Reprinted from JOURNAL OF FOOD SCIENCE, 1965, Vol. 30, No. 2, Pages 288-295

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Identification of Volatile Constituents from Pure-Culture Fermentations of Brined Cucumbers

SUMMARY

A high-vacuum distillation method, with liquid-nitrogen trapping, was used to separate the volatile components present in pure-culture fermentations of cucumbers. The pure cultures used were *Lactobacillus plantarum*, *L. brevis*, *Pediococcus cerevisiae*, and *Leuconostoc mesenteroides*. The vapors were subjected to gas-liquid chromatography and the components identified by comparison of retention times with those of known compounds. Differences in the vapor chromatograms were obtained both between the different species of lactic acid bacteria and with strains of the same species. Comparison of the chromatograms with the organoleptic evaluation of the different pickle samples indicated that the flavor of pickles is due to a blend of volatile components rather than the presence or absence of a single component. Formaldehyde, acetaldehyde, acetone, ethyl alcohol, propionaldehyde, and butyraldehyde were isolated and identified.

A knowledge of the chemical composition of a flavor characteristic of a specific food is important because of the opportunities it offers to produce more uniform and flavorful products, to maintain flavor during processing and storage, and to produce new products.

The flavor of green cucumbers, unidentified as to variety, was attributed by Takei and Ono (1939) to nona-2,6-dienol and nona-2,6-dienal. The alcohol was present in larger amounts but the aldehyde was the primary flavor constituent. More recently, Forss *et al.* (1962) reported that nona-2: trans-6-cis-dienal, nona-2-enol, hex-2-enal, and three saturated aliphatic aldehydes were present in green cucumbers (Marketer, Super Market, Ashley) and several others unidentified as to varietal type. To our knowledge no other reports have appeared dealing with volatile constituents of green cucumbers and none on pure-culture fermentation of brined cucumbers.

The preservation of cucumbers by fermentation processes dates back to antiquity. The effects of fermentation are mediated through the metabolic activities of naturally occurring acid-forming microorganisms. The utilization of pure-culture fermentations appears to offer promise for the improvement of cucumber pickles (Etchells *et al.*, 1963). There is considerable precedent for this kind of work in other areas of food research. For example, Pederson (1960) has made an extensive study of the end products of fermentation of cabbage to sauerkraut by the lactic acid bacteria. Similarly, Vorbeck *et al.* (1961) reported on biochemical changes of minor constituents of cabbage by lactic acid bacteria. Wiseblatt (1960) and Hunter *et al.* (1961) characterized some of the flavor components in fermented dough, bread extracts, oven vapors, and pre-ferments for bread. Flavor and aroma compounds of several types are produced by pure cultures used in meat and dairy products. Single strains of bacteria usually do not give the desired development of flavor and aroma to dairy products, but, instead, a mixture of two or more species or strains of bacteria are needed (Foster *et al.*, 1957). Before pure-culture fermentation as a science can be applied to the production of pickles, more detailed information is needed as to the identity of flavor constituents introduced by organisms used in the fermentation. Consequently this study reports some of the flavor and aroma components produced by lactic acid bacteria normally isolated from natural pickle fermentations, and carried out under pure-culture conditions.

MATERIALS AND METHODS

Preparation of samples. The pure-culture cucumber fermentations were made at the M. A. Gedney Company Plant, Chaska, Minnesota, by means of a special process (Etchells *et al.*, 1963) consisting of the following steps:

1) SMR-15 variety of pickling cucumbers, 7/8–1 1/16 inches in diameter (size No. 1-B), were used for the study. The cucumbers were soaked in tap

water for a few minutes, washed by hand with a vegetable brush and drained.

2) The washed cucumbers, contained in deep-fry wire baskets fitted with hardware-cloth tops, were immersed for 5 min in a steam-jacketed kettle containing about 50 gal. of water maintained at 77°C. The average internal cucumber temperature, based on the 24 bulk heatings required for the experiment, was 63°C.

3) The heated cucumbers were immediately packed by hand, using aseptic precautions, in 48-oz-capacity glass jars which, together with their caps, had been previously rinsed with 70% alcohol and drained. The packed cucumbers were locked in position, to prevent floating, by wedging 2 or 3 larger cucumbers (No. 2) beneath the shoulder of the jar and parallel to the top.

4) Prior to brining, all jars scheduled for inoculation were acidified by the addition of 1.5 ml of 85% lactic acid.

5) The packed jars were then filled with a 30° salometer salt-brine solution (7.9% salt/wt) which had been previously boiled and cooled to approximately 4°C. This resulted in an equalized temperature for the brined material of 30–32°C.

6) The jars of acidified brined cucumbers were inoculated with 2.5 ml of 24–36-hr trypticase sugar broth cultures of the appropriate species of lactic acid bacteria. The jars were hand-sealed with 77-mm-diameter "Twist Off" closures (White Cap Co., Chicago, Illinois), incubated 72 hr at 32°C, and stored at room temperature (21–27°C) until examined.

7) Pasteurized uninoculated controls consisted of three jars of cucumbers prepared as described above except that 2.5 ml of lactic acid was added for acidification. These jars were pasteurized 30 min in a water bath at 77°C and promptly cooled. For unheated or natural controls, two jars were packed with washed cucumbers, acidified with 2.5 ml of 85% lactic acid, brined, and allowed to ferment naturally; two jars were handled in the same manner except that they were not acidified.

Cultures used. The lactic acid bacteria selected for study represented 34 cultures in the following four species: *Lactobacillus plantarum* (10 strains); *L. brevis* (10 strains); *Pediococcus cerevisiae* (11 strains); and *Leuconostoc mesenteroides* (3 strains). Most of the cultures originated from brined cucumber fermentations and were obtained from three sources: Dr. R. N. Costilow, Michigan State University (FBB-designation); Dr. William Haynes, Northern Regional Research Laboratory, Peoria, Illinois (B-designation); and Dr. A. F. Borg, Kansas State University (L-designation). All pure-culture fermentations were made in duplicate.

Distribution of samples. The pack totaled 75 (48-oz) jars. Except for the heated controls, they were divided into two equal lots. One lot was used for gas chromatographic studies, and the other was examined at the plant after 5 months of storage for certain chemical, physical, and organoleptic changes.

Preparation of extracts for analysis. Five hundred grams of pickles and 250 ml of the liquor were blended in a 1-gal-capacity Waring blender. The slurry, with rinsings (250 ml), was poured into a 5-L flask, and the flask was attached to a high-vacuum low-temperature distillation system (Fig. 1). The pressure was slowly lowered to

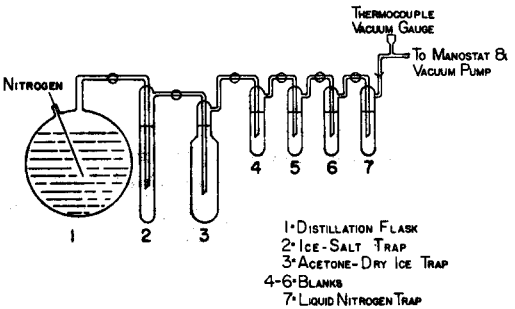

Fig. 1. High-vacuum low-temperature distillation apparatus.

750 μ of mercury, and nitrogen gas was then bubbled into the flask through a capillary tube. The slurry was distilled for 2 hr, during which time the temperature was maintained at 35°C.

Preliminary studies indicated that the flavor components were retained in the liquid nitrogen trap (Fig. 1). Consequently, this trap was removed from the system, fitted with a rubber septum, and allowed to warm to room temperature. A 5-ml vapor sample was removed for gas-liquid chromatographic (GLC) analysis. Immediately following the above step, 30 ml of 2,4-dinitrophenyl hydrazine (DNP) reagent (300 mg in 30 ml concentrated hydrochloric acid) was added to the trap and the reaction mixture allowed to stand overnight. The DNP hydrazones were collected by centrifugation, washed with 2*N* hydrochloric acid and water, and dried under vacuum.

Fractionation of volatile components. A Barber-Colman model 10 chromatograph unit equipped with a flame ionization detector was used for the qualitative determination of the volatile components. The U-shaped columns were heavy-walled glass tubing, 5 mm ID and 6 ft long, packed with Carbowax 20M on 60–80-mesh GC-22 Firebrick (1:10 w/w). The columns were preconditioned by baking at 90°C prior to use. The operating conditions were: column temperature

70°C; detector 155°C; flash heater 190°C; helium pressure 14 psig; sensitivity 10^{-9} amps. Where applicable, the carbonyl derivatives were introduced into the column as described by Ralls (1960).

The resolved components were identified by comparing their retention times with compounds of known composition.

RESULTS

Representative chromatograms of the volatile components present in a natural nonacidified control and an uninoculated acidified pasteurized control are shown in Fig. 2. Differences existed

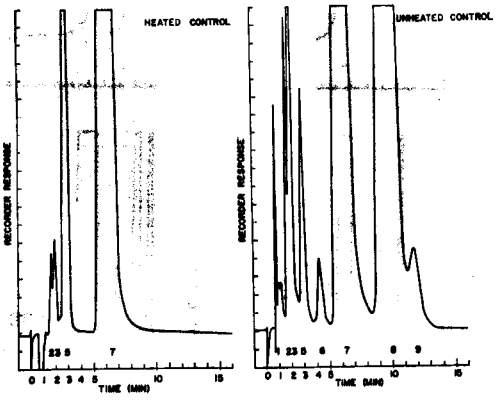

Fig. 2. Gas chromatograms of volatiles from a heated control (unfermented, pasteurized) and unheated control (natural fermentation, nonacidified). The peaks represent formaldehyde (2), acetaldehyde (3), acetone (5), butyraldehyde (6), ethyl alcohol (7), ethyl butyrate (8), isovaleraldehyde (9). Ordinate represents recorder response and abscissa represents time.

between the two control samples in that butyraldehyde, ethyl butyrate, and isovaleraldehyde (Peaks 6, 8, 9) were present in the nonacidified control whereas they were absent in the pasteurized control. This difference was due to the microorganisms present in the natural, unacidified fermented sample.

Representative chromatograms of the volatile components in pure-culture fermentations are presented in Fig. 3. The relative retention volumes of the volatile components in the distillates from the pure-culture fermentations and the controls are presented in Table 1. The data and chromatograms show marked differences between species. For example, formaldehyde and propionaldehyde (Peaks No. 2 and 4) were not detected in *L. brevis*; butyraldehyde (Peak No. 6) was absent in *P. cerevisiae*; all six compounds were present in *L. plantarum* and *Leuc. mesenteroides*. Similarly, differences existed for strains within

Table 1. Relative retention volumes of volatile compounds from cucumbers and pure-culture fermentations of cucumbers.[a,b]

Volatile compounds	Retention volume of known compound	Peak[c]	Natural control	Pasteurized control	Species and strains used for inocula							
					L. plantarum		*P. cerevisiae*		*L. brevis*		*Leuc. mesenteroides*	
					FBB-14	L-313	FBB-40	FBB-63	FBB-50	L-636	FBB-41	FBB-42
Formaldehyde	0.60	2	0.63	0.60	0.60	.....	0.60	.....	.....	.....	0.63	0.60
Acetaldehyde	0.68	3	0.70	0.68	0.70	0.63	0.68	0.68	0.68	0.68	0.70	0.68
Propionaldehyde	0.80	4	.....	.....	0.82	0.80	.....	0.80	.....	.....	.....	0.80
Acetone	1.00	5	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Butyraldehyde	1.40	6	1.48	.....	1.48	1.40	.....	.....	1.40	1.40	1.48	1.40
Ethyl alcohol[d]	2.20	7	2.22	2.20	2.22	2.20	2.20	2.20	2.20	2.20	2.22	2.20
Ethyl butyrate	3.70	8	3.66	.....	.....	.....	.....	.....	.....	.....	.....	.....
Isovaleraldehyde	4.67	9	4.66	.....	.....	.....	.....	.....	.....	.....	.....	.....

[a] Retention volumes relative to acetone.
[b] See Procedure for details.
[c] Peak No. 1 is the air peak.
[d] The jars and caps were rinsed in 70% alcohol and drained (see preparation of samples given under Materials and Methods); this may account in part for this component in the samples.

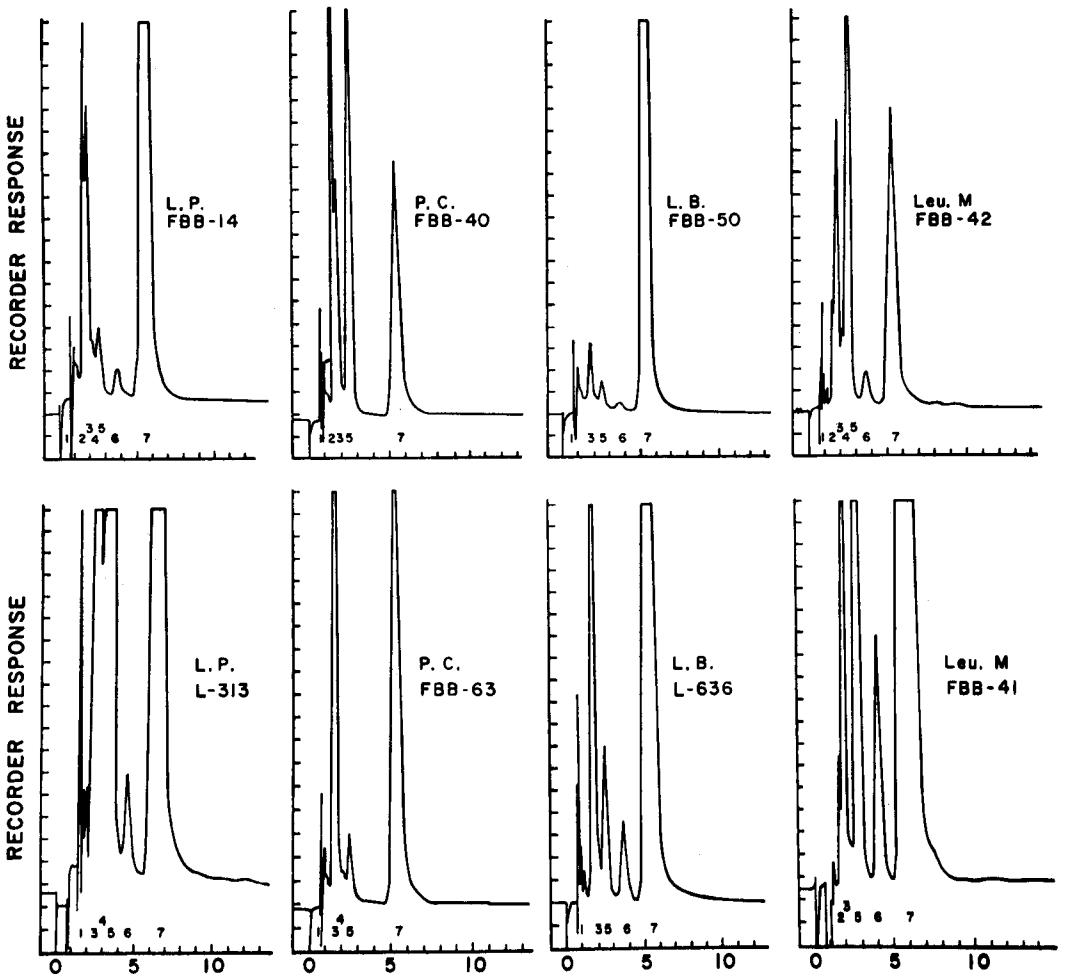

Fig. 3. Gas chromatograms of volatiles from pure-culture cucumber fermentations with lactic acid bacteria are shown with two strains each of four species from left to right; *Lactobacillus plantarum* (L.P.), *Pediococcus cerevisiae* (P.C.), *Lactobacillus brevis* (L.B.), and *Leuconostoc mesenteroides* (Leuc. M). The peaks represent air (1), formaldehyde (2), acetaldehyde (3), propionaldehyde (4), acetone (5), butyraldehyde (6), ethyl alcohol (7). Ordinate represents recorder response, and abscissa represents time.

species. Formaldehyde (Peak No. 2) was not detected in *L. plantarum* (L-313) and *P. cerevisiae* (FBB-63) whereas it was present in *L. plantarum* (FBB-14) and *P. cerevisiae* (FBB-40). Propionaldehyde (Peak No. 4) was absent in *Leuc. mesenteroides* (FBB-41) but present in *Leuc. mesenteroides* (FBB-42).

The chromatograms (Figs. 2, 3) show quantitative as well as qualitative differences for the natural control, pasteurized control, species, and strains within species. A comparison of the chromatograms for the two controls, or of *L. plantarum* and *L. brevis*, or of the two strains of *L. brevis* (FBB-50 and L-636) indicates the magnitude of their differences. Thus, the distinction between the various samples may be a matter of relative concentration of the volatile components rather than the presence or absence of a particular component.

The relative percent concentration of the volatile components from the distillates of the pure-culture fermentations is presented in Table 2. Flavor descriptions, as judged by an experienced taste panel, are presented in Table 3. Little, if any, relationship exists between the relative percent concentrations and flavor descriptions, as

Table 2. Relative percent concentration of volatile compounds present in the pure-culture fermentation samples.[a]

Species	Strain	Volatile compounds							
		Form-aldehyde	Acet-aldehyde	Propion-aldehyde	Acetone	Butyral-dehyde	Ethyl Alcohol	Ethyl Butyrate	Isovaler-aldehyde
		(%[b])	(%)	(%)	(%)	(%)	(%)	(%)	(%)
L. plantarum	FBB-14	12	7	2	4	2	73	0	0
	L-313	0	32	3	13	2	50	0	0
	FBB-12	18	5	3	5	3	68	0	0
	L-346	10	15	3	3	3	65	0	0
P. cerevisiae	FBB-40	23	12	0	26	0	39	0	0
	FBB-63	0	20	1	4	0	75	0	0
	FBB-61	16	12	3	4	0	66	0	0
	L-358	19	7	1	12	0	60	0	0
	B-1325	12	7	0	25	1	56	0	0
L. brevis	FBB-50	0	12	0	12	10	66	0	0
	L-636	0	21	0	9	6	64	0	0
	L-106	0	6	0	6	3	85	0	0
	L-544	0	8	0	1	2	89	0	0
	B-1836	0	22	0	12	23	42	0	0
Leuc. mesenteroides	FBB-42	6	15	5	27	3	44	0	0
	FBB-41	3	9	0	14	8	66	0	0
Controls									
Natural (acidified)		0	27	1	12	6	54	0	0
Natural (nonacidified)		3	8	0	6	3	33	38	8
Pasteurized		3	3	0	24	0	70	0	0

[a] See procedure for treatment details.
[b] Percent to nearest whole number.

illustrated by comparing *L. plantarum* with the pasteurized control. The percent concentration of the several volatile components varied for the strains within *L. plantarum,* and propionaldehyde and butyraldehyde were absent in the pasteurized control. In contrast, the odor and flavor description of the pickles fermented with *L. plantarum* and the unfermented uninoculated pasteurized control were similar; the taste-panel reaction to these particular samples differed chiefly in response to the degree of acid present.

Since no definite correlation could be observed between the relative percent concentration of volatile components (Table 2) and flavor description (Table 3), the chromatograms of the various samples (Fig. 3) were compared with the flavor and odor descriptions of the same sample (Table 3). It appeared that the profiles of the volatile components were related to the odor description of the sample. A comparison of the chromatograms of *L. brevis* and *L. plantarum* indicates a greater amount of volatile components for the latter and a difference in the odor descriptions between the two. Similarly, the same relationship exists for strains within species as illustrated by comparing the chromatograms and odor descriptions of two strains of *L. brevis* (FBB-50 and L-636). The chromatogram of the latter culture (L-636) shows an increase in concentration of acetaldehyde, acetone, and butyraldehyde (Peaks No. 3, 5, 6, resp). The odor description was "aromatic" for this sample (L-636), and "slightly off" for the other strain (FBB-50).

DISCUSSION

McCarthy *et al.* (1963) reported consistent correlations between odor of ripe bananas and chromatographic patterns. Furthermore, it was suggested that identification of each volatile component in a chromatogram would give more precise flavor profiles. In view of those observations and those reported above, it seemed logical to determine, if possible, the relative importance of the sense of smell (odor) and taste in flavor profiles for pure-culture fermentations of brined cucumbers.

It will be recalled that flavor depends upon reactions to the physical senses (sight, taste, odor, touch, and hearing). Strictly

Table 3. Flavor and odor characteristics of the pure-culture fermentations of brined cucumbers.

Species	Strain	Odorant classification[a] Pungent (%)	Rancid (%)	Ethereal (%)	Flavor Description	Odor Description
L. plantarum	FBB-14	21	2	77	Clean, raw cucumber strong acid	Clean, raw cucumber
	L-313	35	2	63	Clean mild acid	Clean, raw cucumber
	FBB-12	26	3	73	Good, clean, acid cucumber	Clean, raw cucumber
	L-346	28	3	68	Very sharp acid	Clean, raw cucumber
P. cerevisiae	FBB-40	35	....	65	Mild acid, slightly bitter	Clean, mild acid cucumber
	FBB-63	21	....	79	Mild acid, bitter after taste	Clean, mild acid
	FBB-61	31	....	70	Mild, slightly aromatic, slightly undesirable after taste	Clean, raw cucumber slightly aromatic
	L-358	27	....	72	Clean, mild acid	Clean, mild fresh cucumber
	B-1325	19	1	80	Mild acid, salt slightly bitter, musty, hay-like	Clean, fresh cucumber
L. brevis	FBB-50	12	10	78	Mild acid, bitter after taste	Slightly off
	L-636	21	6	73	Medium acid, slightly bitter	Aromatic
	L-106	6	3	91	Bitter	Aromatic
	L-544	8	2	90	Raw cucumber taste	Aromatic, clean
	B-1836	22	23	54	Medium acid, slightly bitter	Mild aromatic
Leuc. mesenteroides	FBB-41	12	8	80	Medium acid, slightly fruity (apple), pleasant, clean, excellent	Slightly aromatic
	FBB-42	26	3	71	Slightly acid, salt	Aromatic
Controls						
Natural (acidified)		28	6	66	Predominantly acid secondary cucumber	Salt stock
Natural (nonacidified)		11	49	39	Acid and bitter, with undesirable after taste	Stale, unpleasant
Pasteurized		6	....	94	Mild, very slight acid, bland, raw cucumber	Raw cucumber

[a] Amoore, 1952

speaking, taste is the reaction resulting from stimulation of taste buds on the tongue which detect salt, sweet, sour, and bitter. Odor is determined by the reaction to stimuli on free nerve-ends in a relatively small area high in the nasal cavity. Flavor and odor are detected at the same place, with the distinction generally made that flavor is obtained through the mouth, whereas odor is detected through the nose. When a food is consumed, stimuli from the five physical senses are received simultaneously in the brain. Thus, it is difficult to separate entirely one sense reaction from the others, since they all influence each other. For purposes of discussion, the assumption was made that color (sight), touch, and hearing were common for all samples while odor and

taste were variables. In addition, flavor was defined as the combined effect of the sample on the taste and olfactory nerves.

The data (Fig. 3) indicated that a relationship existed between peak heights and organoleptic evaluation on the samples. The subjective measurements of the various samples (Table 3) were re-examined from the standpoint of comparing odor and flavor descriptions. The taste panel described the odor of the pasteurized unfermented control as "raw cucumber" and the flavor as "mild, very slight acid, bland, raw cucumber." The pasteurized control was slightly acidified with lactic acid to inhibit the growth of sporeformers, especially the anaerobes. Thus, it appears that good agreement exists between odor and flavor descriptions because, in the flavor and description, both taste (very slight acid) and odor (raw cucumber) were detectable. Similarly, the taste and odor description of cucumbers fermented with *L. plantarum* showed good agreement. The odor description was "clean, raw cucumber," and the flavor description was "clean, raw cucumber, strong acid, mild acid, etc." In contrast, the flavor and odor descriptions for the remaining pure-culture fermentations did not show good agreement. The taste of cucumbers fermented with *L. brevis* was described as "mild acid, salty, slightly bitter, etc." while the odor description was "aromatic, slightly off." It was noted above that the profiles of the volatile components appeared to be related to the odor description. Thus, it is apparent that flavor profiles *per se* cannot be used as a subjective measurement unless some other criteria are used in conjunction with the flavor profiles.

The stereochemical theory of olfaction proposed by Amoore (1952) appears to offer an explanation for the results obtained in this study. The theory has two prerequisites for an odorant: volatility and a molecular configuration complementary to the specialized receptors in the nasal cavity. After a comprehensive study of odorants (Amoore, 1952), seven primary odors were identified: camphoraceous, pungent, ethereal, floral, pepperminty, musky, and putrid. Odorants like lemon, garlic, cedar, and rancid were classified as complex odors. Using this classification, the components identified in the chromatograms of the volatiles from the various pure-culture fermentations would be as follows: formaldehyde, pungent; acetaldehyde, pungent; propionaldehyde, pungent; acetone, ethereal; butyraldehyde, rancid; ethyl alcohol, ethereal; ethyl butyrate, rancid; isovaleraldehyde, rancid. The percent concentration of the volatile components was arranged according to the above classification (Table 3). A comparison between the odorants and odor description indicates there is a relationship between pungent and rancid components and odor. In the fermentation with *L. plantarum* the relative concentration of pungent odorants compared to rancid odorants was high, the odor description was "clean, raw cucumber," and flavor description was "clean, raw cucumber, mild to strong acid." There were no rancid odorants in the volatiles from *P. cerevisiae*. The odor was described as "clean, mild acid, raw cucumber," and flavor as "slightly bitter, mild acid, slightly aromatic, musty, hay-like, etc."

In contrast, pure-culture fermentations of cucumbers with *L. brevis* and *Leuc. mesenteroides* had greater concentrations of the rancid odorants and correspondingly lesser concentrations of pungent odorants. The odor descriptions by the taste panel were strikingly different when compared to the fermentations with *L. plantarum* and *P. cerevisiae*. The flavor descriptions for *L. brevis* were "mild acid, raw cucumber, bitter after taste, etc."; and for *Leuc. mesenteroides* "medium acid, slightly fruity, salt." The natural-fermentation control (nonacidified) has a higher concentration of rancid odorants than of pungent odors. The odor description was "stale, undesirable, unpleasant," and the flavor description was "acid and bitter with undesirable aftertaste." It was concluded that good agreement may be obtained between subjective and objective measurements if each volatile component in the chromatogram (objective measurement) is identified and classified according to the Amoore theory. It was also concluded: a) that the odor and flavor associated with an acceptable pure-culture fer-

mentation of brined cucumbers may be described in terms of the primary odorants, particularly the pungent odors; b) when the pungent odorants and rancid odorants (complex) are similar in concentration, the taste component of flavor is predominant; and c) when rancid odorants were in greater concentration than pungent odorants, the odor component is predominant for the flavor description, as illustrated for natural nonacidified control.

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Ms. rec'd 10/5/64.

Published with approval of the Director, North Carolina Agricultural Experiment Station, as Paper No. 1858 of the Journal Series.

J. A. Singleton is a Research Fellow, supported by Pickle Packers International, Inc., St. Charles, Illinois.

The U. S. Food Fermentation Laboratory is one of the laboratories of Southern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

The mention of trade names or products of any company does not imply recommendation; the name is supplied for information only.

The authors gratefully acknowledge the cooperation, assistance and facilities provided by the M. A. Gedney Company, Chaska, Minnesota. We also express sincere thanks to the following persons for generous help in preparing and evaluating the pure-culture pickles: Messrs. I. D. Kittel, Clifford White, Eugene S. Schoene, all of the M. A. Gedney Company, and D. H. Wallace, formerly of this company; and Messrs. J. C. Pacilio and E. J. Pharr, both of the White Cap Company, Chicago, Illinois.

This work was supported in part by a research grant from Pickle Packers International, Inc., St. Charles, Illinois.