im Prozentsatz der Fettsäuren waren gering. Es besteht eine Vermehrung von Palmitin- und Öläure und eine Verminderung von Linol- und Linolensäure beim Höschenpollen gegenüber den handgesammelten Pollenproben. Aus den Pollenproben wurden Sitosterol, Isofucosterol, und 24-Methylencholesterol isoliert. Sitosterol erwies sich als besonders instabil, es zeigte sich in Verlust von etwa 75 % des Gehaltes von handgesammelten bei dem Höschenpollen.

Es wurde der Gehalt an 4 Vitaminen und an Inositol bestimmt; Ascorbinsäure war in den Wabenzellen in besonderem Masse unstabil. Am Ende der Versuche hatte Bienenbrot nur 1/4 des Gehaltes an Ascorbinsäure wie der ursprüngliche Höschenpollen. Auch der Gehalt an Pyridoxin nahm auf die Hälfte des ursprünglichen Wertes ab. Da der Gehalt an tritrierbaren Säuren des Höschenpollens 115 fach gegenüber dem handgesammelten erhöht war, wird angenommen, dass die Bienen dem Pollen saure Sekrete aus den Mandibel- und Hypopharyngealdrüsen zugesetzt haben. Die Lagerung in der Wabe reduzierte die tritrierbare Azidität um 1/3 gegenüber dem Höschenpollen.

Die Mengen and Mg, Ca, K, Zn und Pb wurden sowohl in Höschen- wie in Vorratspollen bestimmt.

Zusammenfassend kann gesagt werden, dass wir mehrere Veränderungen in der chemischen Zusammensetzung von Pollen und Bienenbrot festgestellt haben, welche den Nährwert des entstandenen Bienenbrotes beeinflussen könnten.

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