Heat Treatment of Pollens: Impact on Their Volatile Flavor Constituents

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Three different heat treatments of honeybee-collected pollens were compared. Twenty hours at 30 °C decreases the water activity to below 0.6 without modifying the free amino acid content, thus avoiding further synthesis of undesirable flavor compounds. More drastic treatment (40 or 60 °C) can lead to high amounts of dimethyl sulfide (cooked-onion flavor), mainly when pollens rich in *S*-methylmethionine are used. Moreover, many Strecker aldehydes may be produced in concentrations above their perception thresholds.

Keywords: *Pollen; flavor; water activity; dimethyl sulfide; S-methylmethionine; bee*

INTRODUCTION

Honeybee-collected pollen constitutes a potential source of energy and proteins for human consumption (Chauvin, 1959, 1987; Dillon and Louveaux, 1987; Hügel, 1962; Schmidt and Schmidt, 1984). Over the last decade, the use of pollen, pure or mixed with other food products (honey, baby-foods, ...), has become widespread. It is therefore surprising that few scientific studies have been devoted to quantifying the chemical constituents of pollen in relation to its organoleptic properties. Moreover, few analytical methods have been proposed for this food in order to determine properties such as moisture content, water activity, amino acid distribution, or volatile composition.

Each pollen has its own specificity, mainly linked to the floral species or cultivars (Baker and Baker, 1981; Grunfeld, 1989; Zauralow, 1983). No information is yet available for choosing the best samples, i.e., ones which will not emit unpleasant flavors after the drying process currently applied by beekeepers to achieve longer storage life. The most commonly used preserving methods are open air-drying, artificial heating, freezing, and silica gel treatment (Jéanne, 1983; Serra Bonvehi and Lopez Allegret, 1986; Serra Bonvehi et al., 1987). For commercial scale production, pollen dried by artificial heating is the most favored. A 5–6% water content is usually obtained by raising the temperature to 40–45 °C. Unfortunately in some cases, this temperature tends to alter the organoleptic properties, mainly increasing pungent flavors related to sulfur compounds.

In the present work, we have analyzed moisture levels in two different pollens during drying at 30, 40, and 60 °C, in relation to the water activity, volatile compound content, and free amino acid distribution. One pollen was characterized by an intense passion fruit aroma; the second was more pungent. Our aim was to evaluate how heating affects organoleptic properties before the pollen reaches the 0.62 water activity threshold required to inhibit any osmophilic yeast growth.

MATERIALS AND METHODS

Pollen Samples. Two honeybee-collected pollens were harvested by a beekeeper in 1992, in the region of St. Ambroix (France; two different sites), by using a pollen trap. They were immediately stored in a freezer. After acetolysis, morphological analyses were performed on both samples. Results are given as a percentage of the total number of pollen grains. One pollen sample (I) was composed of sunflower (62%), dandelion (20%), crucifers (8%), graminaceae (7%), chenopodiaceae (1%), and rosaceae (1%), while the second (II) was 41% crucifers, 29% willow, 21% fruit tree, 7% ranunculus, 1% dandelion, and 1% chestnut tree. For heating, the samples were deposited in a 2 cm layer in a 30, 40, or 60 °C oven.

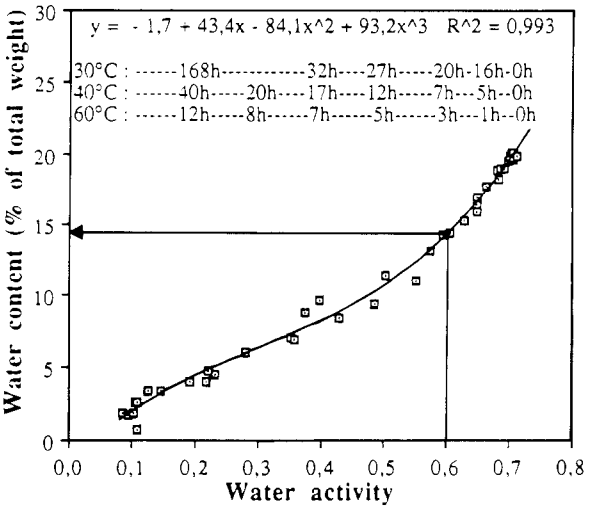

Figure 1. Water desorption isotherm of pollen I.

Reagents. Butyraldehyde, caproaldehyde, dimethyl sulfide, pentane-2,3-dione, poly(ethylene glycol) 2000, and valeraldehyde were purchased from Fluka Chemika. 2-Ethylfuran, heptanal, 2-methylbutyraldehyde, 2-methylvaleraldehyde, pentane, α-pinene, β-pinene, and sabinene came from Aldrich Chemicals. Acetone, butan-2-one, dimethyl disulfide, isobutyraldehyde, 3-methylbutyraldehyde, 2-methylfuran, and octane came from Janssen Chimica. Ethyl acetate, phosphorus pentoxide, and toluene were from UCB Chemicals. Trifluoroacetic acid came from Pierce. Perchloric acid and hexane were from Merck-Belgolabo. Methanol came from Lab-Scan.

Volatile Constituent Analysis. *Sample Preparation.* Four grams of pulverized pollen, mixed with 9 mL of volatile-free deionized water (Milli-Q water purification system, Millipore, Bedford, MA) and 250 μL of poly(ethylene glycol) 2000 (antifoam), was poured into the purge vessel maintained on ice. As an internal standard, 100 μL of 2-methylvaleraldehyde (9 ppm in water) was added. All the preparation steps were carried out at 4 °C.

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Table 1. Free Amino Acid Distributions of Pollens I and II (ppm)[a]

	pollen I						pollen II				
		1 day at			1 week at			1 day at		1 week at	
	fresh	30 °C	40 °C	60 °C	30 °C	40 °C	60 °C	fresh	40 °C	60 °C	40 °C	60 °C
aspartic acid	695 (16)	757	782	607	764	654	167	410	516	445	479	321
glutamic acid	394 (7)	405	473	181	431	350	36	689	654	536	603	303
proline	7373 (7)	8772	9302	6845	9513	7862	4802	7673	8202	3857	7934	2976
glycine	58 (2)	44	67	38	39	53	4	83	90	56	35	24
alanine	357 (3)	374	416	298	375	361	68	408	383	419	386	185
valine	83 (1)	90	104	62	91	84	16	199	227	164	187	97
methionine	12 (1)	12	18	–	17	12	–	–	–	–	–	–
isoleucine	46 (1)	46	55	27	53	39	7	94	98	73	73	10
leucine	50 (1)	51	68	63	56	53	–	71	85	82	74	–
tyrosine	107 (2)	120	139	48	144	107	–	40	39	21	–	–
phenylalanine	70 (0)	76	90	32	77	68	5	77	86	61	61	8
γ-aminobutyric acid	286 (3)	361	328	131	371	198	–	146	235	255	226	42
histidine	2564 (18)	3669	2585	1289	3426	2033	540	868	888	729	732	625
tryptophan	53 (1)	71	38	16	109	56	18	72	81	59	65	21
lysine	237 (1)	271	238	136	261	202	12	156	176	129	136	53
arginine	88 (2)	128	88	32	40	186	–	1368	1470	1179	1311	880
S-methylmethionine	43 (1)	48	43	(X)	46	19	2	421	426	(X)	327	86

[a] Average of heat treatment duplicates. Standard deviations are given for information in the case of fresh sample I (in parentheses). (–) undetected. (X) not measured.

Dynamic Headspace Injector Operating Conditions. A Chrompack purge-and-trap injector was used. Samples were injected into the chromatographic column in three steps as follows. (1) Precooling of the cold trap (CP-SIL8 CB capillary column, 0.53 mm i.d.; film thickness, 5 μm): the trap was cooled for 1 min with a stream of liquid nitrogen. (2) Purging of the sample: the temperature of the purge vessel was set at 40 °C. The sample was purged with nitrogen gas (10 mL/min) for 15 min. The gas stream was successively passed through a condenser kept at −15 °C by means of a cryostat (Colora WK 15) to remove water vapor and then through an oven at 200 °C. The volatiles were finally concentrated in the cold trap maintained at −95 °C (liquid nitrogen). (3) Desorption of the volatiles: cooling was stopped, and the surrounding metal capillary was immediately heated to 220 °C for 5 min. The carrier gas swept the trapped compounds into the analytical column.

Gas Chromatography Analytical Conditions. A Hewlett Packard Model 5890 gas chromatograph equipped with a flame ionization detector and an integrator (Shimadzu C-R3A) was used. Analysis of pollen volatile compounds was carried out on a 50 m × 0.32 mm, wall-coated, open tubular (WCOT) apolar CP-SIL5 CB capillary column (film thickness, 1.2 μm). Oven temperature, initially kept at 30 °C for 15 min, was programmed to rise from 30 to 100 °C at 2 °C/min, remaining at the maximum temperature for 15 min thereafter. Helium carrier gas was used at a flow rate of 1.5 mL/min. Injection and detection temperatures were 200 and 220 °C, respectively. All analyses were done in duplicate. The minimum peak area for data acquisition was set at 500 μV s. The assessment of the technique reproducibility has been previously described (coefficients of variation under 10% for five analyses of the same standard mixture; Collin et al., 1993).

Gas Chromatography–Mass Spectrometry Analytical Conditions. The column (see above) was directly connected to an HP 5988 quadrupole mass spectrometer. Electron impact mass spectra were recorded at 70 eV. Spectral recording throughout elution was automatically performed with the HP59970C MS Chemstation analytical workstation. Identification was done on the basis of peak enrichment by coinjection with authentic standard compounds and with the help of the NBS/EPA/NIH mass spectra library.

Calibration for Quantitative Analysis. Relative response coefficients (relative RC = (compound peak area (μV s)/internal standard peak area (μV s))/(compound concentration (ppb)/internal standard concentration (ppb))) for the various volatile constituents were determined by adding standard amounts (six points, in duplicate) of pure compounds to a pollen sample prior to analysis, from stock solutions in volatile-free deionized water (except for heptanal, toluene, octane, and monoterpenes whose stock solutions were prepared in acetone). Accordingly, added amounts were adjusted to approximate the expected concentrations. All the obtained calibration correlation coefficients were above 0.98.

Table 2. Volatile Compounds Identified in Pollens I and II[a]

	PN	t_R	relative RC	sample I	sample II
aldehydes, ppb					
isobutyraldehyde	2	13.8	1.02	30	124
butyraldehyde	3	17.2	0.46	107	313
3-methylbutyraldehyde	8	24.7	0.58	45	247
2-methylbutyraldehyde	9	26.0	0.93	22	124
valeraldehyde	11	29.8	1.04	167	396
caproaldehyde	15	42.3	1.11	794	2478
heptanal	17	53.9	0.32	67	272
ketones, ppb					
butan-2-one	6	19.2	0.29	23	79
pentane-2,3-dione	10	29.3	0.06	1545	1972
ester, ppb					
ethyl acetate	7	21.8	0.35	162	213
furans, ppb					
2-methylfuran	4	18.2	1.43	6	28
2-ethylfuran	12	30.4	1.45	21	82
sulfur compounds, ppb					
dimethyl sulfide	1	10.6	2.30	25	66
dimethyl disulfide	13	35.0	0.49	–	35
hydrocarbons, ppb					
hexane	5	18.5	1.17	123	96
toluene	14	38.4	2.17	34	42
octane	16	43.4	1.00	51	136
α-pinene	18	60.5	3.70	970	–
camphene	19	62.5	3.37	11	–
sabinene	20	66.2	3.82	90	–
β-pinene	21	67.2	2.78	19	–

[a] Peak numbering (PN) gives the order of elution through the column. All compounds were identified by gas chromatographic retention data compared with those of authentic samples and mass spectral data compared with those of library compounds and/or those of authentic samples. t_R, column retention time (min); relative RC, (compound peak area (μV s)/IST peak area (μV s))/(compound concentration (ppb)/IST concentration (ppb)); concentrations are means of duplicates; (–) area <500 μV s.

Free Amino Acid Determination. One gram of pulverized pollen was vortexed in 10 mL of a 1% (w/v) trifluoroacetic acid (TFA) and 3 × 10^{-4} M norleucine (internal standard) solution and centrifuged at 10000 rpm (12062*g*) in a Sorvall SS-34 rotor for 15 min at 4 °C. A 3 mL mixture including 1 mL of the supernatant, 660 μL of ultrapure water (Milli-Q water purification system, Millipore, Bedford, MA), 140 μL of a 1% (w/v) TFA solution, and 1200 μL of methanol was centrifuged at 10000 rpm for 5 min at 4 °C in an Eppendorf

Table 3. Strecker Aldehydes, Sulfur Compounds, and Furans through the Heat Treatments of Pollens I and II[a]

	pollen I					pollen II				
		1 day at		1 week at			1 day at		1 week at	
	fresh	40 °C	60 °C	40 °C	60 °C	fresh	40 °C	60 °C	40 °C	60 °C
isobutyraldehyde	30	13	66	74	387	124	81	202	314	1464
3-methylbutyraldehyde	45	35	182	180	602	247	174	440	568	1610
2-methylbutyraldehyde	22	20	70	92	378	145	102	212	312	1068
dimethyl sulfide	25	64	508	668	3045	66	1285	7456	9775	31393
dimethyl disulfide	–	–	–	–	36	35	18	14	25	64
2-methylfuran	6	4	7	9	21	28	9	6	13	23
2-ethylfuran	21	35	66	49	97	82	117	112	153	248

[a] Concentrations (ppb) are means of duplicates; (–) area < 500 μV s.

centrifuge. The supernatant was flushed through a C18 Sep-Pak cartridge (Waters) preactivated with 20 mL of methanol, 20 mL of 0.1% (w/v) TFA, 10 mL of an 80/20 0.1% (w/v) TFA/methanol solution, and 30 mL of air. The fraction eluting between 1.5 and 2.4 mL was collected. Amino acids were measured by high-performance liquid chromatography and fluorescence techniques as described by Dethier et al. (1991).

Our sample preparation in TFA was compared with two published methods, preparation in perchloric acid (Grünfeld et al., 1989) and preparation in water only (Bieberdorf et al., 1961; Serra Bonvehi et al., 1991). Surprisingly, the three solvents gave very similar results (data not shown). Crucial to good reproducibility, however, was a high solvent vol/pollen wt ratio.

Water Activity. Water activity (a_w) was determined with a humidistat (HUMIDAT-TH2/TH1).

Water Content. One gram of pollen was placed in a P_2O_5-containing vacuum desiccator and kept for 6 days in a 60 °C oven. The protocol used was similar to that described by Serra Bonvehi and Marti Casanova (1987).

RESULTS AND DISCUSSION

The relation between water content and water activity in pollen I during heat treatment at 30, 40, or 60 °C is depicted in Figure 1. Pollen II dried at 40 and 60 °C yielded the same desorption isotherm. In order to prevent any osmophilic yeast development (water activity below 0.62; Larpent and Larpent-Gourgaud, 1990), the water content should be, therefore, below 14.5% (compared to initial weight).

Pollen I, initially characterized by a 0.71 water activity and a 20.0% water content, reached this threshold after 20 h at 30 °C, 7 h at 40 °C, and only 3 h at 60 °C (Figure 1). We obtained similar results with pollen II, exhibiting a 0.67 initial water activity and a 15.5% initial water content.

The free amino acid distribution (Table 1) of both fresh pollens shows a high predominance of proline (above 7000 ppm, reaching a level as high as 60% of the total free amino acid content). This predominance has already been reported for the total amino acid content (Dillon and Louveaux, 1987) and the free fraction of flower pollens in small grains (Bathurst, 1954; Day et al., 1990; Grunfeld et al., 1989). The two pollens displayed significant differences, however, in their contents in other amino acids (mainly histidine, arginine and *S*-methylmethionine), indicating that the nutritional properties can vary according to the floral origin. In all cases, we observed low amounts of various essential amino acids, including tryptophane, methionine, phenylalanine, and leucine.

The free amino acid composition was strongly influenced by heating conditions. Predictably, levels of most amino acids dropped drastically at 60 °C. On the other hand, slight enrichments in most amino acids were detected both at 40 °C until 24 h and at 30 °C.

Dynamic headspace analysis also revealed significant differences between pollens I and II (see Table 2). The former, characterized by an intense passion fruit aroma (Herderich and Winterhalter, 1991; Shibamoto and Tang, 1990), proved to be rich in various monoterpenes such as α-pinene, camphene, sabinene, and β-pinene. Pollen II, emitting an unpleasant aroma, contained various sulfur compounds such as dimethyl sulfide (66 ppb) and dimethyl disulfide (35 ppb), in concentrations exceeding the detection thresholds (50 ppb for dimethyl sulfide (Meilgaard, 1975), 3 ppb for dimethyl disulfide (Shankaranarayana et al., 1974)). Concentrations of aldehydes, ketones, ethyl acetate, and furans were also much higher.

Despite the expected losses by volatilization, which may be undesirable in the case of pleasant flavors, Maillard compounds and sulfur derivatives were produced during drying at 40 and 60 °C (see Table 3 and Figure 2). In particular, Strecker aldehydes (isobutyraldehyde, 3-methylbutyraldehyde, and 2-methylbutyraldehyde) were derived from valine, leucine, and isoleucine, respectively. Higher amounts of branched aldehydes in pollen II are probably related to the higher initial concentrations of the corresponding amino acids. Aldehyde concentrations measured after a week at 60 °C were in the range of the perception thresholds (respectively 1.0, 0.6, and 1.2 ppm; Meilgaard, 1975). During the first day, losses by volatilization at 40 °C exceeded the production levels (Table 3). Two other Maillard compounds (Hurrell, 1982), 2-methylfuran and 2-ethylfuran, were also synthesized at 40 and 60 °C (Figure 2). Here again, when treatment was very short, the concentrations were found to decrease (see pollen II in Table 3).

High amounts of dimethyl sulfide were produced in both samples by *S*-methylmethionine degradation (Table 3). After 1 week at 40 or 60 °C, pollen II, initially rich in this compound, reached respectively 10 or 31 ppm. Eight hours at 40 °C, which decreased the water activity to below 0.62 (see above), added 35 ppb dimethyl sulfide (101 ppb instead of 66 ppb; data not shown). After 20 h at 40 °C or 7 h at 60 °C, pollen I also developed a cooked-onion aroma due to a dimethyl sulfide concentration above the 50 ppb threshold value (Figure 2). Meanwhile, in both samples, the *S*-methylmethionine content dropped significantly (see Table 1). Predictably, thermal degradation was most noticeable in sample II, with its 421 ppm initial *S*-methylmethionine content. In both pollens, dimethyl disulfide concentration was significantly increased only after 1 week at 60 °C (Table 3), showing that the impact of this compound should be low in properly dried pollen when a low concentration has been measured in the fresh sample.

In conclusion, to prevent any microbiological development in pollen, we recommend a 20 h heat treatment

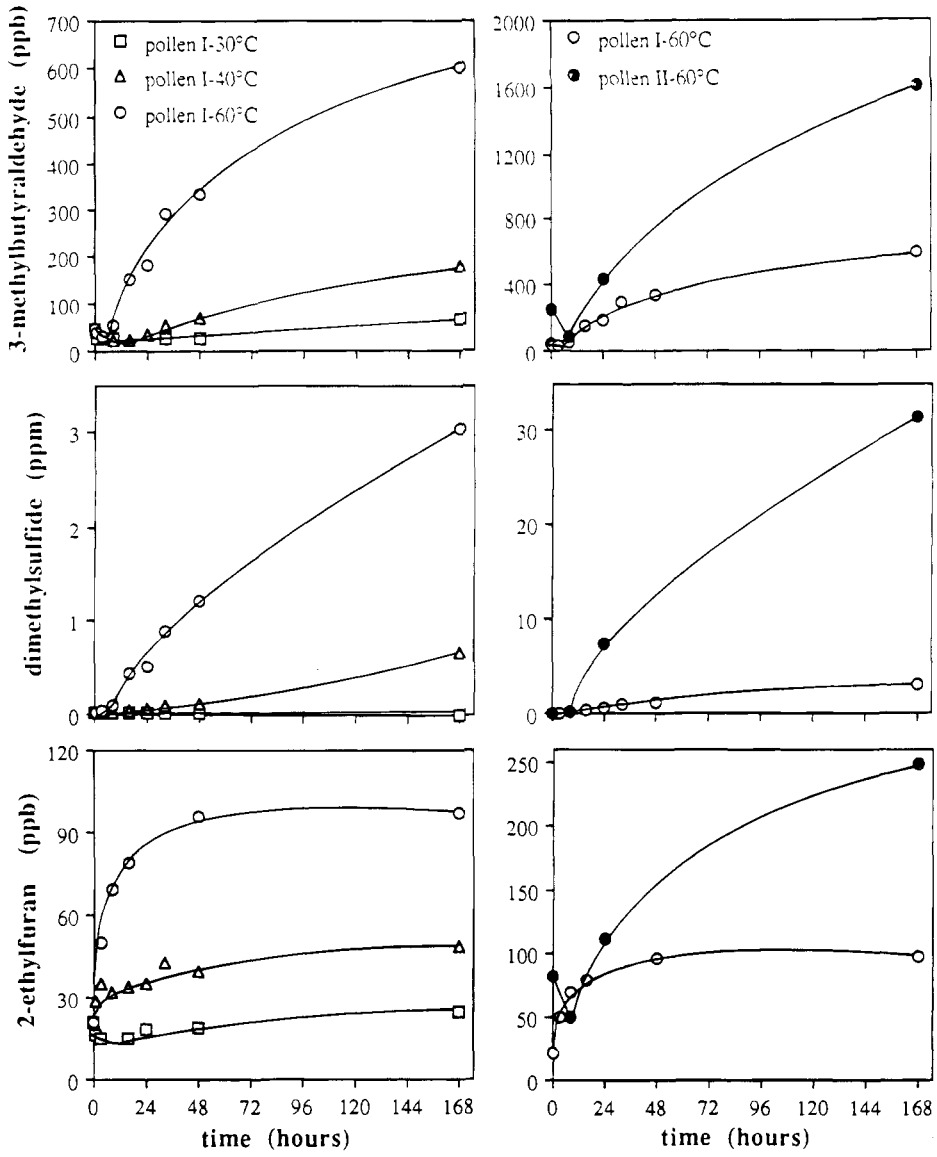

Figure 2. Evolution of 3-methylbutyraldehyde, dimethyl sulfide, and 2-ethylfuran during heat treatments.

at 30 °C. At this temperature, the synthesis of dimethyl sulfide, branched aldehyde, and furan compounds is avoided and pleasant compounds like monoterpenes are not lost. In some cases, however, very short heating at 40 °C could be an effective way to get rid of unpleasant aromas, provided *S*-methylmethionine-rich samples are not used. The initial *S*-methylmethionine content appears to be a very good index for predicting the development of an unpleasant flavor due to thermal degradation. By determining this amino acid in many pollens of different floral species, we should be able to select the best commercial values. Further analyses are necessary to check whether undesirable enzymatic reactions could take place at a water content below 14.5%.

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