Table V. Data for Brewed and Instant Coffee

Preparation	Peaks and Compounds: 44 Hydrogen Sulfide	69 Isoprene	86 Methyl Mercaptan	105 Acetaldehyde	130 Dimethyl Sulfide	150 Methyl Formate	170 Furan	190 Propionaldehyde	220 Isobutyraldehyde	250 Acetone
	Concentration, P.P.M.									
1[a]	0.060	0.0026	0.056	1.74	0.063	0.22	0.045	0.20	0.47	1.95
2[b]	0.054	0.0015	0.053	1.39	0.020	0.13	0.026	0.15	0.42	1.25
3[c]	0.010	0.0000	0.022	0.71	0.000	0.02	0.004	0.038	0.14	0.55

	Peaks and Compounds: 290 Methylfuran	350 Butyraldehyde	450 Methyl Ethyl Ketone	470 Isovaleraldehyde	540 Unknown	630 Methanol	780 Diacetyl	850 Ethanol	1420 Acetyl Propionyl
	Concentration, P.P.M.								
1[a]	0.078	0.006	0.49	0.073	0	0.83	0.64	0.032	0.67
2[b]	0.039	0.000	0.33	0.047	0	0.46	0.41	0.022	0.40
3[c]	0.008	0.000	0.11	0.019	0	0.03	0.24	0.000	0.11

[a] Prepared from fresh coffee using 10.6 grams of coffee per 170 ml. of boiling, distilled water.
[b] Preparred from 6-day-old coffee using same proportion as 1.
[c] Prepared from an instant coffee using 1.91 grams per 170 ml. of distilled water.

well as to differences in analytical methods and procedures.

Acknowledgment

The research reported herein was made possible by funds supplied through a contract with the Coffee Brewing Institute, Inc.

Literature Cited

(1) Campbell, C. L., Dawes, R. K., Deolalkar, S., Merritt, M. C., *Food Research* **23,** 575 (1958).
(2) Patton, S., Josephson, D. V., *Ibid.*, **22,** 316 (1957).
(3) Rhoades, J. W., *Ibid.*, **23,** 254 (1958).
(4) Sullivan, J. H., Robertson, D. H., Merritt, C., Jr., Division of Agricultural and Food Chemistry, 135th Meeting, ACS, Boston, Mass., April 1959.
(5) Wales, C. S., Harmon, L. G., *Food Research* **22,** 170 (1957).
(6) Zlatkis, A., Sivetz, J., Division of Agricultural and Food Chemistry, 135th Meeting, ACS, Boston, Mass., April 1959.

VEGETABLE FLAVORS

Flash Exchange Gas Chromatography for the Analysis of Potential Flavor Components of Peas

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The technique of flash exchange gas chromatography was applied to the determination of aldehydes, ketones, and acids in the steam distillate of peas. The compounds identified were acetaldehyde, acetone, propionaldehyde, *n*-butyraldehyde, *n*-valeraldehyde, biacetyl, formic acid, acetic acid, and isovaleric acid. All these components occurred at a concentration of less than 3 p.p.m. on a fresh-weight basis. A mixture of these compounds at the concentration levels found did not reproduce the characteristic odor of pea steam distillate.

THE LONG-RANGE GOAL of flavor research is to improve flavor so that abundant, nutritious foods will be consumed at an increased rate. It is most desirable that rapid techniques be developed which could be applied to small, experimental quantities of material. Accuracy need not be greater than that required for a starting point in taste panel evaluation. With this type of research tool, studies of plant breeding, crop selection, harvesting practice, processing, and storage could be made with improved flavor as an objective.

A useful technique for the determination of carbonyl compounds and acids was developed which required only a few milligrams of compounds (*3*). Aldehydes, ketones, and acids are isolated as solid derivatives, regenerated in rapid exchange reactions, and volatilized

Table I. 2,4-DNPH Prepared from Steam Distillate of Peas

Run No.	2,4-DNPH, Mg. 1958[a]	1959[b]
1	25.4	11.7
2	21.2	11.5
3	23.6	8.9
4	15.1	11.3

[a] Insoluble 2,4-DNPH collected by filtration.
[b] Extracted. Filtration gave 0.7 to 2.8 mg. of 2,4-DNPH.

Table II. Concentration of Volatile Carbonyl Compounds in Initial Steam Distillate of Peas

Compound	Av. Concn., Mg./Kg. Fresh Wt. 1958	1959
Acetaldehyde	2.4	0.56
Acetone	0.35	0.12
Propionaldehyde	0.17	0.00
n-Butyraldehyde	0.13	0.06
n-Valeraldehyde	0.00	0.44

Table III. Change in Volatile Carbonyl Composition of Steam Distillate of Peas (1958) with Time

Time, Min.	Volume Distillate, Ml.	Weight 2,4-DNPH, Mg.	Weight Due to Biacetyl	Carbonyl Content, Mg./Kg., Fresh Wt.	
				Biacetyl	0.61
				Acetaldehyde	0.62 (0.70)
				Acetone	0.14 (0.52)
				Propionaldehyde	0.046 (0.10)
				n-Butyraldehyde	0.055 (0.11)
33	275	15.1	6.27		
57	263	5.05	4.04	Biacetyl	0.39
81	310	3.59	3.58	Biacetyl	0.34
99	258	1.96	3.02	Biacetyl	0.29

directly into a gas chromatography unit for separation and identification. Aldehydes and ketones are determined by regeneration from 2,4-dinitrophenylhydrazones (2,4-DNPH) with α-ketoglutaric acid. Monobasic, carboxylic acids are transformed from their potassium salts into ethyl esters by heating with potassium ethylsulfate.

Experimental

The peas used were portions of large lots of blanched, frozen, Perfection (canners) peas prepared on June 25, 1958, and June 19, 1959, at Milton, Ore. The only difference in the two lots was the earlier maturity and longer fluming time for the 1959 lot. The peas (2-kg. samples) were thawed for 4 hours, ground with 1000 ml. of distilled water in a food blender, and steam distilled at atmospheric pressure with a boiling water bath as a heat source. A heating period of 1 hour was necessary to reach distillation temperature. A first distillate of 250 ml. was collected by passing in clean steam for about a half hour. A gas trap containing 2,4-dinitrophenylhydrazine reagent was used to ensure that all volatile carbonyl compounds were trapped. The distillate was treated with a solution of 200 mg. of 2,4-dinitrophenylhydrazine in 20 ml. of concentrated hydrochloric acid. The turbid suspension was heated to boiling for 5 minutes, cooled slowly to room temperature, and stored overnight in a refrigerator. The 2,4-DNPH was collected by filtration, washed with 2*N* hydrochloric acid, then water, and dried to constant weight at reduced pressure over phosphorus pentoxide. The volatile C_2 to C_6 aldehydes and ketones in the mixed 2,4-DNPH were determined by the flash exchange reaction with excess α-ketoglutaric acid (*3*).

After the predominant derivatives had been removed by filtration, the filtrate and washings were diluted to 450 ml. and extracted continuously with purified petroleum ether for 16 hours. The yellow-colored extract, 250 ml., was washed with three 50-ml. portions of water, dried over anhydrous sodium sulfate, and evaporated.

For the determination of acids, the steam distillate was treated with 0.1*N* potassium hydroxide to pH 8.5 and evaporated. The resulting mixture of potassium salts was dried over phosphorus pentoxide at room temperature and pressure. The volatile C_1 to C_6 monobasic, carboxylic acids were determined by flash exchange with potassium ethylsulfate.

Results and Discussion

Carbonyl compounds are present in the steam distillate of peas at a very low concentration. Indeed, if blanched, frozen, intact peas are heated with water, there is almost no detectable carbonyl compound in the distillate. It was necessary to steam-distill the pulverized peas to get sufficient carbonyl derivative for analysis. The results of several such steam distillations are shown in Table I. These results are in marked contrast to those reported by Silberstein (*5*) and Wager (*6*). Silberstein obtained 60 to 300 mg. of 2,4-DNPH from 2 kg. of peas, by steam distillation under pressure, which may account for part of the difference in results. The discrepancy is due in part to such factors as variety of pea, maturity at harvest, type of blanching used, and storage conditions. Silberstein demonstrated that blanched peas had a much lower volatile carbonyl content than fresh peas.

The results of the analyses of the mixed 2,4-DNPH from pea distillates are shown in Table II. The predominant carbonyl compounds are acetaldehyde, acetone, propionaldehyde, *n*-butyraldehyde, *n*-valeraldehyde, and biacetyl. The latter compound was determined separately by colorimetric methods and the results are shown in Table III. The difference in amount and distribution of the carbonyl compounds in the 1958 and 1959 pea lots is interesting. The 1959 material had a much lower volatile carbonyl content and was different from the 1958 material by the absence of propionaldehyde and the presence of *n*-valeraldehyde.

A brief study of the variation of carbonyl content with time of distillation was made and the data are shown in Table III. It was found that after the first distillation fraction, the major part of the weight of the precipitated 2,4-DNPH was due to biacetyl bis-(2,4-dinitrophenylhydrazine). The concentration of the biacetyl appears to decrease to a limiting value with time. A separate study (*4*) of acetoin and biacetyl in peas showed that acetoin is the predominant material with a concentration of 260 mg. per kg. of fresh weight. The acetoin is not volatile with steam under the conditions of the distillation.

Extraction of the filtrate after collection of the precipitated 2,4-DNPH produced considerable material (11.7 mg. from the first fraction of 1958 run No. 4). Analysis of the extracted material is shown within parentheses in Table III. It was expected that some trace constituents would appear in the extracted material. This did not occur, because only the four primary carbonyl compounds were found at both the 90° and the 150° C. column temperatures.

Volatile acids in the steam distillate

Table IV. Volatile Acids in Steam Distillate of Peas (1959)

Acid	Concn., Mg./Kg. Fresh Wt. Run No. 1	2	Av.
Formic	0.33	0.46	0.39
Acetic	0.024	0.029	0.027
Isovaleric	0.067	0.060	0.064

of 1959 peas are shown in Table IV. The acid composition is relatively simple. Formic acid is the primary constituent with small amounts of acetic and isovaleric acids also present. Formic acid has been detected previously in canned peas (*1*).

The steam distillate of peas has the pleasant, characteristic odor of cooked peas. If the compounds responsible for the odor can be identified, a substantial part of pea flavor would be understood. Earlier work in this field has indicated that carbonyl compounds are an important part of pea odor (*2*). Mixtures of the carbonyl compounds identified in this study do not approach the cooked pea odor. Work on the isolation and identification of the odorous components of pea steam distillate is being continued.

Literature Cited

(1) Lee, F. A., Shallenberger, R. S., *Food Research* **24,** 68 (1959).
(2) Little, A. D., Inc., "Flavor Research and Food Acceptance," p. 285, Reinhold, New York, 1958.
(3) Ralls, J. W., *Anal. Chem.* **32,** 332 (1960).
(4) Ralls, J. W., J. AGR. FOOD CHEM. **7,** 505 (1959).
(5) Silberstein, O., *Proc. Am. Soc. Hort. Sci.* **63,** 359 (1954).
(6) Wager, H. G., *Analyst* **83,** 291 (1958).

Received for review June 5, 1959. Accepted November 2, 1959. Division of Agricultural and Food Chemistry, 135th Meeting, ACS, Boston, Mass., April 1959. The author as collaborator was employed by the National Canners Association, with which this work was conducted cooperatively.

LACTOSE BINDING IN MILK

Free and Bound Lactose in Milk

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By using both isotope dilution methods and a conventional protein precipitation technique, it was demonstrated that in unheated milk 0.54% lactose is associated with milk protein(s). The association is apparently weak, as all lactose is removed from unheated milk by dialysis for 72 hours. The results suggest an equilibrium, free lactose ⇄ bound lactose, favoring a free-bound ratio of about 8 to 1. Binding of lactose by heated proteins, which is apparently a carbonyl-amino reaction, was investigated at 80° and 100° C. The activation energy of the reaction, probably the first step in the browning reaction, is of the order of 11 × 10^3 calories per mole.

WHILE lactose binding by the proteins of heated milk (browning reaction) has been the object of much research and the subject of a comprehensive review (*10*), little attention has been directed to bound lactose in unheated milk.

Various carbohydrates (glucose and galactose among others) have been reported to be associated with casein (*7, 8*), with some minor protein components (*13*), and with amino acids from tryptic hydrolyzates (*1*). Goulden, studying freeze-dried model systems, interpreted infrared spectra as indicating an association between casein and lactose (*3*). Other workers (*9*) disagreed with this interpretation, and Goulden subsequently attributed the irregularities in the spectra to the polymorphism of lactose (*4*). Schober and Christ (*12*) studied the binding of glucose by casein as influenced by heat, and concluded that two different types of association occurred—a reversible binding in protein that was native or subjected to low heat treatments, and at higher temperatures an irreversible binding that was a part of the browning reaction. The existence of bound lactose in unheated milk has not been definitely established nor has the extent of binding been measured.

The objects of this investigation were to determine the amount of bound lactose in unheated milk, the nature of the binding, and the energy requirements of the process in the temperature range 80° to 100° C.

Materials and Methods

Heating Trials. Aliquots (150 ml.) of fresh, standardized (3.5% fat) mixed-herd raw milk were placed in 14½-ounce evaporated milk cans. The cans were soldered closed, placed in a constant-temperature water bath for the indicated periods, and cooled in running tap water for 15 minutes. They were stored in an ice-water bath overnight and the milk was analyzed the next day.

Dialysis Trials. Separate aliquots of the milk were placed in dialysis bags and dialyzed against a pH 6.60 buffer (3.75 grams of anhydrous monohydrogen sodium phosphate and 5.41 grams of anhydrous dihydrogen potassium phosphate per liter of distilled water). The volume ratio of milk to buffer during these trials was about 1 to 10.

Lactose Determinations. Total lactose was determined by the zinc acetate–phosphotungstic acid reagent method of Grimbleby (*5*). Free lactose was determined by two methods, with excellent agreement in results.

Isotope Dilution Methods. To reduce coincidence losses, a 6-mg. portion