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CHEMICAL COMPOSITION AND NUTRITIVE VALUE OF BEE-COLLECTED AND BEE-STORED POLLEN

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SUMMARY

When fresh pollen and bee-stored pollen extracted from brood combs of free-flying colonies were offered to caged colonies of bees, there appeared to be no difference in nutritive value judged by the number of bees reared to the sealed stage.

Bee bread and pollen from seven locations were analyzed for moisture, protein, reducing and nonreducing sugars, lipids, sulfated ash, starch, pH, pectins and crude fiber. The levels of protein, moisture, and lipids differed little between pollen and bee bread. However, there was no detectable starch in any of the samples of bee bread; and starch was present in all seven samples of pollen (mean value of 1.77 %). In addition, bee bread contained higher levels of reducing sugar and crude fiber than pollen but lower levels of ash. The ash of all pollen samples ranged between 2.4 and 3.4 % of the dry weight. The pH of bee bread averaged 4.1 compared with 4.8 for pollen. The percentage of undetermined matter in pollen and bee bread averaged less than 10 % of the dry weight.

INTRODUCTION

Pollen substitutes formulated and tested at the Beltsville Bioenvironmental Bee Laboratory have been based on the levels of essential nutrients found in pollen, the predominant source of proteins, lipids, minerals, and vitamins in the diet of the honey bee, *Apis mellifera* L. However, the chemical composition of pollen varies with plant species, environment during pollen development, age of plant when pollen developed, nutrient status of the plant, methods of pollen extraction, and storage. Also this large variability in chemical composition is associated with an equally large variability in food value for the bee. For example, pollen of different plants do not have the same physiological effects (MAURIZIO, 1954, TODD and BREATHERICK, 1942, LUNDEN, 1954,

VIVINO and PALMER, 1944, LOUVEAUX, 1963, HUGEL, 1962); and nutritive value was not entirely correlated with protein quantity (TODD and BREThERICK, 1942).

Bees do not consume pollen as collected by foragers. The pollen pellets are stored in cells; and honey, nectar, or glandular secretions are added to the mass of stored pollen. Pollen stored in this way undergoes a lactic acid fermentation and becomes what is called "bee bread". Stored pollen generally has a specific bacterial flora associated with it (EGOROVA, 1971, PAIN and MAUGENET, 1966); and PAIN and MAUGENET (1966) found three microbial genera in bee bread: *Pseudomonas*, *Lactobacillus*, and *Saccharomyces*. This suggests that microorganisms are probably involved in the storage of pollen.

Bee bread stored in combs therefore differs from fresh bee-collected pollen, but the fermentation may be responsible for increased stabilization of the product or may lead to chemical changes that increase digestibility and nutritive value for the bee. BEUTLER and OPFINGER (1949) found that bees lived longer on pollen removed from combs than from pollen traps.

We therefore compared the chemical properties of bee bread and pollen and also fed these materials to caged colonies of newly emerged bees to determine the brood rearing capability of each. A greater nutritive value of bee bread would suggest that our diets should be based on the chemical composition of bee bread, not of pollen.

MATERIALS AND METHODS

Bees

Bee-collected pollen obtained from pollen traps and stored pollen (bee bread) extracted from brood combs of free-flying colonies were offered to test colonies established in small queen-mating nucleus colonies (23 × 19 × 27 cm) located at Beltsville, Maryland. Each nucleus consisted of a mated queen, 400 g of newly emerged bees, and five drawn combs (2.5 × 16 × 23 cm) free of any honey or pollen. As soon as the nuclei were established, the bees were offered the bee bread or pollen diets by inverting petri dish lids (100 × 15 mm) containing 50 g of diet over the brood combs. Then each nucleus was placed in a 2 × 2 × 2 m screen flight cage, and the bees were supplied with a 50% (w/v) sucrose solution that was replenished twice a week when the diets were changed. After the first sealed brood appeared, the number of sealed cells was estimated once a week by using a wire grid of 6.25 cm² divisions.

Chemical Analysis

Bee-collected pollen and stored pollen obtained from seven different geographic locations in the U.S. were analyzed as follows:

The percent moisture in pollen and bee bread was determined by placing 5 g of each sample in a hot-air oven at 100 °C for 24 hr. After the samples were removed from the oven, they were allowed to reach room temperature and then weighed. The difference between dry weight and the initial weight was used to calculate percent moisture.

Total nitrogen content was determined by the AOAC Micro-Kjeldahl method (Association of Official Agricultural Chemists, 1960).

Total reducing and nonreducing sugars were determined by modifying AOAC method No. 13.028 for sugars in cereal foods. The quantity of reducing sugar was calculated as maltose for the Maltose-Sucrose

Conversion Table (No. 13.030). The nonreducing sugar was reduced after hydrolysis and calculated as sucrose by using the same table.

The percent of crude lipids in each sample was determined by using a chloroform-methanol extraction. The chloroform layer was collected and washed three times with 100 ml of a 0.1 % NaCl solution. The sample was dried, and the weight difference was used to calculate percentage crude lipid.

Sulfated ash in samples was determined according to AOAC Method No. 29.014.

The percentage starch in the samples was compared with a standard curve derived by taking measurements of a series of test tubes containing from 10 mg to 100 mg of soluble starch suspended in 20 ml of distilled water to which 2 ml of KI solution were added. The percentage transmittance was measured in a Bausch and Lomb Spectronic 20 at 450 nm and used to plot the standard curve.

The percentage of crude fiber and pectins in the pollen and bee bread sample was determined according to LEES (1971).

RESULTS AND DISCUSSION

There was no difference in the nutritive value of bee bread and pollen when based on the number of bees reared to the sealed stage. Bees fed either the pollen or the bee bread patties reared $7,195.50 \pm 243.75 \text{ cm}^2$ and $7,046.00 \pm 152.93 \text{ cm}^2$ larvae, respectively, to the capped stage. However, bees fed pollen did not initiate brood rearing immediately after they were offered the diet, probably because the bees were all newly emerged. Consumption of the diet was poor for the first 4 to 5 days. Several authors have observed that newly emerged bees do not feed on pollen immediately after they emerge, and this may account for the retarded brood rearing. HAYDAK (1933) found that most newly emerged bees only start to eat pollen between 12 to 18 hr after emergence. HAGEDORN and MØLLER (1967) stated that newly emerged bees consume small amounts of pollen for the first 40 hr; thereafter, the rate of pollen consumption increases greatly. However, by the time the bees were 42 hr old, 90 % had consumed some pollen. In contrast, the first sealed cells in nuclei that were fed bee bread were observed 12 days after the queens were released. The levels of brood rearing remained relatively constant throughout the test period.

The chemical components of bee collected pollen and bee bread are summarized in Table 1 and 2 respectively. Values are expressed on a dry-weight basis. The last column represents the mean value for all seven locations. Most ranges were fairly uniform. Moisture, for example, ranged from 21.7 % in the Wyoming sample to a high of 27.0 % in the California sample; reducing sugars ranged from 12.6 % to 30.2 % (average 20.71 %), and nonreducing sugars ranged from 1.0 to 2.5 %. Our results were therefore similar to those of TODD and BRETHERICK (1942) who analyzed and found that the content of crude protein ranged from 7.02 to 29.87 %.

The values for starch, lipids, and sulfated ash obtained from all pollen samples were remarkably similar; starch was present in all seven samples (mean value of 1.77 %). The total percent of crude lipids ranged from 4.03 % (Arizona) to 5.48 % (Maryland), and the ash content ranged from 2.4 to 3.5 % (mean of 3.17 %).

TABLE 1. — Chemical analyses of bee-collected pollen (a) from seven locations (b)

Test	Md.	N.-J.	Wisc.	Wyo.	Ariz.	La.	Cal.	Avg.
Moisture (%)	24.0	23.1	26.6	21.7	22.6	25.2	27.0	24.31
Protein (%)	21.9	21.3	29.3	22.1	21.0	27.1	25.9	24.1
Reducing sugars (%)	15.9	21.8	21.3	30.2	20.7	22.5	12.6	20.7
Nonreducing sugars (%)	1.0	2.4	2.4	2.5	2.4	2.4	1.9	2.1
Starch (%)	2.9	2.2	2.9	0.8	0.9	1.0	1.8	1.8
Lipids (%)	5.5	5.1	4.2	5.3	4.0	4.9	5.2	4.9
Sulfated ash (%)	3.2	2.4	3.4	3.4	3.5	2.9	3.5	3.2
pH	4.4	4.1	4.5	5.9	5.8	4.6	4.4	4.8
Crude fiber (%)	7.2	8.4	4.2	7.4	11.4	7.9	(c)	7.7
Pectins (%)	1.9	1.3	0.9	1.0	0.9	0.4	(c)	1.6

(a) Determinations on a dry weight basis.
(b) Average of three replications.
(c) Insufficient sample.

TABLE 2. — Chemical analyses of bee-stored pollen (a) from seven locations (b)

Test	Md.	N.-J.	Wisc.	Wyo.	Ariz.	La.	Cal.	Avg.
Moisture (%)	28.0	23.8	21.3	18.8	21.5	27.8	25.4	23.8
Protein (%)	21.1	23.1	26.5	25.8	22.8	19.3	24.5	23.3
Reducing sugars (%)	28.2	23.7	21.8	37.3	34.7	35.3	14.0	27.9
Nonreducing sugars (%)	1.9	1.5	1.0	5.7	2.9	2.9	1.5	2.5
Starch (%)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Lipids (%)	3.9	4.7	4.8	5.8	6.7	6.1	5.6	5.4
Ash (%)	3.0	2.1	3.2	2.5	3.1	2.6	2.9	2.8
pH	4.3	4.1	3.9	3.8	4.2	4.2	4.0	4.1
Crude fiber (%)	7.1	6.6	18.5	9.1	9.3	5.8	17.7	10.6
Pectins (%)	0.2	0.4	0.4	0.7	0.5	0.5	(c)	0.5

(a) Determinations on a dry weight basis.
(b) Average of three replications.
(c) Insufficient sample.

The percentage of ash we found in our study was within the range reported by earlier authors. For example, TODD and BRETHERICK (1942) reported that the ash content of bee-collected pollen ranged from 0.91 to 6.36 % with a mean of 2.70 %. Also, they found no great difference in ash levels between hand- and bee-collected pollens. The higher levels of ash that we found in pollen (3.17 %) compared with that in bee bread (2.76 %) was, however, surprising. One would suspect that bee bread contains higher levels due to the addition of honey. WHITE *et al.* (1962) and WHITE (1965) found levels of ash in excess of 1 % in honey samples.

The lipid content of pollen and bee bread in our seven samples ranged from 3.86 % to 6.73 %. TODD and BREThERICK (1942) analyzed 32 plant species, five hand-collected and 27 bee-collected, and found a wide range in the ether extract of bee-collected pollen (0.94 to 14.44 % with a mean of 4.96 %). The highest values were found in dandelion and mustard, and the lowest in birch brush and buckthorn. STANDIFER (1966) determined the lipid constituents of 16 species of bee-collected and three species of hand-collected pollen and found the total lipid content ranged from 1.5 % in the heartsease to 18.9 % in dandelion, a mean of 9.2 %.

We found starch present in pollen but not in bee bread. However, when 100 mg bee bread or pollen were mixed with concentrations of soluble starch ranging from 10 mg to 100 mg in 20 ml H₂O (Table 3), starch hydrolysis was rapid. After 1 day, five of 10 tubes incubated with bee bread had hydrolyzed the soluble starch, and all the starch was hydrolyzed after 2 days. The results after 1 day were not conclusive. We added the 100 mg aliquots of bee bread to the tubes without regard to the portion of the pellet used, but we subsequently found that most of the enzymatic activity occurred in the top portion of the pellet containing the honey layer. None of the pollen samples hydrolyzed the starch during 12 days of incubation. Thus the lack of starch in bee bread apparently results from the honey and enzymatic secretions added to the pollen as it is packed by the honey bees. Amylase and invertase are, in fact, secreted by the bee food glands (RINAUDO *et al.* 1973), but starch has not been found in nectar (WHITE, 1967); so the function of amylase in honey is not clear. WHITE *et al.* (1962) tested 490 samples of honey and found amylase present in varying amounts in nearly all honeys.

TABLE 3. — *Amylase activity (a) in bee-collected pollen and bee bread*

Amount (mg) soluble starch/20 ml H ₂ O	Bee bread			Pollen							
	Day			Day							
	0	1	2	0	1	2	5	7	9	12	
10	—	+	+	—	—	—	—	—	—	—	—
20	—	+	+	—	—	—	—	—	—	—	—
30	—	—	+	—	—	—	—	—	—	—	—
40	—	+	+	—	—	—	—	—	—	—	—
50	—	—	+	—	—	—	—	—	—	—	—
60	—	+	+	—	—	—	—	—	—	—	—
70	—	—	+	—	—	—	—	—	—	—	—
80	—	—	+	—	—	—	—	—	—	—	—
90	—	+	+	—	—	—	—	—	—	—	—
100	—	—	+	—	—	—	—	—	—	—	—

(a) — : no amylase activity; + : starch hydrolysis.

The concentration of reducing sugars in pollen shown in Table 2 is not a true value for pollen as produced by the plant. Also the content of sugars in pollen from pollen traps is considerably greater than the content in pollen gathered by hand. LUNDEN (1954) reported that bee-collected pollens always contain larger amounts of reducing sugars because of the honey or nectar in the fluid used to cement the grains together. Maltose is the most common reducing disaccharide found in nature as a constituent of the polysaccharides, starch, and glycogen. Sucrose is the only important nonreducing disaccharide.

The higher level of reducing sugar in bee bread therefore probably results from the addition of honey or nectar as the pollen is prepared for storage. The added honey reduces the pH from 4.8 in pollen to an average of 4.07 in bee bread, which facilitates the growth of some apparently desirable microorganisms (EGOROVA, 1971).

A considerable percentage of undetermined matter remained after removal of water, ash, lipids, protein, sugars, and starch from pollen. Even after an additional analysis for crude fiber (mean 7.73 %) and pectins (mean 1.56 %), there were still some undetermined materials. Some authors have assumed these additional undetermined materials to be pigments, waxes, and resins. However, the percentage reported in this study (mean 9.59 % for pollen) was less than the values reported by TODD and BRETHERICK (1942). They found a range from 21.65 to 35.87 %, with a mean of 28.55 %. These authors attributed these high percentages in part to substances called "pollenin", which builds up the extremely resistant exterior pollen membranes. Pollenin contents were determined and found to be 21.9 % in spruce, 20 % in silver pine, and 7.3 % in hazelnut.

In sum, there was no observable nutritional difference between pollen and bee bread fed to caged colonies of bees based on number of larvae reared to pupation. Bee bread and pollen had similar levels of protein, moisture, and lipid. Starch was present in all pollen samples, but absent from bee bread. The levels of reducing and nonreducing sugar, ash, and crude fiber were within the range of experimental error. This information suggests that either pollen or bee bread might be used as a standard in the formulation of our future basal diets.

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ZUSAMMENFASSUNG

CHEMISCHE ZUSAMMENSETZUNG UND NÄHRWERT VON POLLEN, DER VON BIENEN GESAMMELT ODER GESPEICHERT WURDE

Wenn frisch gesammelter Pollen und aus Brutwaben freiliegender Völker entnommener, gespeicherter Pollen (Bienenbrot) an gekäfigte Bienenvölkchen verfüttert wurde, zeigte sich kein Unterschied des Nährwertes in bezug auf die Menge der bis zur Verdeckelung aufgezogenen Brut. Bienen, die entweder mit Pollen oder mit Bienenbrot gefüttert wurden, zogen 7,19550 cm² und 7,04600 cm² Brutflächen bis zur Verdeckelung auf.

Bienenbrot und Pollen aus sieben geographisch verschiedenen Orten der U.S.A. wurden auf Feuchtigkeitsgehalt, Proteine, reduzierende und nicht reduzierende Zucker, auf Lipide, sulfatierte Asche, Stärke, pH, Pektine und feste Bestandteile untersucht. Der Gehalt an Feuchtigkeit, Proteinen und Lipiden wies in Pollen und Bienenbrot nur geringe Unterschiede auf. In keiner der Bienenbrotproben war Stärke nachzuweisen, aber in allen sieben Pollenproben war Stärke vorhanden (Durchschnittswert = 1,77 %). Bienenbrot enthielt ausserdem mehr reduzierende Zucker und feste Bestandteile als der Pollen, aber weniger Asche. Die Asche aller Pollenproben betrug zwischen 2,4 % und 3,4 % des Trockengewichtes. Der höhere Gehalt des Bienenbrotes an reduzierendem Zucker ist vermutlich auf den Honig- oder Nektarzusatz bei der Pollenverarbeitung für die Speicherung zurückzuführen. Der zugesetzte Honig verringerte das pH von 4,8 im Pollen auf durchschnittlich 4,07 im Bienenbrot. Der Prozentsatz nicht bestimmter Substanzen in Pollen und Bienenbrot betrug durchschnittlich weniger als 10 % des Trockengewichtes.

RÉSUMÉ**COMPOSITION CHIMIQUE ET VALEUR NUTRITIVE DU POLLEN
RÉCOLTÉ PAR LES ABEILLES ET DU POLLEN STOCKÉ DANS LA RUCHE**

Après avoir offert à des nucléi d'abeilles en cagettes du pollen frais et du pollen stocké, extrait de rayons de couvain de colonies volant librement, on n'a noté aucune différence dans la valeur nutritive évaluée d'après le nombre d'abeilles élevées jusqu'au stade de couvain operculé. Les abeilles ayant reçu soit du pollen, soit du pain d'abeilles ont élevé respectivement 7 195,50 cm² et 7 046,00 cm² de larves jusqu'au stade operculé.

On a analysé dans du pain d'abeilles et du pollen provenant de 7 localités différentes des États-Unis la teneur en eau, les protéines, les sucres réducteurs et non-réducteurs, les lipides, les cendres sulfatées, l'amidon, le pH, les pectines et les constituants bruts. Les teneurs en protéine et en eau et les lipides diffèrent peu du pollen au pain d'abeilles. Pourtant, on n'a décelé aucune trace d'amidon dans aucun des échantillons de pain d'abeilles, alors que l'amidon était présent dans les 7 échantillons de pollen (valeur moyenne de 1,77 %). De plus, le pain d'abeilles contenait de plus fortes teneurs en sucres réducteurs et en constituants bruts, mais des teneurs plus faibles en cendres. Les cendres de tous les échantillons de pollen représentaient de 2,4 à 3,4 % du poids sec. La teneur plus élevée en sucres réducteurs du pain d'abeilles résulte probablement de l'addition de miel ou de nectar au moment où le pollen est préparé à être stocké. Le miel ainsi ajouté abaisse le pH de 4,8 dans le pollen à 4,07 en moyenne dans le pain d'abeilles. Le pourcentage de substances non déterminées dans le pollen et le pain d'abeilles est en moyenne inférieur à 10 % du poids sec.