the higher the temperature. At 80° C. there is a maximum activity only at pH 6.0 with very little stability on either the acid or base side of this pH. The optimum pH will depend on the duration and temperature of the reaction. For higher temperatures the pH must be carefully controlled to as near 6.0 as possible, whereas at lower temperatures a much wider range of pH without any specific optimum may be used. The higher the reaction temperatures, the easier it is to determine the optimum pH of maximum stability closely.

Influence of Acidity on Heat Stability of Sweet Potato α-Amylase. Preheating solutions of sweet potato amylase which had been adjusted to different pH's (range pH 3.6 to 8.1 at 0.2 pH intervals) for 15 minutes at 70° C. demonstrated that minimum destruction (maximum heat stability) of the enzyme occurred at pH 6.0 (Figure 3). The pH data shown are those determined after heating. The chart showing the influence of pH at 70° C. (Figure 2) is reproduced in Figure 3 for comparison. These data confirm greater stability of the sweet potato α-amylase at pH 6.0 than at the other pH's investigated. Destruction of the enzyme is progressively greater on either side of the optimum but more so on the basic side. No activity was detected in the preheated mixtures in which the pH was adjusted to pH 6.6 and higher, or in those adjusted to pH 4.5 and lower. As the curves in Figure 3 show, preheating the enzyme solution for 15 minutes at 70° C. does damage the enzyme, although the extent of damage at pH 6.0 may not be detectable by the methods employed. Adjustment of the pH's to the optimum prior to performing the activity test indicated that the inactivation effect of

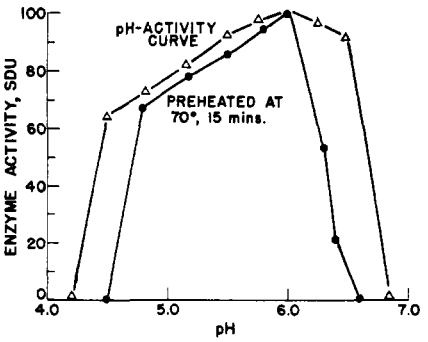

Figure 3. Effect of pH on heat stability and pH-activity curve

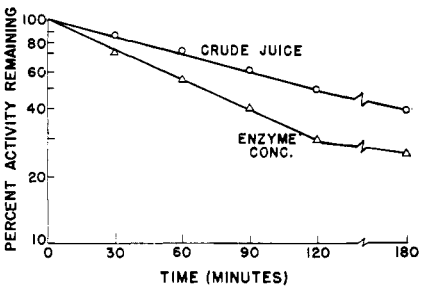

Figure 4. Effect of heating time on inactivation of α-amylase

pH on the stability of the enzyme at the preheat and test temperatures was irreversible. Nakayama and Kono (*10*) reported similar findings for sweet potato β-amylase. Employing a similar technique, they observed that β-amylase was most stable against heat inactivation at pH 5.4.

Heating the sweet potato α-amylase preparations for long periods at 70° C. causes a progressive destruction of the enzyme (Figure 4). The residual activity plotted on a logarithm scale against time of heating resulted in a straight line for 120 minutes for either the crude or the purified sweet potato α-amylase, indicating that the heat inactivation of the α-amylase follows first-order kinetics with respect to time. This has already been reported for β-amylase in the sweet potato (*10*). These results suggest that heat inactivation in both of these enzymes may be an intramolecular denaturation phenomenon. Further work will be needed to indicate the nature of the breakdown of these protein molecules.

Literature Cited

(1) Assoc. Offic. Agr. Chemists, Washington, D. C., "Official Methods of Analysis," 9th ed., p. 129, 1960.
(2) *Ibid.*, p. 432.
(3) Balls, A. K., Thompson, R. R., Walden, M. K., *J. Biol. Chem.* **173,** 9 (1948).
(4) Culpepper, C. W., Magoon, C. A., *J. Agr. Res.* **33,** No. 7, 627 (1926).
(5) Giri, K. V., *Current Sci.* **2,** 128 (1933).
(6) Giri, K. V., *J. Indian Chem. Soc.* **11,** 339 (1934).
(7) Gore, H. C., *J. Biol. Chem.* **44,** 19 (1920).
(8) Heinze, P. H., Appleman, C. O., *Plant Physiol.* **18,** 548 (1943).
(9) Ikemiya, M., *J. Ferment. Technol. (Japan)* **28,** 265, 392 (1950); **29,** 241 (1951).
(10) Nakayama, Shigeru, Kono, Yoshiko, *J. Biochem. (Tokyo)* **44,** 25 (1957).
(11) Sandstedt, R. M., Kneen, E., Blish, M. J., *Cereal Chem.* **16,** 712 (1939).

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CHEESE FLAVORS

Quantitation, Evaluation, and Effect of Certain Microorganisms on Flavor Components of Blue Cheese

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EFFORTS to characterize the unique flavor properties of Blue-veined cheese and to define the microbiology of ripening have been the subject of numerous investigations. The list of compounds comprising Blue cheese volatiles has become extensive (*2*) and limited quantitative data have been reported on a few classes of compounds—i.e., methyl ketones (*11*) and fatty acids (*1*).

This paper reports on the quantitative analysis of two major classes of compounds in Blue cheese volatiles. The quantitative data were evaluated by preparing mixtures of selected compounds and determining their similarity to Blue cheese flavor. Since the ratios of the major classes of compounds might be altered by the microflora of the cheese, the effect of bacteria, yeasts, and molds, common to the cheese during ripening, upon ketone-alcohol interconversion was determined.

Experimental Procedure

Quantitation of Methyl Ketones. Hexane, benzene, and chloroform solvents were treated to remove carbonyls and redistilled. Nitromethane was distilled over boric acid, and ethylene chloride was distilled and stored over anhydrous potassium carbonate.

The quantitation procedure was similar to that described for fat and oils (*10*) and cheese (*11*). Ten grams of cheese and 15 grams of Celite 545 were ground with a mortar and pestle, and the mixture was placed in a chromatographic column plugged with glass

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Quantitative data on the C_3, C_5, C_7, C_9, and C_{11} methyl ketones in Blue and Roquefort cheese showed considerable variation among samples, but no consistent differences between Blue and Roquefort cheese. There appears to be a selective conversion of the 8:0 and, to a lesser extent, the 6:0 and 10:0 fatty acids to methyl ketones during cheese curing. The C_5, C_7, and C_9 secondary alcohols were measured in Blue cheese by gas chromatography using the methyl ketones as internal standards. The alcohols were present in lower concentrations than the ketones, but in approximately the same ratios. A synthetic flavor closely resembling normal Blue cheese was prepared. The compounds could not be used at the same concentrations found in cheese. Methyl and ethyl esters and 2-phenylethanol were important in Blue cheese flavor. The mycelia of *Penicillium roqueforti* are more active than the spores in reducing methyl ketones to secondary alcohols. Yeasts associated with Blue cheese are capable of reducing methyl ketones to secondary alcohols and may play a role in flavor development by producing ethanol, other alcohols, and certain esters.

wool at the bottom. Two hundred milliliters of hexane was passed through the column to extract the fat from the cheese-Celite mixture. The hexane-fat solution was passed over a 10-gram reaction column to form the 2,4-dinitrophenylhydrazones of the methyl ketones. The yield of fat was determined after removing the hexane under reduced pressure. The fat was removed from the hydrazones using a modified procedure of Schwartz, Haller, and Keeney (*10*). Fourteen grams of Sea Sorb 43 and 28 grams of Celite 545 were slurried in hexane and poured into a chromatographic column. The fat-hydrazone mixture was dissolved in 5 ml. of hexane and applied to the column. Two hundred milliliters of hexane, followed by 100 ml. of benzene-hexane (1 to 1) and then 200 ml. of benzene, was passed through the column to remove the fat. The hydrazones were then eluted with 150 ml. of chloroform-nitromethane (3 to 1). After the chloroform-nitromethane had been removed, the hydrazones were taken up in 4 ml. of ethylene chloride and applied to a column consisting of 15 grams of Sea Sorb 43 (heated 400° C. per 48 hours) and 30 grams of Celite 545 (heated 150° C. per 24 hours) to separate the methyl ketone derivatives from other hydrazones. Excellent separation of the aldehyde and methyl ketone hydrazones was achieved using ethylene chloride as the developing solvent.

The column (Method B, 25 grams) of Day, Bassette, and Keeney (*3*) was utilized to separate the methyl ketone hydrazone class into its individual members. The column eluate (hexane as solvent) was monitored for absorption at 345 mμ and 5-ml. fractions of eluate were collected with an automatic fraction collector. Concentrations of the C_3, C_5, C_7, C_9, and C_{11} hydrazones were determined by measuring their absorbance in chloroform at 363 mμ. Percentage recovery for the isolation and identification procedures was determined using a standard mixture of methyl ketones in steam-stripped milk fat. All recovery determinations and cheese analyses were done in duplicate.

Quantitation of Secondary Alcohols. Gas chromatography was used to obtain semiquantitative data on 2-pentanol, 2-heptanol, and 2-nonanol in Blue cheese fat. The analysis was made using the headspace entrainment and on-column trapping technique described by Morgan and Day (*8*). Chromatographic conditions were established so that peak areas of the methyl ketones (quantitated previously) served as internal standards and enabled calculation of secondary alcohol concentrations by measurement of peak areas.

Relative recoveries of each ketone and alcohol analog were determined using standard solutions with paraffin oil as the diluent. A series of three different concentrations (5, 10, and 20 μmoles per 10 grams of oil) containing equimolar quantities of 2-heptanone and 2-heptanol and a series of 2-nonanone and 2-nonanol were prepared. The 2-pentanone and 2-pentanol standards were made up using 60 parts of paraffin oil and 40 parts of saline solution (12% NaCl). The 2-pentanol could then partition between an oil-water system similar to that present when cheese samples were centrifuged to obtain fat for analysis. Partitioning was not a problem with the C_7 and C_9 alcohols because of their limited solubility in water. Recorder response was linear within the range of concentrations studied. All recoveries and all cheese samples, except two samples which were in limited supply, were analyzed in duplicate.

Five grams of cheese fat obtained by high speed centrifugation was placed in a screw-capped vial containing 2 grams of anhydrous $NaSO_4$ and the volatiles were chromatographed according to Morgan and Day (*8*). An F & M Model 810 gas chromatograph equipped with a hydrogen flame detector and a 12-foot × 1/8-inch o. d. column packed with 20% 1,2,3-tris (2-cyanoethoxy)-propane on 60/80 mesh acid-alkali-washed Celite 545 was used. Conditions used for chromatography and for purging the cheese fat are given in Table I.

Evaluation of Synthetic Flavor. The medium used for evaluation of the synthetic flavor consisted of 110 grams of dry curd cottage cheese, 40 grams of fat from melted sweet cream butter, 100 grams of cream, and 3.5% salt. Water or paraffin oil solutions of the flavoring components were mixed into the medium in a Waring Blendor. The samples were placed in plastic cottage cheese containers and stored overnight at refrigeration temperatures before evaluation by experienced judges.

Table I. Conditions for Quantitating Secondary Alcohols in Blue Cheese Fat

Compounds to Be Identified	GLC Column Temp., ° C.	Water Bath Temp., ° C.	Purge Time, Min.
2-Pentanone 2-Pentanol	50	70	2
2-Heptanone 2-Heptanol	80	70	4
2-Nonanone 2-Nonanol	90	80	10

Effect of Selected Microorganisms on Methyl Ketones and Secondary Alcohols. Stock cultures were obtained as follows: American Type Culture Collection, *Penicillium roqueforti* (10110), *Torulopsis sphaerica* (2504), *Mycoderma sp.* (6432); *Geotrichum candidum* (12784), *Bacterium linens* (9175); department stocks, *Streptococcus lactis* C2-F.

The *Penicillium*, *Geotrichum*, *Torulopsis*, and *Mycoderma* were grown in fortified malt extract broth (*6*), *B. linens* in tryptone broth (*9*), and *S. lactis* in lactic broth (*4*). A synthetic medium (*7*) was used for growth of nonsporulating hypal cells of *P. roqueforti*. Spore suspensions were prepared by the method of Gehrig and Knight (*5*). Fifty milliliters of medium was dispensed into 250-ml. long-necked culture flasks and sterilized 20 minutes at 121° C.

One milliliter of spore suspension or 18-hour culture was added to the media containing approximately 0.001*M* concentrations of the appropriate substrate (2-pentanone, 2-pentanol, acetone, or 2-propanol). All cultures were incubated 48 hours at 25° C. with agitation, except *S. lactis* which was incubated at 21° C. without agitation. Spores and mycelia were incubated in a pH 6.8 phosphate buffer (*12*) for 12 hours at 25° C. to determine their individual action on the substrates.

Headspace samples of the incubated cultures were analyzed by gas-liquid chromatography to check for substrate conversion. The cultures were trans-

ferred into a 125-ml. Erlenmeyer flask equipped with a 24/40 ground-glass joint. Fifty grams of anhydrous Na_2SO_4 were added and a standard-taper ground glass joint with the top drawn out to accommodate a Barber Colman septum was used to stopper the flask. One-half-milliliter samples were used for the *Mycoderma* and *Torulopsis* cultures, and 2-ml. samples were used for all other cultures. A 9-foot × 1/8-inch o. d. column packed with 15% Carbowax 1500 on 80/100-mesh acid-alkali washed Celite 545 and a 12-foot × 1/8-inch o. d. column packed with 20% 1,2,4-butanetriol on 80/100-mesh acid-alkali washed Celite 545 operated at 70° C. in an Aerograph Model 600 were used for the analysis. Peaks in the chromatograms were tentatively identified by retention times and confirmed by mass spectrometry. The 1,2,4-butanetriol column was used in a Barber Colman 5000 in conjunction with an Atlas-MAT CH-4 mass spectrometer. The column effluent was split 1 to 1 between the flame detector of the chromatograph and the EC-1 inlet of the mass spectrometer.

Results and Discussion

The results of the ketone quantitation are presented in Table II. Samples A, B, E, and F were the cheeses used for fatty acid quantitation (*1*). A large variation in the quantity of ketones in the cheeses is evident; however, 2-heptanone was the predominant ketone in all samples. No consistent difference between Blue and Roquefort cheese was noted; however, no acetone was found in one Roquefort sample.

The methyl ketones have been shown to be produced from fatty acids by *P. roqueforti* mold spores (*5*). Results of the present investigation indicate that the quantity of each ketone produced does not depend directly on the amount of available fatty acid precursor. This is illustrated in Figure 1 by a plot of the average mole per cent of the individual methyl ketones and their fatty acid precursors (*1*) found in cheeses A, B, and E. The proportion of acetone is relatively low compared to its precursor, butyric acid. Conversely, the concentration of 2-heptanone is high relative to its precursor, octanoic acid. During curing, the mold spores appear to convert the 8:0 acid to the C_7 methyl ketone most readily, while the 4:0, 6:0, 10:0, and 12:0 acids are converted to the C_5, C_7, C_9, and C_{11} methyl ketones, respectively, to a lesser extent.

Quantitation of Secondary Alcohols. The concentrations of the C_5, C_7, and C_9 secondary alcohols are given in Table III as milligrams of alcohol per kilogram of cheese. The chromatograms of sample E shown in Figure 2 are representative of those used in the quantitation procedure. The alcohols were found in much lower concentrations than the methyl ketones. The alcohols were present in approximately the same ratios as the ketones; however, 2-pentanol was in lower proportion than 2-pentanone. This might be because a greater portion of the 2-pentanol partitioned into the aqueous phase of the cheese than into the aqueous phase of the standards used for determining recovery factors. A large peak between 2-nonanone and 2-nonanol interfered with measurement of the alcohol peak in the Roquefort samples (F and G). The peak appeared to be unique to the Roquefort samples; however, its identity was not established.

Evaluation of Synthetic Flavor. The quantitative data obtained in this study coupled with fatty acid data (*1*) were utilized as a starting point for compounding a synthetic Blue cheese flavor. It was found that incorporation of the 10:0 and higher acids imparted an objectionable soapy flavor. Furthermore, in order to eliminate soapiness, it was necessary to reduce the total quantity of the 2:0 to 8:0 acids to 2/3 that found in the cheese. The ketones and alcohols were used at double the concentrations found in cheese. The mixture containing only the acids, ketones, and alcohols was similar to Blue cheese flavor, but was not considered typical. Large quantities of methyl and ethyl esters have been identified in Blue cheese (*2*); however, their importance in flavor has not been reported. Incorporation of ethyl butanoate, methyl hexanoate, and methyl octanoate greatly improved the flavor and aroma of the synthetic mixture. Addition of 2-phenylethanol imparted a yeasty background flavor which resulted in a more typical Blue cheese flavor. The addition to the medium of the compounds given in Table IV yielded a flavor resembling but not completely typical of Blue cheese. Several reasons

Table II. Concentration of Methyl Ketones in Blue-Vein Type Cheese

Methyl Ketone Chain Length	Mg. Ketone/Kg. Cheese[a]						
	A[b]	B	C	D	E	F	G
3	3.4	2.8	3.9	1.7	2.7	2.7	0.0
5	18.4	7.2	20.9	6.5	17.5	19.2	3.6
7	40.8	19.0	71.8	17.9	39.1	69.9	17.6
9	28.0	22.3	88.3	19.8	42.5	78.9	13.9
11	6.4	6.0	29.9	4.9	12.3	6.7	2.4

[a] Average of duplicate analyses.
[b] Samples A–E were domestic Blue, samples F and G were imported Roquefort.

Table III. Concentration of Secondary Alcohols in Blue-Vein Type Cheese

Secondary Alcohol Chain Length	Mg. Alcohol/Kg. Cheese[a]						
	A[b]	B	C[c]	D	E	F[c]	G
5	0.6	0.3	1.9	1.3	0.3	0.6	0.2
7	6.3	8.2	9.1	9.8	3.0	3.4	4.2
9	3.9	3.7	4.3	2.5	2.5	...[d]	...[d]

[a] Average of duplicate analysis.
[b] Samples A–E were domestic Blue, samples F and G were imported Roquefort.
[c] Single analysis due to limited sample.
[d] Peak area could not be measured.

Table IV. Compounds Used in Synthetic Blue Cheese Flavor

Compounds	Concentration, Mg./Kg.	
	Added to mixture	Found in cheese
Acetic acid	550	826
Butanoic acid	964	1448
Hexanoic acid	606	909
Octanoic acid	514	771
Acetone	6.2	3.1
2-Pentanone	30.3	15.2
2-Heptanone	69.5	34.8
2-Nonanone	66.3	33.1
2-Undecanone	17.0	8.5
2-Pentanol	0.9	0.4
2-Heptanol	12.1	6.1
2-Nonanol	7.0	3.5
2-Phenylethanol	2.0	...
Ethyl butanoate	1.5	...
Methyl hexanoate	6.0	...
Methyl octanoate	6.0	...

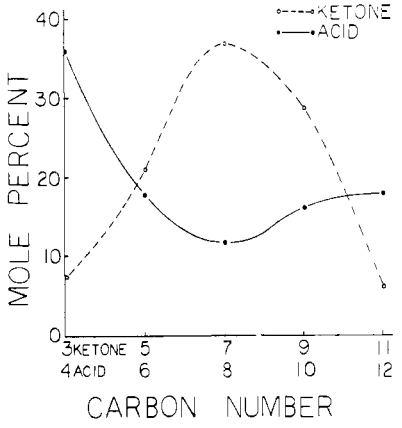

Figure 1. Mole percentages of methyl ketones and their fatty acid precursors in Blue cheese

Table V. Compounds Identified and Ketone-Alcohol Interconversions Observed with Certain Microorganisms Associated with Blue Cheese

Compound Identity	Growing Culture: Mycoderma	Torulopsis sphaerica	Geotrichum candidum	Bacterium linens	Streptococcus lactis	Penicillium roqueforti	In Phosphate Buffer, Penicillium roqueforti: Spores	Mycelia
Ethyl acetate	+	+	−	−	−	−		
Ethanol	+	+	+	−	−	+		
2-Methyl propanol	+	+	+	−	−	+		
2-Methylbutanol	+	+	+	−	−	+		
3-Methylbutanol	+	+	+	−	−	+		
Conversion Observed								
2-Pentanone to 2-pentanol	+	+	+	−	−	+	+ (weak)	+
2-Pentanol to 2-pentanone	+	+	+	+	−	+	+	+
Acetone to 2-propanol	+	+	+	−	−	+		
2-Propanol to acetone	+	+	+	−	−	+		

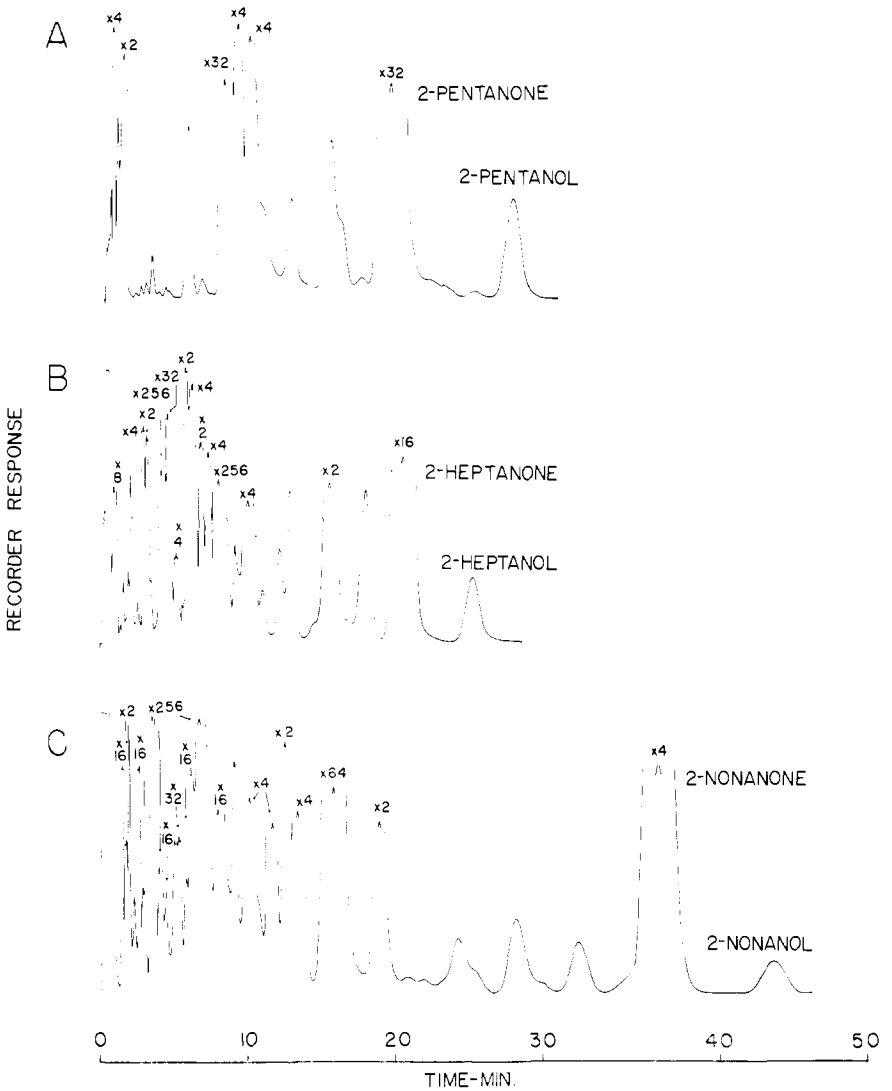

Figure 2. Gas chromatograms of Blue cheese fat showing the C_5, C_7, and C_9 methyl ketone and secondary alcohol peaks

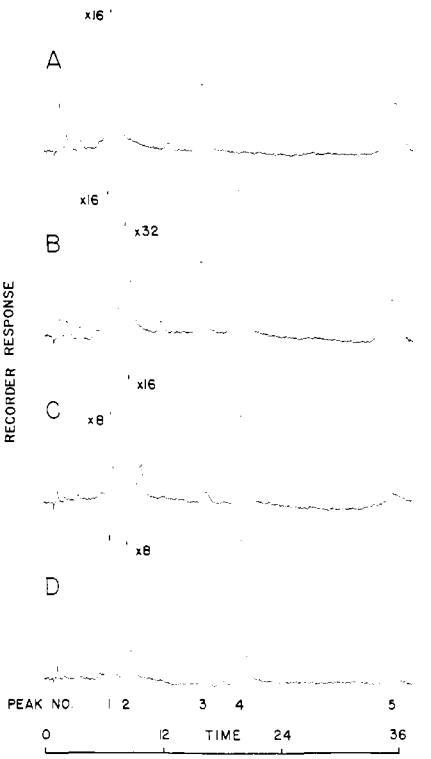

Figure 3. Headspace chromatograms of *Penicillium roqueforti* cultures

A. *P. roqueforti* only
B. Contained added hexanoic acid
C. Contained added 2-pentonone
D. Contained added 2-pentanol
Peak 1, ethanol; 2, 2-pentanone; 3, 2-methylpropanol; 4, 2-pentanol; 5, 2-methyl- and 3-methylbutanol

for this difference are obvious. No proteolysis occurred in the synthetic medium; therefore, no significant amounts of free amino acids or sulfur compounds were present. The solids content of the mixture was lower than cheese, and the complete complement of compounds identified in cheese was not added. Further refinement of the flavor mixture and its use in conjunction with some type of short term curing might lead to the production of a high quality Blue cheese at a lower cost than with conventional curing methods.

Influence of Microorganisms on Ketones. Reduction of methyl ketones to secondary alcohols could affect cheese flavor by lowering the total ketone concentration and by producing a new class of flavor compounds. Yeasts, bacteria, and molds are associated with ripening and all could influence reduction of the ketones to alcohols.

Figure 3 shows the headspace chromatograms of a *P. roqueforti* culture. When hexanoic acid was present (chromatogram B), 2-pentanone and 2-pentanol

were produced. Chromatogram C shows that a portion of the added 2-pentanone was converted to 2-pentanol, and in chromatogram D it can be seen that 2-pentanol was oxidized to 2-pentanone.

Table V summarizes the ketone-alcohol conversions observed and the compounds identified in the culture headspaces. The *Mycoderma*, *Geotrichum*, *Torulopsis*, and *Penicillium* cultures actively interconverted 2-pentanone and 2-pentanol, and acetone and 2-propanol. Headspace of the *Mycoderma* and *Torulopsis* cultures contained large amounts of ethyl acetate. *S. lactis* had no effect on any substrate, and *B. linens* could only convert 2-pentanol to 2-pentanone. The *B. linens* cultures had a very strong putrid aroma; however, no peaks other than the added substrates were present in the chromatograms. *P. roqueforti* mycelia were much more active than the spores in reducing the ketone to the alcohol. It appears that yeasts associated with Blue cheese ripening could influence the formation of secondary alcohols as well as produce certain esters and alcohols.

Literature Cited

(1) Anderson, D. F., Day, E. A., *J. Dairy Sci.* **48,** 248 (1965).
(2) Day, E. A., Anderson, D. F., J. AGR. FOOD CHEM. **13,** 2 (1965).
(3) Day, E. A., Bassette, R., Keeney, M., *J. Dairy Sci.* **43,** 463 (1960).
(4) Elliker, P. R., Anderson, A. W., Hannesson, G., *Ibid.*, **39,** 1611 (1956).
(5) Gehrig, R. F., Knight, S. G., *Appl. Microbiol.* **11,** 166 (1963).
(6) Jackson, H. W., Hussong, R. V., *J. Dairy Sci.* **41,** 920 (1958).
(7) Meyers, E., Knight, S. G., *Appl. Microbiol.* **6,** 174 (1958).
(8) Morgan, M. E., Day, E. A., *J. Dairy Sci.*, **48,** 1382 (1965).
(9) Purko, M., Nelson, W. O., Wood, W. A., *Ibid.*, **34,** 699 (1951).
(10) Schwartz, D. P., Haller, H. S., Keeney, M., *Anal. Chem.* **35,** 2191 (1963).
(11) Schwartz, D. P., Parks, O. W., *J. Dairy Sci.* **46,** 989 (1963).
(12) Umbreit, W. W., Burris, R. H., Stauffer, J. F., "Manometric Techniques and Tissue Metabolism," p. 168, Burgess, Minneapolis, 1957.

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BEEF COMPONENTS

Effect of Cooking Procedure on Flavor Components of Beef. Carbonyl Compounds

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Gas-liquid chromatography was employed to determine the carbonyl compounds resulting from beef cooked by two procedures. Aldehydes and ketones were collected as their 2,4-dinitrophenylhydrazine derivatives and regenerated as carbonyls with levulinic acid for injection into the chromatograph. Tentative identifications were made by comparing the retention times with known carbonyl compounds using two different column packings. Beef cooked with water gave the same number of aldehydes and ketones as beef cooked in fat, although in varying amounts. Results suggest that the characteristic differences in flavor and aroma of roasted and boiled beef may arise from the volatile carbonyl compounds.

THE question of origin of volatile flavor components has been a topic of wide speculation. Many workers agree that the flavor precursors can be extracted from raw meat with water (*1, 3, 5, 8*), and that the characteristic meat flavors can be produced by heating the isolated precursors with fat (*1, 3, 4*). Hornstein and Crowe (*3, 6*) suggested that the isolated precursors from most meats produce a similar aroma regardless of the species, while the characteristic odor of a particular meat is due to the fat. Researchers have isolated and identified volatile carbonyl compounds during cooking of beef (*6, 9*), chicken (*10, 12, 13*), pork (*3*), cured meats (*2, 11*), and lamb (*4*). Hornstein and his associates (*3, 4, 6*) determined the volatile carbonyl compounds evolved on heating the water-soluble precursors and then the previously separated fat.

The present investigation was undertaken to compare the amount and nature of the carbonyl compounds produced upon cooking beef in water and fat.

Apparatus and Materials

The meat used in this study was removed from the lumbar region of the longissimus dorsi muscle of 20-month-old cattle (U. S. Choice and Prime grade). The meat was finely ground and stored at −20° C. prior to cooking.

Figure 1 shows the cooking apparatus used for water-cooked beef. The apparatus for fat-cooked beef was identical, except that no condenser was necessary. After cooking, the regenerated carbonyl compounds were chromatographed on a Barber-Colman Model 20 gas chromatograph equipped with a radium ionization detector. A 6-foot, $^1/_4$-inch o.d. copper column packed with 10% diisodecyl phthalate on acid-washed Diaport W (60/80-mesh) separated mixtures of known aldehydes and ketones satisfactorily. Ockerman, Blumer, and Craig (*11*) reported good results using similar procedures for separating regenerated carbonyl compounds from cured ham. The column was operated at 100° C., with the flash heater and detector cell temperatures at 150° C. Argon was utilized as the carrier gas at 8 p.s.i., giving a calculated flow rate of 43.5 ml. per minute through the column. A voltage setting of 1250 volts was used.

A second column of 6-foot, $^1/_4$-inch o.d. copper tubing packed with 10% Carbowax 20M on acid-washed Diaport W (60/80-mesh) was prepared for confirmatory identification of the volatile aldehydes and ketones from cooked beef. It was operated at 75° C. with the detector cell and flash heater at 155° C. As before, the cell voltage was 1250 volts and the argon carrier gas was 8 p.s.i., giving a flow rate of 43.5 ml. per minute through the column.

Experimental Procedure

A total of 500 grams of ground beef was cooked with 1000 ml. of distilled water in the cooking apparatus (Figure 1). All volatile constituents were removed by constantly sweeping the surface of the cooking slurry with nitrogen gas and then trapping the volatiles in a series of reagent traps. The volatiles were bubbled through a capillary tube into two traps containing a saturated solution of