Journal of
FOOD SCIENCE

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Non-Member Subscriptions, Back Volumes, Single Copies, and Reprints: JOURNAL OF FOOD SCIENCE 510-524 N. Hickory Street, Champaign, Ill.

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Published by the Institute of Food Technologists six times a year. Printed by The Garrard Press, 510-524 N. Hickory Street, Champaign, Illinois. Entered as second-class matter at the Post Office at Champaign, Illinois, under the act of March 3, 1879. Accepted for mailing at special rate of postage provided for in amended Section 34.40(e), pursuant to the provisions of Public Law 233, approved October 8, 1951.

JULY-AUGUST, 1963 **Volume 28, Number 4**

An Official Publication of the Institute of Food Technologists

Journal of FOOD SCIENCE

JULY-AUGUST, 1963 Volume 28, Number 4

CONTENTS

The Relative Perception of Weak Sucrose-Citric Acid Mixtures

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(Manuscript received October 15, 1962)

SUMMARY

The perception of changes in the relative intensity of sweetness and acidity was investigated with series of sucrose-citric acid mixtures at near-threshold intensities—up to 2% sucrose and up to .0885% citric acid. Changes in citric acid concentration were generally perceived by some tasters to increase sweetness and by other tasters to decrease sweetness. Similarly, changes in sucrose concentration increased or decreased perceived acidity. It is suggested that contradictions between this and previous studies might be due to differences in procedure, which could affect the relative perception of taste intensities. Perceptual processes play as great a part in determining responses to very weak tastes as do the stimuli themselves. Controls and measures of tasters' prior relevant behavior are suggested to be necessary for proper interpretation of their responses.

INTRODUCTION

Recent studies by Kamen *et al.* (1961) and Pangborn (1960, 1962), following Fabian and Blum (1943), have cited apparently contradictory evidence concerning the effects of the presence of citric acid on the perceived sweetness of a sucrose-citric acid mixture. The citric acid may make the mixture more (Kamen *et al.*, 1961) or less (Panghorn, 1960) sweet than a sucrose solution of comparable concentration. Panghorn (1962) has given evidence that the effect of one component on the perceived intensity of the other in a taste mixture depends in some consistent but unexplained manner on the method of presentation used; the methods of paired comparisons and single-stimulus intensity ratings have both been employed, yielding different results for the stimuli of weaker intensity.

This paper reports an experiment designed to investigate some effects that might be considered to modify the perception of weak sucrose-citric acid mixtures, and thus suggest what might be controlled experimentally in order to reconcile divergent findings. The experiment is concerned solely with near-threshold stimulus intensities, and differences between them. In this study, unlike any previous reported work of which we are aware, ratings of relative intensities on both real and imaginary differences between two stimuli were sought. Previous work may therefore be comparable with our findings only where the method is similar.

THEORY

We wish to try to relate the problem of the perception of taste mixtures to precedents that have extensive experimental support in the literature of perception in other senses. To this end we cite, and define formally in the context of the perception of taste mixtures, two sorts of behavior that may arise in responding to complex stimuli. These theories are not original to this paper but are being advanced here in order to derive corollaries that make predictions about the perception of taste mixtures when such mixtures are compared in series.

CONCORDANT RESPONDING

Given a complex stimulus made up of a set of component stimuli each at some measurable intensity, and a measure of intensity that permits comparison between components, such a mixture may have a gestalt quality recognized by a common

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name. For example, a mixture of appropriate intensities of salt, sugar, and tannin in water apparently tastes like sherry to some judges. If the mixture is perceived as a gestalt, then small changes in intensity of one component relative to the rest will not be noticed, because they will tend to be assimilated into the whole pattern of sensations by a process called "closure" (Koffka, 1935). In formal terms, if a_i is the intensity of the ith component, and a_j the intensity of some other jth component, then if we take all possible ratios of the intensities of two components from the total number of components (in the example of the imitation sherry, three components and three ratios) then the matrix

$$|| \; a_i \, a_j^{-1} \; ||$$

which is the matrix of all such ratios, remains (as perceived) unchanged.

For convenience we will call this "concordant" responding. The corollary to this theory is that a small increase or decrease in concentration of acid added to sucrose should respectively increase or decrease perceived sweetness provided the previous experience of the taster has led him to have an expectation of the balance of relative intensities of components appropriate to the mixture in question: such an expectation may be reinforced by giving the mixture a name, say "lemonade" (Carmichael *et al.*, 1932).

CONTRASTING RESPONDING

An alternative theory, after Helson (1959), is that the subject has a subjective average level of intensity that he uses as a reference against which to judge the intensity of any other stimulus. The subjective average is termed the adaptation level (A.L.) and is postulated to be the geometric mean of the different relevant stimuli to which the subject has previously been exposed. In the case of a taste mixture followed by a taste mixture, A.L. is formally

$$\left(\prod_{i=1}^{n} a_j \right)^{\frac{1}{n}}$$

for a mixture with n components, and on this theory a real small change in intensity of the ith component, which we may write as δa_i, results in a perceived change in the other, actually unchanged, components in the opposite direction, which for the jth component we may write as $-\delta_j$, because the tasters' A.L. has been moved by the real change δ_i, and the other components are now judged from a new reference level, from which they appear less intense. On this theory we move the A.L. every time we expose the subject to a new different mixture. A crucial test of the theory demands a quantification of the subjective magnitudes of the intensities concerned.

The corollary to this theory assumes that subjects can break down the gestalt quality of the sucrose-citric acid mixture and experience it as a mixture of two parts. This leads to what we will call "contrasting" responses; a small increase or decrease in concentration of acid added to sucrose should respectively decrease or increase perceived sweetness, again provided the previous experience of the taster has been such that he has formed an A.L. The term "contrast effect" has been used in a similar sense by Kamenetzky (1959) to refer to hedonic judgments.

In both the above paradigms the effects of acid on sucrose and sucrose on acid are postulated to be symmetrical.

EXPERIMENT

The object of the experiment was to investigate whether judgments of the apparent direction of intensity change over two successive tastings of the constant-intensity component of a sucrose-citric acid mixture were concordant with or contrasting to real changes in the intensity of the other component present, over the same successive tastings. The design of the experiment also made possible responses indicating that neither concordant nor contrasting effects were occurring for a given taster. The mixtures chosen were tap-water solutions of sucrose and citric acid. Three intensities of each component were involved in the experimental design: $A_1 = .0440\%$, $A_2 = .0625\%$, and $A_3 = .0885\%$ citric acid, and $S_1 = .5\%$, $S_2 = 1.0\%$, and $S_3 = 2.0\%$ sucrose. Only four mixtures of the nine possible were employed, $a = A_2 \, S_1$, $b = A_2 \, S_3$, $c = A_1 \, S_2$, and $d = A_3 \, S_2$. (This notation is used throughout the tables.) The mixtures were tasted in runs of five stimuli, and length of run was chosen as long enough to establish subjective expectancies but at the same time not so long as to induce fatigue or loss of motiva-

tion in the taster. The design of the experiment was chosen to reflect the sequential character of most taste experiences, which in the normal consumption situation are the basis of comparative judgments.

There are eight basic runs of five stimuli; the complete paradigm is included in Table 1. The runs designated E1 to E8 correspond to: E1) acid held constant at middle intensity and sucrose increased on the last stimulus, i.e., *aaaab*; E2) the same with sucrose decreased on the last stimulus, i.e., *bbbba*; E3) the same with sucrose increased on the penultimate stimulus and then decreased; i.e., *aaaba*; E4) the same with sucrose decreased on the penultimate stimulus and then increased, i.e., *bbbab*; and E5 to E8) all these four paradigms again but with the roles of citric acid and sucrose interchanged; i.e., with *c* for *a* and *d* for *b*.

In the notation of the tables we have used capital letters to mean sucrose or acid in the case of mixtures, and sweetness or acidity in the case of responses to those mixtures, number suffixes refer to component intensities as chemically defined; lower-case letter suffixes to mixtures as perceived and rated. For example, $S_{3.1}$ is to be read "Sucrose at level S_3 presented after sucrose at level S_1," whereas $A_{a.b}$ is to be read "the acidity of mixture *a* judged relative to the acidity of mixture *b*." Hence, columns headed, say, $S_{c.c}$ or $A_{b.b}$ are comparisons of two identical mixtures tasted one immediately after the other.

For each experimental run there was also a corresponding control run, using the same stimulus

Table 1. Each value in the table is based on 14 responses in the scale range + 3 to − 3.[a]

		Stimuli involved in comparisons			
		1st + 2nd	2nd + 3rd	3rd + 4th	4th + 5th
		Comparisons in sequence			
Run	Variable	1st	2nd	3rd	4th
E1	Stimulus	$(A_2\ S_1) = a$	$(A_2\ S_1)$	$(A_2\ S_1)$	$(A_2)\ S_{3.1}$
	judgment	$A_{a.a}$	$A_{a.a}$	$A_{a.a}$	$A_{b.a}$
	judgment mean	1.00	0.50	0.36	0.14
	judgment s.d.	1.20	1.84	1.76	2.53
E2	Stimulus	$(A_2\ S_3) = b$	$(A_2\ S_3)$	$(A_2\ S_3)$	$(A_2)\ S_{1.3}$
	judgment	$A_{b.b}$	$A_{b.b}$	$A_{b.b}$	$A_{a.b}$
	judgment mean	−0.14	0.21	−0.79	−0.57
	judgment s.d.	1.30	1.26	1.32	2.58
E3	Stimulus	$(A_2\ S_1) = a$	$(A_2\ S_1)$	$(A_2)\ S_{3.1}$	$(A_2)\ S_{1.3}$
	judgment	$A_{a.a}$	$A_{a.a}$	$A_{b.a}$	$A_{a.b}$
	judgment mean	0.57	0.43	0.36	0.43
	judgment s.d.	1.18	1.40	2.52	2.44
E4	Stimulus	$(A_2\ S_3) = b$	$(A_2\ S_3)$	$(A_2)\ S_{1.3}$	$(A_2)\ S_{3.1}$
	judgment	$A_{b.b}$	$A_{b.b}$	$A_{a.b}$	$A_{b.a}$
	judgment mean	0.36	−0.29	−0.21	1.07
	judgment s.d.	1.95	1.58	2.34	1.98
E5	Stimulus	$(A_1\ S_2) = c$	$(A_1\ S_2)$	$(A_1\ S_2)$	$(S_2)\ A_{3.1}$
	judgment	$S_{c.c}$	$S_{c.c}$	$S_{c.c}$	$S_{d.c}$
	judgment mean	0.29	0.57	−0.36	−0.29
	judgment s.d.	1.58	1.55	2.12	2.60
E6	Stimulus	$(A_3\ S_2) = d$	$(A_3\ S_2)$	$(A_3\ S_2)$	$(S_2)\ A_{1.3}$
	judgment	$S_{d.d}$	$S_{d.d}$	$S_{d.d}$	$S_{c.d}$
	judgment mean	−0.43	−0.71	0	−0.57
	judgment s.d.	2.19	1.62	2.04	2.19
E7	Stimulus	$(A_1\ S_2) = c$	$(A_1\ S_2)$	$(S_2)\ A_{3.1}$	$(S_2)\ A_{1.3}$
	judgment	$S_{c.c}$	$S_{c.c}$	$S_{d.c}$	$S_{c.d}$
	judgment mean	−0.07	−0.93	−1.21	0.14
	judgment s.d.	1.98	1.49	2.21	2.29
E8	Stimulus	$(A_3\ S_2) = d$	$(A_3\ S_2)$	$(S_2)\ A_{1.3}$	$(S_2)\ A_{3.1}$
	judgment	$S_{d.d}$	$S_{d.d}$	$S_{c.d}$	$S_{d.c}$
	judgment mean	−0.71	0	−0.71	0.21
	judgment s.d.	1.44	1.73	1.83	2.24

[a] The stimulus components constant over a given comparison are shown in parentheses.

sequence, but the control-group subjects were asked to make judgments of the component that in fact varied on the last or last two comparisons, whereas the experimental-group subjects judged the relative intensity of the component that in fact stayed constant over the series. It is necessary for the controls to taste the whole stimulus series and not just the comparisons between the two actually different stimuli, because of time-error effects in the series (Borak, 1922).

Each subject did two runs in sequence, and the runs were paired in the design so that the eight run combinations: *E*1 then *E*3, *E*3 then *E*1, *E*2 then *E*4, *E*4 then *E*2, *E*5 then *E*7, *E*7 then *E*5, *E*6 then *E*8, and *E*8 then *E*6 occurred equally often. The total number of subjects was 72, 16 controls and 56 experimentals, which gives four replications of each stimulus run in the controls and fourteen replications of each stimulus run in the experimentals. The subjects were factory and office staff: both sexes and a wide age range were represented, and probably a fair range of intelligence as inferred from their occupations and behavior in previous experiments of varying complexity. They also varied in their experience of food-tasting experiments, but the majority of such experiments in which they had participated were not concerned with psychophysical research.

The intensities of the sucrose and citric acid were chosen on the basis of previous experiments on absolute taste thresholds, some results of which are reported in Gregson (1962), and were intended to represent 2, 3½, and 5 jnd units above absolute threshold estimates for the population of tasters from which the 72 were drawn.

Samples were made in concentrated form and allowed to stand for about ½ hr after dilution to tasting concentrations on the day of use, but were always used within a day of making. Each subject started each tasting run with a tap-water mouth rinse. All tasting was performed individually in closed ventilated tasting cubicles, about 6-ft.-side cubes, the experimenter administering samples via a hatchway and rotary table. Subjects did the runs at their own pace. Illumination was daylight or daylight supplemented by artificial light via "daylight" color filter. Stimuli were colorless liquids in clear-glass tumblers; each portion was about 30 cc.

PROCEDURE

The samples were set before the subject, and the following instructions given: "Here are five samples of a diluted lemon drink. They vary in their sweetness (acidity) and I want you to say how each varies compared to the one you tasted before, using this card." (A card was shown bearing seven statements as follows: MORE SWEET (+3), SLIGHTLY MORE SWEET (+2), VERY SLIGHTLY MORE SWEET (+1), THE SAME AS THE LAST (0), VERY SLIGHTLY LESS SWEET (−1), SLIGHTLY LESS SWEET (−2), LESS SWEET (−3). The word "sweet" was changed to "acid" where required. The numerical values indicated were never shown to subjects, but were used throughout analysis of the results.)

"You will see that a sample will either be more sweet (acid) or less sweet (acid) or the same as the one before it. Would you taste the first two and say how the second compares with the first. Now would you taste the third and say how it compares with the second" (etc.).

When the control subjects were being used they alternated with the experimental subjects.

RESULTS

The mean and standard deviations of the relative intensity ratings for each comparison on each experimental run are set out in Table 1. It will be noticed that the mean values of all comparisons, including those associated with a change in the component not being judged, $A_{a.b.}$, $A_{b.a.}$, $S_{a.c.}$, $S_{d.c.}$, are near to zero, suggesting that neither concordant nor contrasting responses were occurring. This interpretation makes the assumption that mean ratings are an adequate measure of the situation, an assumption that has been made in previous studies. However, examination of the standard deviations of the relative-intensity comparisons reveals that they are greater for those comparisons involving a change in the component not being judged than for those comparisons in which the total mixture actually remained unchanged. The application of a test of homoscedasticity (Bartlett, 1934) reveals that the results of all judgments are heteroscedastic ($p<.02$), but when the two groups of comparisons are considered separately they are both homoscedastic. In other words the distributions of the relative intensity ratings of pairs of identical stimuli, regardless of which component is being compared, are similar whereas the distributions of the relative intensity ratings of the constant component in those instances where the other component varied, regardless of which it was or in which direction it moved, are also similar to each other but significantly different from the former distributions.

Table 2 shows the frequencies of the ratings for the two types of comparison. Comparisons of identical stimulus mixtures (Table 2-A) produce unimodal distributions with the erratic exception of judgment $S_{d.d.}$. On the other hand, comparisons in which the component not being rated was changed (Table 2-B) resulted in bimodal distributions. If either contrasting or concordant responding was occurring alone, then these latter distribu-

Table 2. Pooled frequencies of ratings, (2nd stimulus compared with 1st tasted within a comparison), over similar stimulus comparisons from experimental runs.

A. Comparisons of identical stimulus mixtures, based on 1st and 2nd comparisons.

Rating scale value	Judgment elicited			
	$A_{a.a}$	$A_{b.b}$	$S_{c.c}$	$S_{d.d}$
+3	3	1	4	1
+2	16	11	9	11
+1	12	10	7	6
0	15	17	17	11
−1	3	6	6	5
−2	6	6	6	14
−3	1	5	7	8
Σ	56	56	56	56
mean	0.63	0.04	−0.04	−0.46
s.d.	1.44	1.57	1.75	1.79

B. Comparisons of stimulus mixtures differing in one component only, based on 3rd and 4th comparisons where appropriate.

Rating scale value	Judgment elicited			
	$A_{a.b}$	$A_{b.a}$	$S_{c.d}$	$S_{d.c}$
+3	11	11	6	8
+2	5	12	4	8
+1	4	0	7	0
0	1	0	4	2
−1	2	2	2	3
−2	7	5	10	8
−3	12	12	9	13
Σ	42	42	42	42
mean	−0.12	0.21	−0.38	−0.43
s.d.	2.49	2.56	2.15	2.43

tions would also be unimodal with the mode situated towards one or other extreme. In fact both effects have occurred. The population of subjects is not responding in a homogeneous manner and it is for this reason that the variances of the two groups are so different.

The distributions of ratings made by the control group are shown in Table 3. The results for comparisons of identical stimulus mixtures are homoscedastic and so are those for comparisons of the component that varied, but all the results for the control group when considered together are heteroscedastic. In this group, unlike the experimental subjects, there is more agreement between subjects when they are making comparisons of the component that is in fact varying than when comparing identical stimulus mixtures. These results indicate that the changes in concentration of both stimulus components are being adequately detected by the subjects in spite of the presence of the other component and in spite of any residual masking from the previously tasted stimuli in the series.

It might be argued that the distributions in Table 2-B are due to some kind of guessing strategy on the part of the subjects, that is, subjects can detect some kind of difference when the component other than the one they are comparing is changed but are not sure as to what it is and consequently allocate their responses randomly (Cheatham 1952). Inspection of Table 4 reveals that subjects are being more consistent from one trial to the next than would be expected if they were producing contrasting or concordant responses by chance. The indeterminate subjects are those who made the same response to no stimulus change as they made to a real change in one of the components, and consequently cannot be considered as exhibiting either type of behavior. If the indeterminate responses are equally allocated to the four other cells the value

Table 3. Pooled frequencies of ratings, (2nd stimulus compared with 1st tasted within a comparison), over similar stimulus comparisons from control runs.

A. Comparisons of identical stimulus mixtures, based on 1st and 2nd comparisons.

Rating scale value	Judgment elicited			
	$S_{a.a}$	$S_{b.b}$	$A_{c.c}$	$A_{d.d}$
+3	1	1	0	2
+2	3	4	2	1
+1	1	0	0	5
0	7	5	6	2
−1	2	1	3	3
−2	1	3	4	2
−3	1	2	1	1
Σ	16	16	16	16
mean	0.19	−0.13	−0.63	0.19
s.d.	1.51	1.93	1.36	1.71

B. Comparisons of stimulus mixtures differing in one component only, based on 3rd and 4th comparisons where appropriate.

Rating scale value	Judgment elicited			
	$S_{a.b}$	$S_{b.a}$	$A_{c.d}$	$A_{d.c}$
+3	0	10	0	4
+2	0	2	0	6
+1	1	0	1	1
0	0	0	1	0
−1	0	0	1	0
−2	2	0	3	1
−3	9	0	6	0
Σ	12	12	12	12
mean	−2.50	+2.83	−2.00	+1.92
s.d.	1.12	0.41	1.29	1.31

Table 4. Frequency table showing the relation between the types of responses made by subjects on the first and the second trial runs.

First trial	Second trial			
	Concordant	Contrasting	Indeterminate	Σ
Concordant	19	3	1	23
Contrasting	5	17	1	23
Indeterminate	4	1	5	10
Σ	28	21	7	56

of the phi-coefficient is 0.5, which is significant beyond 1% level.

The actual amount of the perceived changes in intensity cannot be measured in absolute terms, because the amount is relative and affected by previously encountered changes in the series of mixtures tasted.

The series of five stimuli gives four comparative ratings between stimuli, and hence three intervals between ratings. Changes in ratings over these three intervals, irrespective of their direction, reflect changes in the perceived differences between stimuli. Taking runs *E*1, *E*2, *E*5, and *E*6 as one group, *t*-tests between the three intervals show differences significant beyond the 5% level in the mean moduli of changes in ratings between the second and third intervals, but not between the first and second, although the second interval shows a greater shift than the first, probably from the negative recency effect (Jarvik, 1951). This is consistent with expectations, for the first three comparisons are between identical pairs of stimuli. For the runs *E*3, *E*4, *E*7, and *E*8 (runs in which intensity reversals occurred) differences in mean moduli of shifts between all three intervals are significant beyond the 1% level by *t*-test. This means that a contrast effect is occurring over the last comparative rating; subjects who detect a change—in whatever direction—between the third and fourth stimulus detect a much bigger opposite change between the fourth and fifth, although the actual change is equal and opposite to the one preceding. This behavior is consistent with the A.L. theory, but only if the actual direction of the changes for half the subjects (the concordant subjects) is ignored. The perceived magnitude of the shifts in sweetness due to acid changes or acidity due to sucrose changes is clearly influenced by immediately preceding taste experience, for the previous stimuli serve as subjective reference points.

DISCUSSION

The results show that both concordant and contrasting responses can occur as types of response behavior, so either theory is excluded as an adequate general description of responses over the near-threshold range. The effects of changes in one component of a sucrose-citric acid mixture on changes in the perceived intensity of sensations associated with the other constant component are as great, when expressed on a rating scale, as the perceived actual changes associated with the variable component itself (compare Tables 2-B and 3-B). On the basis of our results, at least one sort of serial comparative judgment can be elicited in which acid can make sucrose more or less sweet, and sucrose make citric acid more or less sharp: the two sorts of change appear to be associated with two separate groups of tasters, so that it is misleading to treat all tasters as equivalent, as previous workers have done. The evidence does not tell us what the two groups of tasters are; in similar studies in sensory modalities other than taste, comparable divisions of subjects have been made on personality variables (Eysenck, 1942) and on strategies that a subject might adopt in ambiguous situations (Vanderplas and Blake, 1949; Broadbent, 1958).

If the results of Kamen *et al.* held in our experiment we would expect to get contrasting responses when sucrose concentration was increased, but concordant responses when acid is increased. If Pangborn's (1960) results held, we would expect to get contrasting responses throughout. Since previous studies use analyses based simply on mean responses to stimuli, we cannot disentangle all the factors that could account for differences between their studies and our own, but we question the generality of their findings and the validity of their conclusions over the range of concentrations used in our experiment precisely because they have neglected variables that are known to affect the perception of complex stimuli or stimuli encountered in series (George, 1917; Jarvik, 1951; Estes, 1954; Lawrence and Laberge, 1956).

It may be argued that we have not been entirely fair to the A.L. theory, since Engel and Parducci (1961) have revised it and put it into a different and more complex form to cover the case of a variable stimulus judged against a variable background.

This is an extension of the A.L. theory in that one component is identified as "figure" and the other as "background." This third theory might be reconciled with, but only with, the contrasting responses, for if the subject picks on the variable component as his background, the constant component should shift in contrasting fashion, whereas if he has picked on the constant component as background then prediction is indeterminate.

CONCLUSIONS

Current psychological theories can be employed to predict the occurrence of either concordant or contrasting responses, but the fact that we have found both indicates that the situation is more complex than can be accounted for by either theory taken on its own. The conflicting results between previous studies and our own can be the result of inadequate analysis of results or differences between the tasters in their previous experience of similar stimuli, either on trial sessions, or as reference stimuli, or on early experimental tests as compared with later ones.

We conclude that, in the near-threshold range of stimulus intensities, perceptual processes are as important as the stimulus identities or intensities themselves in determining what subjects will say they can taste in a mixture. In order to specify the interaction effects of taste mixture components we must therefore hold constant, or separate out in analysis, the various sources of bias such as time-errors or the subjects' selective expectations about what stimulus mixtures are to be encountered (Gregson, 1961). Also, if possible, we should screen the subjects to determine whether they have a predilection to respond in some habitually erroneous fashion when complex judgments are evoked.

The food technologist needs information on the perception of taste mixtures as a rational basis for product formulation and development. Such information should indicate what generality can be claimed for it: if potential tasters perceive small changes in different ways because they do not constitute a homogeneous group of tasters, then product changes of the perceived magnitudes involved in our experiment cannot be expected to lead to consistent changes in acceptability.

ACKNOWLEDGMENTS

We thank the Directors of J. Lyons and Company Limited, who provided research facilities.

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Correlation of Gas Chromatographic Data with Flavor Profiles of Fresh Banana Fruit [a]

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(Manuscript received November 19, 1962)

SUMMARY

Data obtained with several banana varieties show excellent qualitative and quantitative correlations between the development of flavor and odor notes and chemical analysis for volatiles by gas-liquid chromatography. The characteristic "banana-like" flavor is due to the amyl esters of acetic, propionic, and butyric acids. The distinctive "fruity" and "estery" notes are attributed to butyl acetate, butyl butyrate, hexyl acetate, and amyl butyrate. It is evident from these studies that determination of the chemical composition of the volatiles has permitted a much more precise characterization of flavor and odor notes, and, conversely, that these flavor profiles contribute greatly to interpretation of the significance of chemical constituents. The correlations found also substantiate the potential value of gas chromatographic analysis for routine evaluation of banana fruit quality. It is anticipated that the combined use of these two techniques will enhance the value of both in their application to numerous flavor and odor problems.

INTRODUCTION

Modern techniques of flavor evaluation have relied heavily upon sensory methods designed to eliminate preference judgments. Among these methods, the flavor profile technique of Cairncross and Sjöström (1950) has proved both objective and flexible in its application to a wide range of flavor and odor problems.

The more recent advent of highly sensitive instruments for analyzing the volatile components contributing to flavor and odor has given new impetus to research in this field. In particular, the technique of gas-liquid partition chromatography has provided a rapid and discriminating method of analysis.

Previous studies have been reported in which many of the volatile constituents of the banana fruit have been identified (Hultin and Proctor, 1961; Issenberg and Wick, 1963; Kleber, 1912; Rothenbach and Eberlein, 1905; Von Loesecke, 1950), but the significance of these compounds in relation to the flavor and aroma properties of the fruit has not been investigated. The present study was made to relate the gas chromatographic patterns of banana volatiles to the flavor profiles of the fruit, and to determine the contribution of certain compounds to the characteristic banana flavor. A total of 55 flavor profiles and 200 chromatographic analyses were conducted on several banana varieties at successive stages of ripening under controlled conditions.

EXPERIMENTAL

The varieties of banana fruit (*Musa acuminata*) used in this study were selected and shipped by Dr. Hugh T. Freebairn, Vining C. Dunlap Laboratories, La Lima, Honduras, C. A. Upon arrival from Honduras, approximately 10 hands (bunches) of each variety were placed in a ripening chamber maintained at 16.5°C and 90% relative humidity. Ethylene was introduced through a port in the closed chamber to give a concentration of 0.1% for 18 hr. At intervals during the ripening period

[a] Presented at the 22nd Annual Meeting of the Institute of Food Technologists, Miami Beach, Florida, June, 1962.

of 5–10 days, adjacent banana fingers were removed from different hands of a given variety for concurrent gas chromatographic and flavor profile analyses.

Gas-liquid chromatography. A β-ionization chromatograph employing 10 mc of strontium-90 in the detector was operated isothermally at a constant sensitivity throughout the investigation. Glass columns, 6 ft long and ¼-inch in outside diameter, were packed with Chromosorb W (80–100-mesh) coated with 5% UCON polar. Argon was used as the carrier gas at a rate of 50 ml/min, and column temperatures ranged from 50 to 70°C.

In preparing samples for vapor analysis, the peeled fruit of a single banana was split longitudinally, sliced, and placed in a 250-ml Erlenmeyer flask. The flask was then sealed with a rubber serum cap and allowed to stand at room temperature for 1 hr prior to analysis of the head space above the fruit. For each analysis, an appropriate volume of the vapor (0.5–10 ml) was withdrawn by gas-tight syringe and introduced into the chromatographic column.

Flavor profile analysis. Flavor profiles were determined by an established procedure (Cairncross and Sjöström, 1950; Caul, 1957) in which the individual components or notes of the aroma and flavor of a given product are described in semi-quantitative fashion. The flavor profile panel consisted of five members previously trained in the examination of bananas. Using standardized techniques, samples were prepared by peeling the banana, removing 1 inch of the fruit from the blossom and stem ends, and cutting the remaining pulp longitudinally into five portions.

Each panel member examined a freshly cut surface of one longitudinal section of the banana for aroma and flavor characteristics, and recorded: 1) the over-all impression of aroma and flavor, referred to as amplitude and expressed by a numerical scale ranging from very low, through low, moderate, and high; 2) the individual character notes expressed in descriptive terms such as sweet, sour, amyl acetate, etc.; 3) the intensity or strength of each character note graded on a numerical scale indicating threshold or just detectable, slight, moderate, and strong; and 4) the order in which each character note appears in the aroma and flavor complex.

Individual examination of the first sample by each member was followed by open discussion and formation of composite aroma and flavor profiles. Analysis of a second sample served to confirm initial findings and to resolve minor differences that might exist. If the second banana sample was significantly different from the first, additional bananas were examined.

Precise, descriptive terminology was agreed upon, using specific chemical compounds as reference standards whenever possible, although descriptive terms such as "green," "woody," and "estery" were also used. In this investigation, standards were examined undiluted, on blotter strips, and in a prepared fondant base. The *n*- and iso-forms of the compounds examined were so similar in flavor and odor that no attempt was made to distinguish between them.

RESULTS AND DISCUSSION

Fig. 1 is a composite chromatogram of

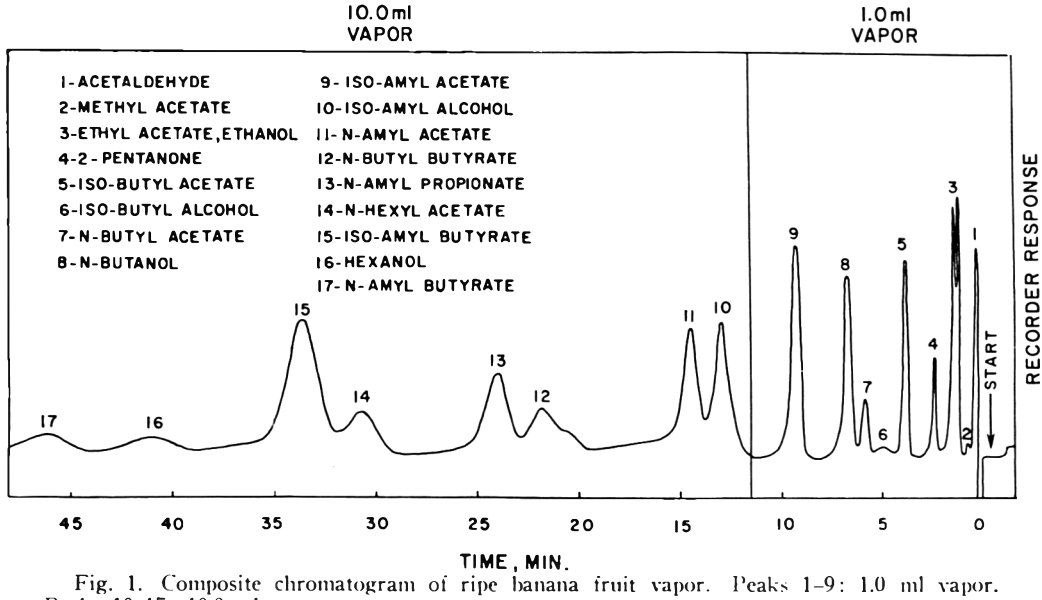


Fig. 1. Composite chromatogram of ripe banana fruit vapor. Peaks 1–9: 1.0 ml vapor. Peaks 10–17: 10.0 ml vapor.

the vapor from a ripe fruit. The identifications are based on retention values compared with those of pure standards at temperatures ranging from 50 to 70°C on three different column substrates: 5% UCON polar, 5% Carbowax 4000, and 5% β,β'-oxydipropionitrile, all on Chromosorb W, 80–100-mesh. With the exception of *n*-amyl propionate and *n*-butyl butyrate, the presence of these compounds in extracts and concentrates of banana fruit has been reported by other investigators (Hultin and Proctor, 1961; Kleber, 1912; Rothenbach and Eberlein, 1905; Von Loesecke, 1950). Issenberg and Wick (1963) recently completed a comprehensive study on the isolation, separation, and identification of these compounds.

Table 1 gives typical flavor profiles of two varieties of fully yellow banana fruit. Fig. 2 shows chromatograms of the vapor from comparable fruits. The profiles of the Gros Michel variety revealed a low flavor amplitude and various characteristic notes ranging in intensity from threshold to slight to moderate. The typical Gros Michel banana flavor was described as "estery" (slight to moderate), with amyl acetate the only recognizable ester. The Valery banana fruit, in contrast, had a moderate to high amplitude, with a more complex "estery" note at a moderate to strong intensity. The panel described the esters as a combination of amyl acetate in moderate amounts, amyl propionate and amyl butyrate in threshold quantities, and a slight to moderate "fruity" note, probably consisting of a butyl ester of butyric and/or valeric acid. Other esters that give a "fruity" impression, such as butyl and hexyl acetates and butyl butyrate, are probably also reflected in this "fruity" complex but could not be specifically recognized. The amplitudes of the flavor profiles of the Valery fruit reflected a much fuller and more interesting flavor than did those of the Gros Michel fruit. The flavorful constituents were more intense and complex, and the presence of a "fruity" complex added significantly to the over-all flavor impression without loss of banana identity.

Confirming the flavor-panel results, both qualitative and quantitative differences were observed in the gas chromatograms, with the fruit of low flavor amplitude (Gros Michel) having fewer compounds and less total volatile material than the fruit of moderate to high amplitude (Valery).

Following identification of the volatiles by gas chromatography, the flavor panel ex-

Table 1. Flavor profiles of ripe banana fruit varieties.

Gros Michel				Valery			
Aroma		Flavor		Aroma		Flavor	
Amplitude 1		Amplitude 1		Amplitude 2		Amplitude 2½	
Sweet fragrance		Sweet	1½	Sweet fragrance		Sweet	2
(estery)	1½	Starchy-mealy	1½	(estery)	2	Sour	1½
Sour	1½	Sour	1½	Sour	1½	Creamy	½
Starchy	1	Estery	1½	Estery	2	Estery	2½
Estery	1½	Amyl acetate	1	Amyl acetate	1½	Amyl acetate	2
Amyl acetate	1	Green	1	Fruity complex[a]	1	Fruity complex	1½
Other ester?		Woody	)(	Amyl butyrate?		Amyl butyrate	)(
Green	1	Drying	1½	Estery		Amyl propionate	)(
Woody	½	Bitter	½	pungency	2	Estery	
Oily-creamy	)(	Oily	)(	Creamy	½	pungency	1½
				Green	½	Tongue sting	1
						Drying	1
						Bitter	½

Intensity
)(—threshold
½—very slight
1 —slight
1½—slight-moderate
2 —moderate
–strong

Amplitude
)(—very low
1 —low
1½—low-moderate
2 —moderate
2½—moderate-high
3 —high

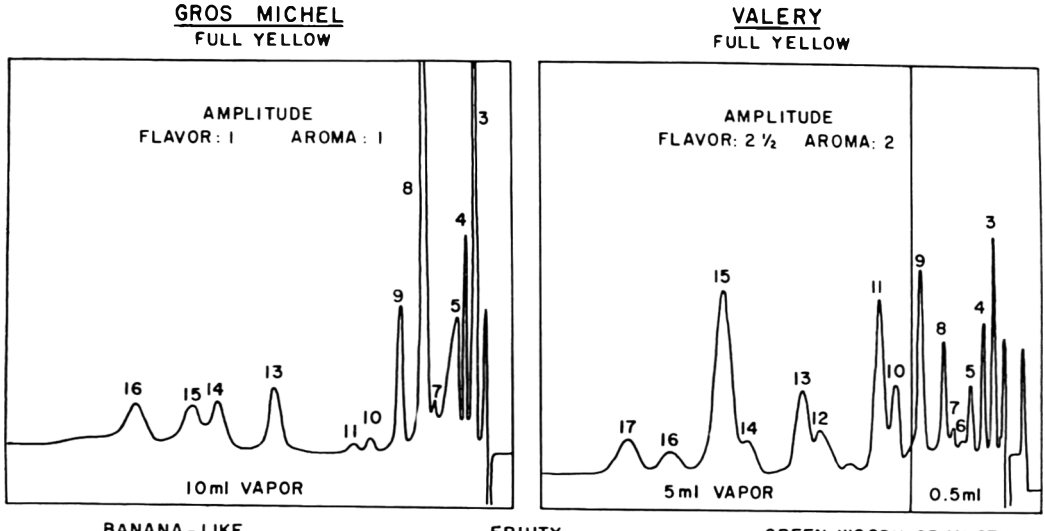
[a] Probably consisting of butyl esters of butyric and/or valeric acids and possibly reflecting the presence of other esters such as butyl and hexyl acetates.

amined each compound for its predominant odor characteristics, and subsequently classified them according to three general sensory impressions, as shown in the lower portion of Fig. 2. Chromatograms of the Gros Michel fruit revealed the presence of those compounds responsible for the "banana-like" flavor, but showed little or none of the volatiles associated with "fruitiness." The Valery fruit, however, contained substantial amounts of the compounds contributing to both "banana" and "fruity" impressions. Additionally, certain alcohols and carbonyl compounds were found to possess "green" and "woody" characteristics and are believed to contribute in part to these notes. The flavor panel found that the "green" note was very similar to trans-2-hexenal, a compound previously reported in the banana fruit by other investigators (Hultin and Proctor, 1961; Issenberg and Wick, 1963) and occasionally observed in the chromatograms during this study. The compounds responsible for other profile notes (sweet, sour, starchy, etc.) were not studied in this program; however, the presence of sugars, starches, free acids, and other nonvolatile compounds undoubtedly contributes to the total flavor picture.

The marked variation in production of volatiles by the banana fruit during ripening has been reported earlier (McCarthy and Palmer, 1962). To confirm and extend the results described above, identical studies were conducted at successive ripening stages as indicated by the peel color of the fruit.

Fruit at the early ripening stage (Fig. 3) showed both a simple gas chromatographic pattern and a flavor profile of low banana identity (Table 2). The amplitudes were very low, and the major notes were described as "sour," "green," and "woody," with only threshold intensity of the typical banana flavor. As ripening proceeded, characteristic notes of varying intensities were observed by the panel. This development of flavor was accompanied by increasingly complex chromatograms. Although precise quantitative data are not yet available, increases in the concentrations of individual components are indicated by increases in their chromatographic peak areas at each stage of ripening.

In the early stages of flavor development, the individual esters were present in low concentrations and are described by the flavor-profile panel as a general "banana" or "estery" impression. As the ester levels in-



BANANA-LIKE	FRUITY	GREEN, WOODY, OR MUSTY
9-ISO-AMYL ACETATE	5-7-BUTYL ACETATE	2-METHYL ACETATE
11-AMYL ACETATE	12-BUTYL BUTYRATE	4-PENTANONE
13-AMYL PROPIONATE	14-HEXYL ACETATE	8-BUTYL ALCOHOL
15-17-AMYL BUTYRATE	15-17-AMYL BUTYRATE	10-AMYL ALCOHOL
		16-HEXYL ALCOHOL

Fig. 2. Vapor chromatograms of Gros Michel and Valery banana varieties. Peaks correspond to those of Fig. 1.

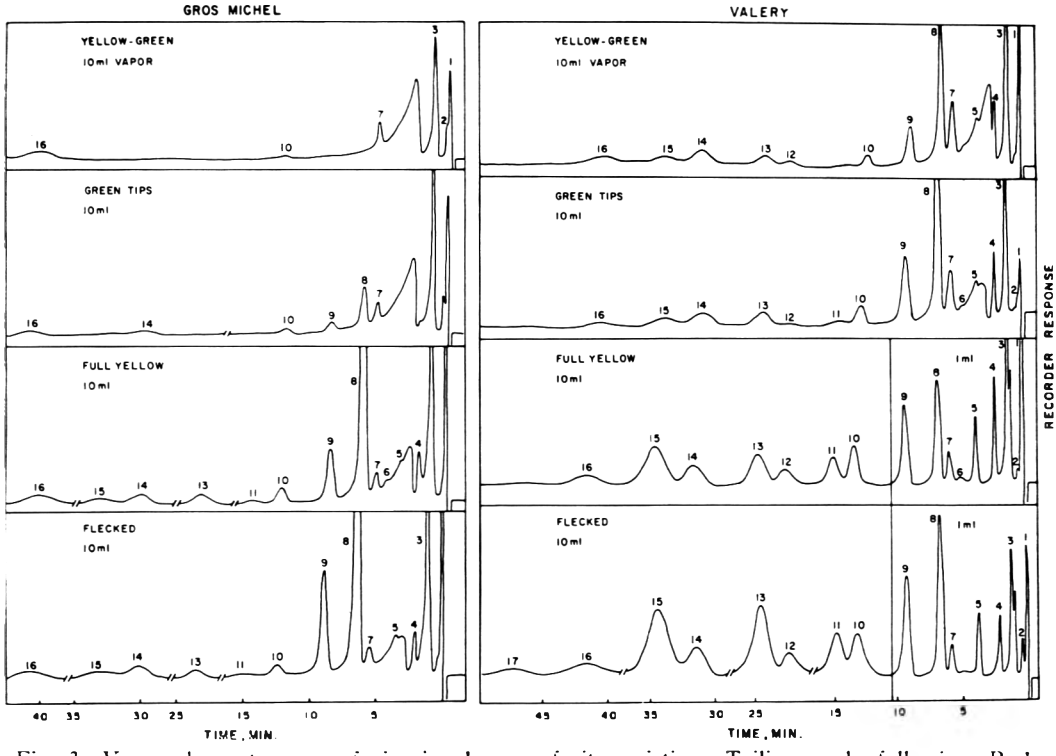


Fig. 3. Vapor chromatograms of ripening banana fruit varieties. Tailing peak following Peak No. 4 is water vapor.

creased, the panel could recognize specific esters and describe them as individual notes. The intensities of these notes correlated with relative concentrations of the esters in the fruit vapor. Panel results showed that "green" and "woody" notes were reduced or absent in the ripe fruit, although chromatographic analyses showed the continuing presence of certain compounds possibly related to these notes. This observation sug-

Table 2. Development of amplitude levels and characteristic flavor notes during ripening of banana fruit varieties.

	Gros Michel				Valery			
	Yellow-green	Green tips	Full yellow	Flecked w/brown	Yellow-green	Green tips	Full yellow	Flecked w/brown
Amplitude	)([a]	½	1	1	1	1	2	2
Flavor notes								
Sweet	0	½	1½	1½	1	1	2	2
Sour	2½	2	1½	1	2	2	1	1
Banana	)(	½	1	1	1	1½	2	2
Amyl acetate	0	0	1	½	½	½	1	1
Amyl propionate	0	0	0	½	0	)(	1½	1½
Butyl butyrate	0	0	0	0	0	0	)(	½
Amyl butyrate	0	0	0	)(	)(	)(	½	1
Green	2	1½	1	0	1½	1½	1	0
Woody	1	½	)(	0	½	0	0	0

[a] Threshold.

gests that the sensory stimuli of the responsible compounds may be suppressed by the higher concentration of esters.

Correlations between flavor and odor profiles and chromatographic patterns were consistently observed throughout the investigation and have resulted in the more effective use of both techniques, individually and in combination. Identification of each constituent by chromatography has resulted in more precise flavor profiles. The development of flavor profiles has made it possible to interpret the significance of compounds found on the chromatographic patterns. Once the relationship of flavor profiles and gas chromatograms has been established for a given product, the gas chromatograph can be used more effectively for routine analyses. In the present study, the combined use of these methods has provided both a definition of the characteristic banana flavor and a distinction between "banana" and "fruity" flavor impressions.

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Classification of Textural Characteristics[a]

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(Manuscript received July 23, 1962)

SUMMARY

Definition of texture is reviewed, and a system for classification of textural characteristics of foods is described. The system is based on fundamental rheological principles, and at the same time is suitable for routine use. Textural characteristics are defined and classified into mechanical and geometrical qualities as well as those related to the moisture and fat content of a product. The mechanical characteristics are subdivided into the primary parameters of hardness, cohesiveness, viscosity, elasticity, and adhesiveness, and into the secondary parameters of brittleness, chewiness, and gumminess. It is pointed out that popular terms used to describe texture often denote degrees of intensity of these characteristics. The proposed classification lends itself to use with both objective and subjective methods of texture characterization.

INTRODUCTION

Texture is the least well described of the many organoleptic food attributes of concern to a food scientist. One should mention, of the several reasons that caused this situation, the lack of an adequate bridge between theoretical rheology and practical applications, and the fact that most of the work in food texture reported to date dealt with a specific textural characteristic in a specific food product. This led to confusion even regarding the definition of texture itself. Only in the last few years have some attempts been made to define texture, and these are not devoid of the influence of the prevailing trend toward identifying texture with just a few characteristics most common to a particular food in question. For example, in discussing the texture of frozen peas the food scientist will usually mean toughness of the skin; in referring to the texture of meat he will usually mean tenderness versus toughness and chewiness; in describing the texture of canned cherries he will mean freedom from cracks and the wholeness of the fruit.

One of the most urgent problems in texture technology is the development of a rational system and nomenclature for describing and translating textural qualities into precisely defined, measurable properties. Foods represent very complex rheological systems, and applications of the principles of theoretical rheology (Burgers and Scott Blair, 1949; Eirich, 1958; Scott Blair, 1949, 1953; Scott Blair and Reiner, 1957) lead, in many cases, to difficult theoretical and experimental problems that are too time-consuming for applied industrial research. This situation is further complicated by the fact that, once obtained, rheological description of foods must be translated into practical terms. Laboratory and field practice has resulted in the creation of a popular nomenclature that serves to describe textural characteristics in terms of simple words. However, this nomenclature has several inherent problems. In many cases, a number of terms are used to describe the same characteristic. In other cases, the same term is used to describe several characteristics. Also, the same word may have different meanings to different people.

This study was made to review the definitions of texture used by workers in the field, and to develop a system of texture nomenclature that could serve as a bridge between fundamental rheological principles and popular nomenclature and lend itself to applied research on a vast variety of food products.

Definition of Texture

Webster's dictionary defines texture as "the characteristic disposition or connection of threads

[a] Presented at the 22nd Annual Meeting of the Institute of Food Technologists, Miami Beach, Florida, June 10–14, 1962.

in a woven fabric or the disposition or manner of union of the particles of a body or substance."

Hall and Fryer (1953) considered texture and consistency as components of mouthfeel. They did not give clear definitions of these terms, but said: "It . . . appears that the term *texture* has come to mean how hard or how soft as well as how large or how small the individual kernels in the mass of . . . food actually are. If the individual kernels are large and hard, the texture is said to be grainy or gritty. If the individual kernels are small and soft, the texture is said to be smooth. *Consistency* has come to refer to the condition of the individual kernels cohering together and adhering to foreign substances, that is, the stickiness, gumminess or viscosity, of the . . . food . . ."

Smith (1947) distinguished between texture and consistency in a similar manner, although his definitions are even less specific. He considered texture to be "the rigidity of solid units of food," and consistency, "a combination of the size and texture of the solid units, the viscosity of the fluid, and the proportions of solids to fluid." The term "texture" is thus applied to solids while "consistency" refers to a mixture of fluid and solid foods.

Consistency is often termed "body." Davis (1937) wrote: ". . . it is preferable to define 'body' as that quality which is perceptible to touch; 'texture' as that which is evident to the eye, excluding color. Under the term 'body' are included several factors of which firmness (viscosity and modulus), springiness (elasticity) and smoothness (homogeneity) are most important. . . ."

Martin (1955) defined the texture of candy as "the sum or resultant of several physical properties including density, hardness, plasticity or elasticity, and consistency." Isherwood (1960) included in the texture of fruit and vegetables such characteristics as toughness, stringiness, slicing quality, and crispness.

Ball *et al.* (1957) stressed the need for a good definition of texture. In an attempt to find a definition that could be used dependably in grading meat for quality by the usual organoleptic procedures, they constructed two tentative definitions: the "sight" and the "feel" definitions. The "sight" definition reads: "Texture of meat is the microscopic appearance of muscle tissue from the standpoint of smoothness or fineness of grain. . . ." The "feel" definition is worded: "The texture of cooked meat is the feel of smoothness or fineness of the muscle tissue in the mouth."

These definitions point out two important elements of texture: the physical structure of the material (its geometry) and the way the material handles and feels in the mouth (its mechanical and surface properties).

A rigorous definition of texture will have to await a better understanding of the basic principles involved, especially those concerned with rheological or mechanical properties of foods. For the purpose of this work, texture was considered as the composite of the structural elements of food and the manner in which it registers with physiological senses. Thus, this definition includes the concepts of "texture" and "consistency" (or "body") as defined by other workers. The term "structural elements" as used in this definition refers to the microscopic and molecular structures as well as to the macroscopic structure that can be sensed visually. Sound effects, such as those occurring during eating of popcorn or celery, are related to the physical constitution of the food and should be considered as part of the over-all textural effect.

Classification of Textural Characteristics

A compilation of terms used in popular texture terminology, an analysis of their meanings, and the definitions of rheological concepts served as a background for the development of an organized system for classification of textural characteristics. In studying the definitions of terms popularly used in description of texture, one finds that whereas some refer to what might be called "primary" characteristics, many refer to "secondary" characteristics, i.e., those that could be adequately described by two or more of the primary terms. In addition, many popular terms actually denote degrees of the same characteristic and could be considered points on a scale.

When terms used to describe texture were compiled and their dictionary and rheological definitions were studied, it became apparent that textural characteristics could be grouped into three main classes:

1) Mechanical characteristics
2) Geometrical characteristics
3) Other characteristics (referring mainly to moisture and fat content of the food)

Mechanical characteristics are manifested by the reaction of the food to stress. They are measured organoleptically by pressures exerted on the teeth, tongue, and roof of the mouth during eating. Geometrical characteristics refer to the arrangement of the constituents of the food, and are reflected mainly in the appearance of the food product. They are mostly sensed visually. However, these characteristics are often sufficiently pronounced to produce an oral sensation through the sense of touch and pressure. In the category of "other characteristics" are included mouthfeel

factors that cannot be easily resolved on the basis of mechanical and geometrical properties.

Mechanical characteristics. The mechanical characteristics are probably the most important in determining the manner in which the food handles and behaves in the mouth. They can be divided into the following five basic parameters:

A) *Hardness*, defined as the force necessary to attain a given deformation.

B) *Cohesiveness*, defined as the strength of the internal bonds making up the body of the product.

C) *Viscosity*, defined as the rate of flow per unit force.

D) *Elasticity*, defined as the rate at which a deformed material goes back to its undeformed condition after the deforming force is removed.

E) *Adhesiveness*, defined as the work necessary to overcome the attractive forces between the surface of the food and the surface of other materials with which the food comes in contact (e.g., tongue, teeth, palate, etc.)

The first four characteristics are related to forces of attraction acting between particles of food and opposing disintegration, whereas adhesiveness is related to surface properties.

In addition to the above five primary parameters, it appears to be desirable to characterize food texture in terms of three additional secondary parameters in order to make the characterization as meaningful as possible to individuals accustomed to popular terminology, while at the same time keeping it in agreement with basic rheological principles. These secondary parameters are:

B-1) *Brittleness*, defined as the force with which the material fractures. It is related to the primary parameters of hardness and cohesiveness. In brittle materials, cohesiveness is low and hardness can vary from low to high. Brittle materials, especially when possessing a substantial degree of hardness, often produce sound effects on mastication (e.g., celery, toasted bread)

B-2) *Chewiness*, defined as the energy required to masticate a solid food product to a state ready for swallowing. It is related to the primary parameters of hardness, cohesiveness, and elasticity.

B-3) *Gumminess*, defined as the energy required to disintegrate a semisolid food product to a state ready for swallowing. It is related to the primary parameters of hardness and cohesiveness. With semisolid food products, hardness is low.

Geometrical characteristics. Geometrical characteristics do not lend themselves to clear-cut divisions. There are, however, two general groups of qualities: A) those related to size and shape of the particles, and B) those related to shape and orientation. A number of characteristics in the first group could be placed on a hardness scale.

While some of the geometrical characteristics (e.g., smooth) refer to homogeneous food products, many are applicable to systems containing more than one phase. For example, "cellular" refers to a highly organized structure composed of cell walls filled with a gas (e.g., egg-white foam) or with a liquid (e.g., watermelon). The mechanical characteristics of each one of the phases should be considered for a thorough texture analysis of such a food.

Other characteristics. This group comprises mouthfeel qualities related to the perception of the moisture and fat content of the food:

A) Moisture content

B) Fat content

1) Oiliness

2) Greasiness

These qualities may also be considered to be concerned with the lubricating properties of the product. The popular terms used to describe the moisture content of a food reflect not only the total amount of moisture perceived but its rate and manner of release. With fat content, the total amount of fat and its melting point, as related to mouth-coating properties, become important. The secondary parameters of oiliness and greasiness have been set up to distinguish between these two characteristics. The secondary parameter of oiliness refers to the intensity of the "oily" feeling in the mouth, which might be related to the surface tension and changes in viscosity of the product affected by movements of the tongue. The secondary parameter of greasiness reflects the solidity and difficulty of removal of a fatty film coating the mouth cavity, both of which, in turn, are related to the melting point of the component fat.

Popular Nomenclature and the Developed Classification

Table 1 shows the relations between the popular nomenclature of food texture and the developed classification of textural characteristics. Because of the large number of terms used in popular terminology, only the most characteristic ones are included in this table to illustrate the principle. It will thus be seen that the terms soft, firm, and hard are actually degrees of the characteristic of hardness, and the terms crumbly, crunchy, and brittle are degrees of the characteristic of brittleness. Dry, moist, wet, and watery are terms denoting different levels of moisture. No popular

Table 1. Relations between textural parameters and popular nomenclature.

MECHANICAL CHARACTERISTICS		
Primary parameters	*Secondary parameters*	*Popular terms*
Hardness		Soft→Firm→Hard
Cohesiveness	Brittleness	Crumbly→Crunchy→Brittle
	Chewiness	Tender→Chewy→Tough
	Gumminess	Short→Mealy→Pasty→Gummy
Viscosity		Thin→Viscous
Elasticity		Plastic→Elastic
Adhesiveness		Sticky→Tacky→Gooey
GEOMETRICAL CHARACTERISTICS		
Class		*Examples*
Particle size and shape		Gritty, Grainy, Coarse, etc.
Particle shape and orientation		Fibrous, Cellular, Crystalline, etc.
OTHER CHARACTERISTICS		
Primary parameters	*Secondary parameters*	*Popular terms*
Moisture content		Dry→Moist→Wet→Watery
Fat content	Oiliness	Oily
	Greasiness	Greasy

terms are available to describe different degrees of oil or fat content. This problem is solved in practice by adding qualifying words such as "slightly," "moderately," "very," etc., to the terms "oily" and "greasy." Combinations of several parameters result in popular terms like "juicy," which is a combination of the geometrical characteristic of cellularity and a high moisture content.

Adaptation to Objective and Subjective Evaluation of Texture

The proposed classification of textural characteristics lends itself to use with instrumental (Friedman *et al.*, 1963) and organoleptic (Brandt *et al.*, 1963) methods of texture evaluation, and has served as a basis for the development of quantitative rating scales (Szczesniak *et al.*, 1963). Use of the same nomenclature for both objective and subjective evaluation of texture facilitated establishment of a correlation between the two methods (*ibid*). In addition, experience has indicated that the proposed classification can be used with ordinary laboratory panels provided definition of the basic parameters is properly understood.

To determine the adequacy of the developed classification and the relation between the individual parameters, quantitative description of the texture of 22 selected food products using this system and the standard scales (Szczesniak *et al.*, 1963) was subjected to scatter-diagram analysis. The results indicated that there was no duplication between the parameters and that the ratings followed the established definitions. A relation was found between hardness and brittleness: products noted for brittleness always fell on the upper portion of the hardness scale. Similarly, an inverse relation was found between hardness and moisture content: relatively moist materials fell on the lower portion of the hardness scale, whereas only low-moisture materials possessed high degrees of hardness. The parameters of viscosity and brittleness were found to be mutually exclusive. This is understandable since only solids can have the characteristic of brittleness, whereas the characteristic of viscosity is limited, in this classification, to liquid and semisolid products. Scatter diagrams of viscosity and brittleness versus hardness show that the brittleness scale may be a continuation of the viscosity scale. A strong positive relation was found between gumminess and adhesiveness. This does not mean that the two parameters are mutually exclusive, but that these two characteristics often occur simultaneously. An interesting correlation was obtained between gumminess and moisture content, and adhesiveness and moisture content. In both cases, a high rating on either one of the scales could be obtained only with a low rating on the other scale. For example, a highly adhesive material was always rated as being low in moisture, whereas products with a low degree of

adhesiveness could fall on any part of the moisture scale. The same situation existed when scatter diagrams were prepared for the elasticity scale versus the adhesiveness and gumminess scales.

Concluding Remarks

A more rigorous evaluation of the proposed classification of textural characteristics will have to await the accumulation of more data and a more extensive use of the system. No doubt, slight modification of this classification may become necessary as problems are encountered in its practical use. It is believed, however, that the basic concepts described may be a step in the direction of supplying the food scientist with a rational tool for a scientific description of food texture.

ACKNOWLEDGMENT

Sincere appreciation is expressed to Dr. Robert V. MacAllister, presently with Clinton Foods, Clinton, Iowa, for inspiring discussions and constructive criticism that made possible the final formulation of the thoughts described in this paper, and to the late Professor Bernard E. Proctor, Massachusetts Institute of Technology, whose interest and encouragement contributed to bringing this work to its present stage of development.

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The Texturometer—A New Instrument for Objective Texture Measurement

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(Manuscript received July 23, 1962)

SUMMARY

A new recording instrument, the "texturometer," gave good correlation between instrumental values and subjective evaluation by a trained texture profile panel. It was applied to measurement of the mechanical textural parameters: hardness, cohesiveness, viscosity, elasticity, adhesiveness, brittleness, chewiness, and gumminess. Subjective definitions of these parameters are interpreted in terms of physical measurement characterized by the texture "profile." Examples of representative profiles are included.

INTRODUCTION

An ever-growing need for a comprehensive objective method for characterizing food texture led to an investigation of the mechanical parameters significant to the definition of texture. A thorough analysis of the subject resulted in the elimination of a vast series of descriptive terms and the acceptance of five basic independent mechanical parameters (hardness, cohesiveness, adhesiveness, viscosity, and elasticity) and three dependent parameters (brittleness, chewiness, and gumminess). The results of that investigation are reported elsewhere (Szczesniak, 1963a).

With establishment of the basic definitions of the textural paramaters, it was possible to design an instrumental unit capable of translating the definitions into the reality of physical measurement. It is certainly true that an objective method can never exclusively replace the sensory evaluation of food texture, but it does offer the opportunity of recording in an unbiased manner the physical condition of a sample for future reference. Because of its dependence upon the reproducibility of standard reference materials and variable errors, the sensory method lacks absolute quality, a lack that is characteristic of any procedure relying on individual experience.

Our investigation of the literature revealed numerous instruments that had been used for objective measurement of selected textural properties of food products. Among them were the shear-press (Decker *et al.*, 1957; Kramer, 1961), gelometers (Schachat and Nacci, 1960; Porette and Billheimer, 1956; Braun, 1954; Doesburg, 1950; Dorner, 1955; Fellers and Griffiths, 1928; Pintauro and Lang, 1959), various makes of viscometers (Barthel, 1951; Bauer, 1959; Becker and Clemens, 1956; Brunelli and Mengozzi, 1951; Cunningham *et al.*, 1953), compressimeters (Combs, 1944; Crossland and Favor, 1950; Kattan, 1957), consistometers (Birdsall, 1946; Bloom, 1938; Clardy *et al.*, 1952; Cohee and Goodale, 1939; Eolkin, 1957), and tenderometers (Lovegren *et al.*, 1958; Clarke, 1951; Cain, 1951; Doesburg, 1954; Kramer, 1948; Bohn and Baily, 1936; Proctor *et al.*, 1955, 1956a,b; Davison, 1959), to mention just a few of the vast number of current instruments available for characterizing texture. Objective methods of texture measurement were recently reviewed by Szczesniak (1963b) and Kramer and Twigg (1959). The chief shortcoming found in most of the instrumentation was a concentration on only a small phase of the mechanical properties rather than a physical representation of all the parameters necessary to the complete description of texture. Thus, the shear press and the tenderometers measure tenderness (or toughness) by recording the force necessary to shear the test materials, the gelometers measure the firmness and strength of gels, the viscosimeters and the consistometers measure resistance to flow, and

the compressimeters determine hardness by measuring resistance to a compressing force. Although these instruments have value in practical applications when the textural quality of the food can be related to the measured property, they fall short when a complete picture is desired of the mechanical parameters of texture and the way these are sensed in the mouth.

At the initiation of the investigation, an instrumental unit called the denture tenderometer, built and used by the Food Technology Laboratory of the Massachusetts Institute of Technology (Proctor *et al.*, 1956a,b), came to our attention because of its potential applicability to the over-all measurement of the mechanical parameters of texture. It was designed to simulate the masticatory action of the human mouth, using a mechanical device called a Hanau articulator. It was made up of a drive motor to activate the upper jaw of the articulator. A pair of sensitive strain gauges in the driving arm of the upper jaw transmitted the response of the chewing action to an amplifier unit, which in turn fed the signal to a cathode-ray oscilloscope. The force-penetration relationship was then photographed with a Polaroid camera. In addition to the driving system and recording facilities, the chewing terminus consisted of a set of plastic dentures secured to the jaws of the unit. These were constructed to simulate a buccal cavity, complete with simulated gums and cheeks, to aid in maintaining the position of the food sample between the teeth during mastication.

It was felt that the M.I.T. tenderometer most closely approximated the requirements of a good instrument for measuring the mechanical properties of texture. As a result, the M.I.T. instrument was used as a basic prototype for the construction of a modified texture-measuring unit, called "texturometer," which would meet our requirements and eliminate some of the basic difficulties encountered with the tenderometer.

DESCRIPTION OF THE TEXTUROMETER

The significant modifications of the M.I.T. prototype were: 1) substitution of a strip-chart recorder for the oscilloscope; 2) replacement of the dentures by a plunger and plate; 3) removal of the strain-gauge sensing unit from the articulator arm and repositioning on the plate support arm; 4) provision for several chewing speeds; and 5) construction of a viscosity attachment.

It was felt that a fast-recording strip-chart unit, such as a Leeds and Northrup Speedomax Model G, ¼-second pen speed recorder, chart speed 60 in. per minute, was preferable to an oscilloscope because it permits a presentation of any number of individual chews in place of the single recording available with the oscilloscopic unit.

Difficulty was experienced in maintaining samples on the teeth, because of slipping during mastication. As a result, it was felt that the dentures should be replaced with a plunger and plate to simulate their function. Plungers of various sizes were constructed, depending upon the characteristics of the samples to be evaluated. In place of the lower jaw, a platform was substituted on which could be mounted cups varying in size and shape.

It was found that the position of the sensing element on the activating arm of the upper jaw was a poor one because of the disturbing effects on the force-penetration relationship produced by inertia of the arm movement. Consequently, the strain-gauge unit was repositioned on the stationary arm of the sample plate, thus eliminating the spurious effects mentioned above. In place of the single speed available on the older unit, provision was made for changing the articulation rate by introduction of a system of two sets of tooth gears to permit any of four speeds. A standard speed of 42 bites per minute gave reproducible results.

The texturometer comprises four basic units: a Hanau articulator, including motor, variable-pulley drive, and strain-gauge sensing plate; a fast-response pen recorder; a variable-voltage power supply; and a Wheatstone bridge circuit with balancing potentiometer for zero adjustment of the strain-gauge circuit. Plungers have been constructed in five sizes and made up of three different materials. The sizes vary from ⅝ inch to 2 inches in diameter. All plungers are 1 inch in height. The materials

used are Lucite, aluminum, and brass. For larger plungers it is preferable to use Lucite, because of the excessive weight of the metal plungers.

Fig. 1 is a front view of the texturometer, showing the Hanau articulator and the strip-chart recorder. Fig. 2 is a side view of the articulator, including the driving arm, gear train, and drive motor, all mounted rigidly on a composition base. The sample cell and plunger are also shown.

The instrument is standardized by positioning the articulator arm for a clearance of ⅛ inch between plunger and sample stage at the point of maximum drive. A standard sponge-rubber sample, ½ inch in height, is maintained for standardization of the bridge circuit and voltage input. The standard is referred to at intervals during a day's run of samples to ascertain continued standardization of the instrument. All samples are made up wherever possible to a height of ½ inch and an area at least that of the plunger base. If other sample heights are used, corrections have to be made, as explained below in the interpretation section.

To provide for measurement of viscosity, a modified cup was constructed with strain-gauge sensing elements mounted on a fixed baffle arm (see Fig. 3). The cup fits easily into position on the lower platform, and the sensing element can be connected to the electrical bridge circuit in place of the strain-gauge element normally used. A Lucite paddle has been substituted for the conventional plunger, mounted on a small motor, which rotates the paddle at a constant torque (see Fig. 4).

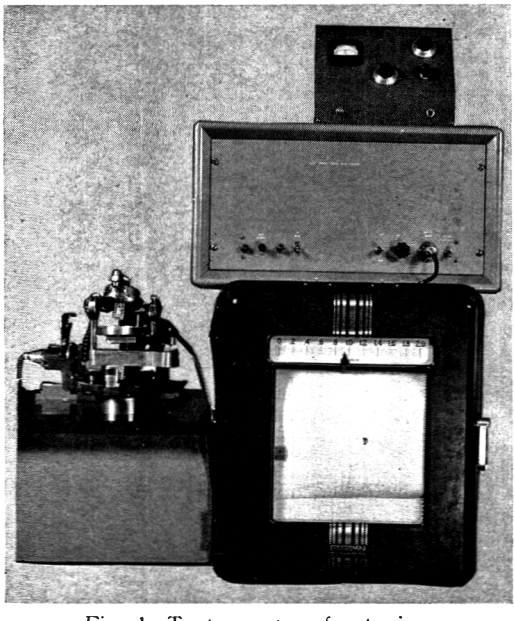

Fig. 1. Texturometer—front view.

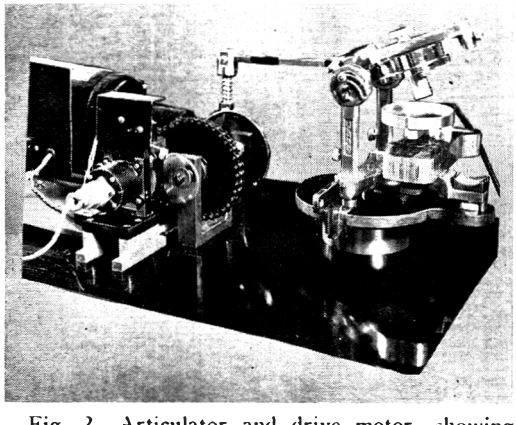

Fig. 2. Articulator and drive motor—showing sample cell and plunger.

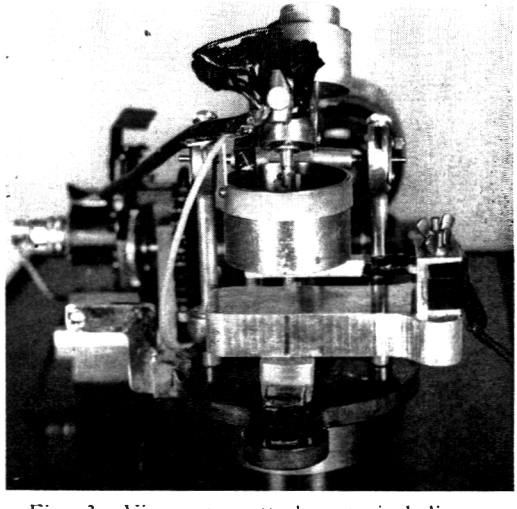

Fig. 3. Viscometer attachment, including cup and paddle.

INTERPRETATION OF THE RECORDED CURVES

The recorded force-distance relations represent an integral picture of the textural characteristics of the product sample under test, and may thus be called texture "pro-

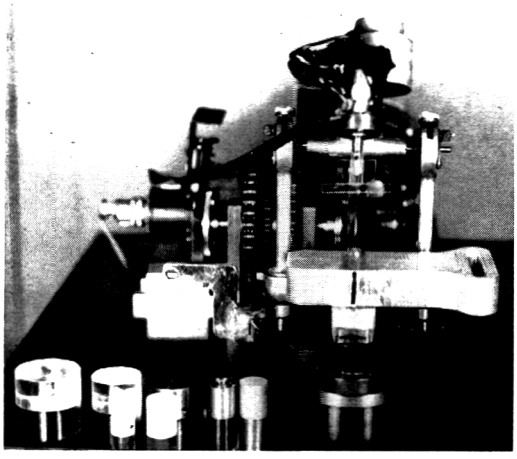

Fig. 4. Viscometer attachment, with cup removed to show Lucite paddle. In the foreground, representative plungers are displayed.

files." Their interpretation depends upon the adaptation of the previously developed definitions (Szczesniak, 1962a) of the mechanical parameters of texture to the conditions of measurement by the texturometer. The mathematical formulation of each of the parameters has been developed after careful experimentation and consideration of the important dependent variables.

Fig. 5 illustrates a typical texture-profile curve obtained on the instrument, indicating the methods of measuring the mechanical parameters as discussed below. Brittleness is not shown, since brittleness and adhesiveness are never encountered in the same food product. The curve should he read from

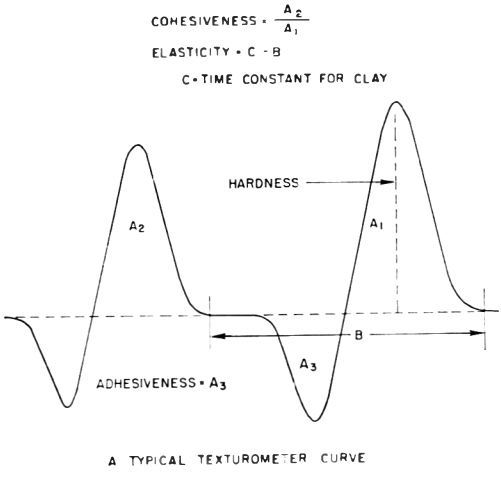

Fig. 5. A typical texturometer curve.

right to left, with peak A_1 being the first chew, and peak A_2 the second chew.

Hardness is measured from the profile as the height of the first chew. Since the height of each sample is standardized to ½ inch and the plunger-travel fixed to reach within ⅛ inch of the plate, this measurement is consistent with our definition of hardness. All hardness values are normalized to a one-volt input by the following method:

$$\text{hardness} = \frac{\text{height of first peak}}{\text{volts input}}$$

Should the sample have a depth of less than ½ inch, extra slices are added to make up the standard height.

Cohesiveness is measured as a ratio of the area, in arbitrary units, under the second peak and the area under the first peak A_2/A_1 in Fig. 5. Since this parameter is measured as a ratio of two areas, the input voltage is not critical. However, the voltage used for measurement should be sufficient to keep the peak heights within the confines of the recorder paper. The area under each peak is the integral of force over a distance, and therefore represents the work done in each chew. This value is a direct function of the work needed to overcome the internal bonds of the material.

If the food sample exhibits adhesiveness, it is necessary to coat the material with talcum powder before recording the cohesiveness profile, because of the distortion created by the negative adhesiveness peak. Sample size is preferably kept at ½-inch height.

Elasticity is measured as the difference between the distance B, measured from the initial sample contact to the contact on the second chew, and the distance C, the same measurement made on a completely inelastic standard material such as clay. With our instrument, the value of C is 68.5. Preferable sample height for this parameter measurement is 1 inch.

$$\text{Elasticity} = C - B$$

Adhesiveness is measured as the area, in arbitrary instrumental units, A_3, of the negative peak beneath the base line of the profile, and represents the work necessary to pull the plunger from the sample. Adhesive-

ness measurements are made on samples of ½-inch height and input of 5 volts.

In addition to the independent mechanical parameters, we have defined three subparameters as functions of the independent variables described above.

Brittleness is easily characterized by the multipeak shape of the first chew, and is measured as the height of the first significant break in the peak. Conditions for making this measurement are the same as for hardness; samples are of ½-inch height and corrected to an input of 1 volt.

Chewiness is expressed mathematically as the product of hardness, cohesiveness, and elasticity.

Gumminess is expressed as the product of hardness and cohesiveness. The result is multiplied by 100 to eliminate decimals.

Viscosity measurement is confined to fluids and recorded as a series of sinusoidal curves (Fig. 6), using the viscosity attachment (paddle and cup) as shown in Figs. 3 and 4.

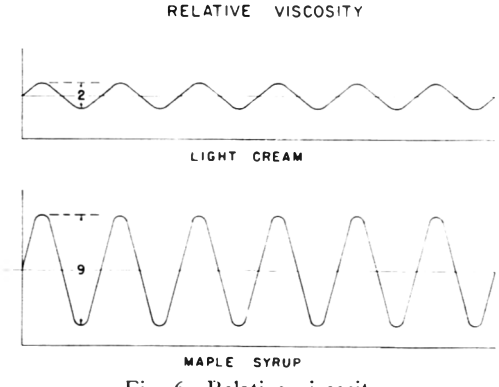

Fig. 6. Relative viscosity.

Fig. 7 presents a few representative texture profiles of food materials. White cake without crust produces an objective picture of low cohesiveness with low hardness. The adhesiveness parameter is quite apparent in the profile of lemon chiffon pudding, indicated by the negative peak below the base line. Bran flakes are characterized by hardness and brittleness. The profile of dry dog food illustrates a high degree of brittleness, as indicated by the sharp breaks in the peaks of both the first and second chews.

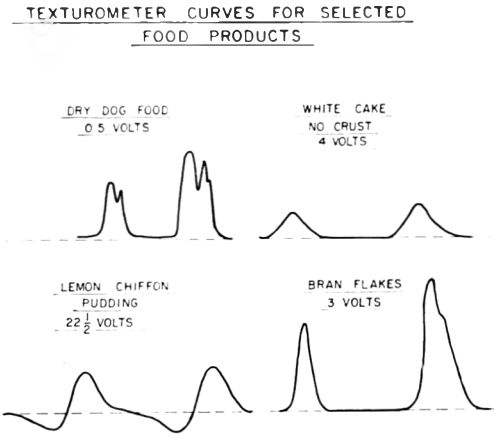

Fig. 7. Texturometer curves for selected food products.

PRECISION OF MEASUREMENTS

The instrumental error inherent in texturometer measurements is small. The following coefficients of variation were found using a piece of hard foam rubber as the test material: hardness 0.045%, cohesiveness 2.64%, elasticity 1.27%, and chewiness 2.98%. Hard foam rubber was selected because of its uniformity and because it did not disintegrate under the test conditions.

Variability greater than that indicated above can be attributed to the heterogeneity of the tested materials. Since most foods vary significantly in textural characteristics from sample to sample and even within a sample, replicate measurements show greater spreads. Mean coefficients of variation calculated for different food products were as follows: hardness 16.1%, cohesiveness 11.8%, elasticity 11.2%, adhesiveness 22.5%, chewiness 22.1%, and gumminess 6.6%. The exact coefficient of variation of any parameter depended on the type of food product under analysis.

DISCUSSION

Measurements made on the texturometer are reported in arbitrary instrumental units that can be converted into cgs units by the use of standard references. Since reference materials are used to calibrate the instrument, absolute values are not necessary. A strip of foam rubber weather stripping was found suitable for calibration.

Foods that change in texture with moisture content are evaluated after equilibration

to reach the required moisture value. The objective texture measurement of cold, dry cereals does not correlate well with the subjective panel evaluation. However, the addition of moisture to the cereal results in excellent agreement between instrumental hardness measurement and the subjective rating. Similar difficulty is experienced in the case of marshmallows, where discrepancy exists with respect to the parameter of adhesiveness. Sensory evaluation indicates that the center of the marshmallow is not adhesive, while texturometer curves clearly show the negative adhesiveness peak. Placing a few drops of water on the surface of the marshmallow eliminates the adhesiveness peak and results in a perfect agreement with the panel. We are currently considering further modification of the texturometer to provide for intermittent synthetic saliva injection to simulate the salivary action during mastication.

In addition, we have been studying the inclusion of a temperature-control unit for the texturometer. When temperature is found to be a factor in the measurement of textural properties of foods, instrumental profiles are made at a range of temperature levels. Parameter values vary with temperature in foods such as hard and soft cheeses and chocolate products, where melting occurs naturally in the mouth during mastication. In this way, a more complete picture can be obtained of the textural characteristics.

The translation of mechanical pressure into electrical impulse by way of paired strain-gauge units depends upon a slight movement of the arm supporting the sample platform. As a result, a correctible error is introduced in calculating sample penetration. This is inherent in the nature of strain-gauge operation. It has also been found that, after long use, the strain-gauges require replacement because of wear and tear produced by continuous mechanical action of the articulator. We have investigated means of monitoring pressure changes that are more stable and can provide the same kind of sensitivity found in strain-gauge circuits. We recently encountered a new technique (for continuously measuring pressure changes) in the form of a series of pressure-sensitive paints, which respond by altering internal resistance to electrical flow.

Texturometer characterization of the mechanical parameters of texture of selected food products correlated well with subjective evaluation by a trained texture-profile panel. These data are reported elsewhere (Szczesniak *et al.*, 1963). The good correlation indicates that the texturometer is able to measure the same type and intensity of mechanical characteristics of texture as are perceived in the mouth.

In addition to mechanical parameters, foods also have geometrical (fibrousness, graininess, grittiness, etc.) and lubricating (dryness, juiciness, oiliness, etc.) qualities (Szczesniak, 1963a). Geometrical characteristics can be detected with the texturometer only inasmuch as they contribute to the heterogeneity of the product in terms of its mechanical properties. No controlled methods of measuring these geometrical properties on the texturometer have been worked out. Application of the instrument to juiciness measurements will be the subject of a separate communication.

ACKNOWLEDGMENT

The authors thank Dr. Thomas L. Fazzina, presently with the Maxwell House Division of General Foods Corporation, who, as a graduate student at the Department of Nutrition, Food Science, and Technology at M.I.T., built the described instrument for our use and contributed significantly to its present state of modification. A special word of appreciation is also due Professor Samuel Goldblith for cooperation and many courtesies extended to us during construction of the instrument.

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Development of Standard Rating Scales for Mechanical Parameters of Texture and Correlation Between the Objective and the Sensory Methods of Texture Evaluation

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(Manuscript received July 23, 1962)

SUMMARY

Standard rating scales of hardness, brittleness, chewiness, gumminess, viscosity, and adhesiveness were established for quantitative evaluation of food texture. The scales cover the entire intensity range found in food products and may be expanded at any desired point for greater precision in a narrower range. Each point on the scale is represented by a food product selected on the basis of availability, familiarity, constancy of textural characteristics, and other criteria. Using the developed scales, correlation was good between sensory and instrumental (texturometer and viscosimeter) evaluations of texture.

INTRODUCTION

Most reported work on sensory evaluation of food texture has used an arbitrary scoring system and has been limited to one product or one class of products. The measured textural characteristics were restricted to those believed most important for the food in question. Thus, Bockian *et al.* (1958) and Deatherage and Garnatz (1952) reported on subjective determination of meat tenderness, Lowe and Stewart (1947) on evaluation of poultry meat, and Longrée and Fenton (1950) on the toughness and stringiness of kale. Bliss *et al.* (1953) compared scoring methods for mealiness of potatoes. Cover described a paired-eating method as a tool for subjective testing of tenderness in meat (1936) and a method of scoring for three components of beef steak tenderness (1959). These components were softness, friability of muscle fibers, and tenderness of connective tissue. Hopkins (1950) attempted to quantify subjective appraisals of texture, and of other food attributes. He differentiated between the intensity of undesirable characteristics and the intensity or absence of desirable characteristics. The report by Raffensperger *et al.* (1956) on the structured toughness-tenderness scale for grading beef is one of the few attempts at a logical development of sensory texture-rating-scale formats.

The present work was done to develop standard rating scales based on the proposed classification of textural characteristics (Szczesniak, 1963) in order to provide a defined quantitative method of evaluation of the mechanical parameters of texture. The desirability of rating scales in organoleptic evaluation of foods has been pointed out by Sheppard (1953, 1955). It was intended to have the scales encompass the entire range of intensity of textural characteristics rather than a selected few. Another objective was to select specific examples for each point on the scale so that proper reference standards could be available and the degree of intensity of a given textural parameter in an "unknown" product could be illustrated in terms of a known product. This would also eliminate the problem of drifting discussed by Tarver and Schenck (1958). A third objective was to use the developed standard scales in seeking a correlation between organoleptic and instrumental methods of texture evaluation.

Presented at the 22nd Annual Meeting of the Institute of Food Technologists, Miami Beach, Florida, June 10–14, 1962.

TECHNIQUE

Criteria for selection of standards for the scale points. Several criteria were used in selecting reference standards for the individual points on the scales. The most important consideration was that the selected food items possess the desired intensity of the textural characteristic and that this characteristic not be overshadowed by other textural parameters, i.e., it is an outstanding property of the selected food and is easy to perceive organoleptically. Other important criteria were the availability, constant quality, and general familiarity of the foods. Specialty items and laboratory preparations were avoided as much as possible, and attempts were made to select well-known commercial products. The commercial brands were selected solely on the basis of the desired intensity of the particular characteristic and its reproducibility from batch to batch. In addition, the chosen foods had to meet the requirements of minimum handling necessary to ready the product for evaluation, and minimum change in textural characteristics from small temperature variations. We avoided fresh fruits and vegetables, whose texture varies greatly with variety, degree of maturity, and other factors, and items that require cooking, baking, etc. Foods that did not meet one or more of the above requirements were selected only when serious difficulties were encountered in finding a product that would meet all of the above requirements.

Judging the prospective standards for textural characteristics. The prospective reference foods were standardized as to size and temperature. They were then evaluated by 9 panel members trained in flavor profile methods and thus possessing an analytical approach to organoleptic evaluation of food attributes. The panel was also thoroughly familiar with the definitions of textural parameters developed earlier (Szczesniak, 1963). Judging was done individually, followed by a round-table discussion to arrive at an average rating and to clarify any possible large discrepancies or misunderstandings of definitions. The prospective reference materials were first judged semiquantitatively and ranked in the order of increasing intensity of the particular textural parameter under consideration. The distances between the materials were then evaluated, and products that showed equal intensity differences from their neighbors on each side were selected for the final scale. Thus, the developed scales are characterized by equivalent distances from point to point. An exception is the chewiness scale, where it has not yet been possible to select foods showing equivalent differences in the characteristic of chewiness.

RESULTS

Development of rating scales. *Hardness scale.* Hardness is judged organoleptically as the force required to penetrate a substance with molar teeth. The evaluation is restricted to solids and some semisolids because human perception of hardness is limited to samples that can be confined between the teeth.

Nine points were selected to represent the scale of hardness. These are shown in Table 1, together with information on sample size and the temperature of the product at evaluation.

Brittleness scale. Brittleness is judged organoleptically as the ease or force with which a sample crumbles, cracks, or shatters. It is a secondary parameter, encompassing the primary parameters of hardness and cohesiveness.

Table 1. Standard hardness scale.

Panel rating	Product	Brand or type	Manufacturer	Sample size	Temp.
1	Cream cheese	Philadelphia	Kraft Foods	½"	45–55°F
2	Egg white	hard-cooked 5 min	.....	½" tip	room
3	Frankfurters	large, uncooked, skinless	Mogen David Kosher Meat Products Corp.	½"	50–65°F
4	Cheese	yellow, American, pasteurized process	Kraft Foods	½"	50–65°F
5	Olives	exquisite giant size, stuffed	Cresca Co.	1 olive	50–65°F
6	Peanuts	cocktail type in vacuum tin	Planters Peanuts	1 nut	room
7	Carrots	uncooked, fresh	.....	½"	room
8	Peanut brittle	candy part	Kraft Foods	.....	room
9	Rock candy	.....	Dryden & Palmer	.....	room

Table 2. Standard brittleness scale.

Panel rating	Product	Brand or type	Manufacturer	Sample size	Temp.
1	Corn muffin	Finast	First National Stores	½"	room
2	Angel puffs	dietetic, heated for 5 min. at 190°F	Stella D'Oro Biscuit Co.	1 puff	room
3	Graham crackers	Nabisco	National Biscuit Co.	½ cracker	room
4	Melba toast	inside piece	Devonsheer Melba Corp.	½"	room
5	Jan Hazel cookies	.....	Keebler Biscuit Co.	½"	room
6	Ginger snaps	Nabisco	National Biscuit Co.	½"	room
7	Peanut brittle	candy part	Kraft Foods	½"	room

The seven-point scale of brittleness is shown in Table 2. It will be noticed that products at the lower end of the scale are soft and are popularly described as "crumbly," whereas those at the upper end of the scale are hard and are popularly known as "brittle."

Chewiness scale. Chewiness was rated organoleptically in terms of the length of time in seconds required to masticate a sample at the rate of one chew per second in order to reduce it to the consistency satisfactory for swallowing. Similarly to brittleness, chewiness is a secondary parameter. It encompasses the primary parameters of hardness, cohesiveness, and elasticity.

The seven-point chewiness scale appears in Table 3. It includes products that would popularly be called tender, chewy and tough.

Gumminess scale. Gumminess is a secondary parameter and is the product of a low degree of hardness and a high degree of cohesiveness. It refers to semisolid materials, and, organoleptically, it can be best described as a denseness that persists throughout mastication.

The five-point gumminess scale is shown in Table 4. It will be noticed that points on this scale are represented by laboratory samples rather than commercial food products. The reason is that the panel was unable to find suitable examples among the available food products. Easily prepared flour pastes of various flour-to-water ratios provided a uniform series of reference points possessing the required intensity of the studied characteristic. The pastes were made by mixing, e.g., 40 g of all-purpose flour with 60 ml room-temperature tap water to obtain a 40% flour paste.

Table 3. Standard chewiness scale.

Product rating	Av. no. of chews	Product	Brand or type	Manufacturer	Sample size	Temp.
1	10.3	Rye bread	fresh, center cut	Pechter Baking Co.	½"	room
2	17.1	Frankfurter	large, uncooked skinless	Mogen David Kosher Meat Products Corp.	½"	50–70°F
3	25.0	Gum drops	Chuckle	Fred W. Amend Co.	½"	room
4	31.8	Steak	round, ½" thick, broiled on each side for 10 min.	.....	½" square	140–185°F
5	33.6	Black Crows candy	.....	Mason Candy Corp.	1 piece	room
6	37.3	Peanut chews	.....	Whitman Co.	1 piece	room
7	56.7	Tootsie rolls	midget size	Sweets Co. of America	1 piece	room

Table 4. Standard gumminess scale.

Panel rating	Product	Brand or type	Manufacturer	Sample size	Temp.
1	40% flour paste	Gold Medal	General Mills	1 tbs.	room
2	45% flour paste	Gold Medal	General Mills	1 tbs.	room
3	50% flour paste	Gold Medal	General Mills	1 tbs.	room
4	55% flour paste	Gold Medal	General Mills	1 tbs.	room
5	60% flour paste	Gold Medal	General Mills	1 tbs.	room

Adhesiveness scale. Organoleptically, adhesiveness is judged as the force required to remove the material that adheres to the mouth (generally to the palate) during normal eating.

Five products were chosen to represent the scale of adhesiveness, which appears in Table 5.

Viscosity scale. Viscosity is perceived organoleptically as the force required to draw a liquid from a spoon over the tongue.

The eight-point viscosity scale appears in Table 6.

Correlation with objective measurements. Figs. 1–6 show the correlation between panel and objective evaluations on the developed standard scales. Foods selected to represent the individual points on the scales of hardness, brittleness, chewiness, gumminess, and adhesiveness were evaluated objectively on the texturometer with techniques described by Friedman *et al.* (1963). Examples on the viscosity scale were tested objectively with a HAT Brookfield Viscosimeter at 100 rpm and 75°F. It was recognized that the panel measured linear-flow viscosity whereas the instrument measured rotary-flow viscosity. However, no linear-flow viscosimeter, which would cover the entire range of viscosity represented by the selected standards, was available. The Bostwick Viscosimeter was found unsuitable because of excessively fast or excessively slow flow at the extremes of the scale. Speeds of 20, 50, and 100 rpm and temperatures of 45, 75, and 100°F were investigated with the Brookfield viscosimeter, and 100 rpm and 75°F were found to give the best correlation with sensory evaluation. It should be noted (Table 6) that the panel evaluated the standards on the viscosity scale at 45–55°F, but the foods undoubtedly underwent some increase in temperature as they were sucked into the mouth.

As shown in Figs. 1–6, correlation is very good between panel and objective evaluations on the developed standard scales. Hardness and brittleness showed a curvilinear relation between the instrument and the panel when the data were plotted on rectangular graph paper. Viscosity exhibited the same relation when the data, converted to centipoises, were plotted on semilog paper. The other parameters showed straight lines when plotted on either rectangular (chewiness and adhesiveness) or semilog (gumminess) paper. These correlations indicate that the panel was well able to distinguish between the individual mechanical parameters of texture and judge them quantitatively. They also indicate that the objective methods used, especially the texturometer, were able to measure the same intensity of textural characteristics as perceived organoleptically. It should be kept in mind, however, that the standards on the scales were carefully selected for the absence of overshadowing characteristics, which facilitated their sensory evaluation and undoubtedly contributed to the good correlation.

DISCUSSION

The classification of textural characteristics developed by Szczesniak (1963) included the mechanical parameters of co-

Table 5. Standard adhesiveness scale.

Panel rating	Product	Brand or type	Manufacturer	Sample size	Temp.
1	Hydrogenated vegetable oil	Crisco	Procter & Gamble Co.	½ tsp	45–55°F
2	Buttermilk biscuit dough	.....	Pillsbury Mills	¼ biscuit	45–55°F
3	Cream cheese	Philadelphia	Kraft Foods	½ tsp	45–55°F
4	Marshmallow topping	Fluff	Durkee-Mower	½ tsp	45–55°F
5	Peanut butter	Skippy, smooth	Best Foods	½ tsp	45–55°F

Table 6. Standard viscosity scale.

Panel rating	Product	Brand or type	Manufacturer	Sample size	Temp.
1	Water	spring	Crystal Spring Co.	½ tsp	room
2	Light cream	Sealtest	Sealtest Foods	½ tsp	45–55°F
3	Heavy cream	Sealtest	Sealtest Foods	½ tsp	45–55°F
4	Evaporated milk	.....	Carnation Co.	½ tsp	45–55°F
5	Maple syrup	Premier 100%	Francis H. Leggett & Co.	½ tsp	45–55°F
6	Chocolate syrup		Hershey Chocolate Corp.	½ tsp	45–55°F
7	Mixture: ½ cup mayonnaise and	Hellman's	Best Foods	½ tsp	
	2 tbs. heavy cream	Sealtest	Sealtest Foods	2 tbs	45–55°F
8	Condensed milk	Magnolia sweetened	Borden Foods	½ tsp	45–55°F

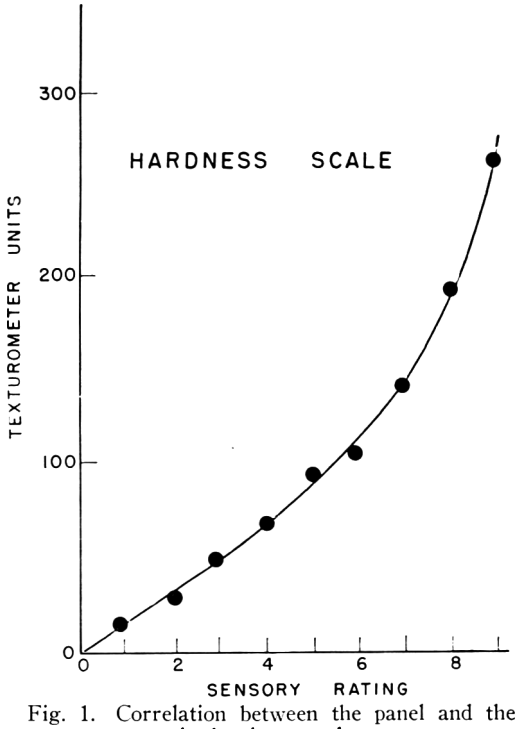

Fig. 1. Correlation between the panel and the texturometer on the hardness scale.

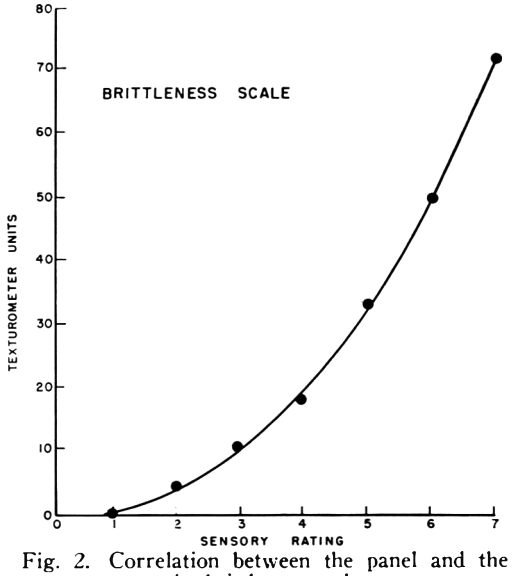

Fig. 2. Correlation between the panel and the texturometer on the brittleness scale.

hesiveness and elasticity, in addition to the parameters quantified by the presently described scales. Organoleptically, both cohesiveness and elasticity were difficult to perceive as such. Elasticity was the most difficult parameter to evaluate in the mouth, because of the sharp edges and relative insensitivity of the teeth. Because of this, it was not possible to construct standard scales for these two primary parameters, and their evaluation is included in the ratings for the secondary mechanical parameters.

The described scales offer a basis for quantitative organoleptic texture evaluation in a rational and relatively complete man-

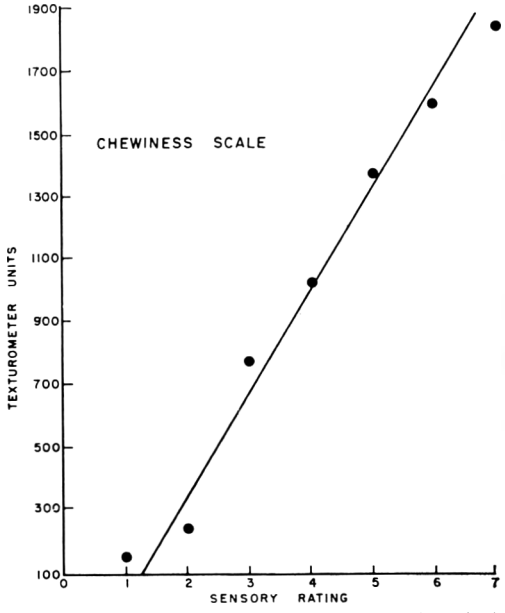

Fig. 3. Correlation between the panel and the texturometer on the chewiness scale.

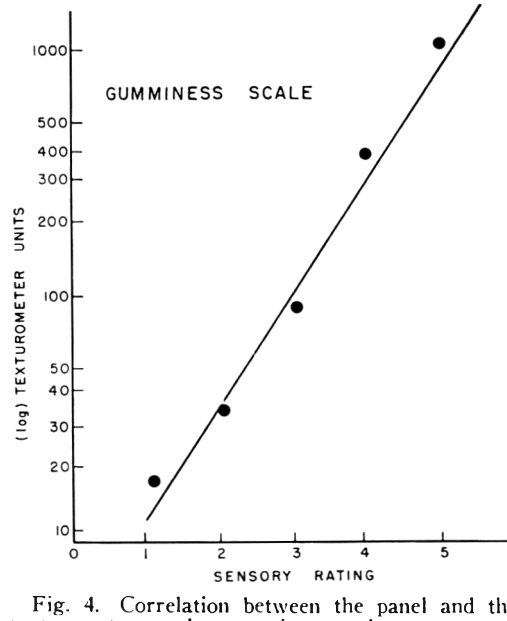

Fig. 4. Correlation between the panel and the texturometer on the gumminess scale.

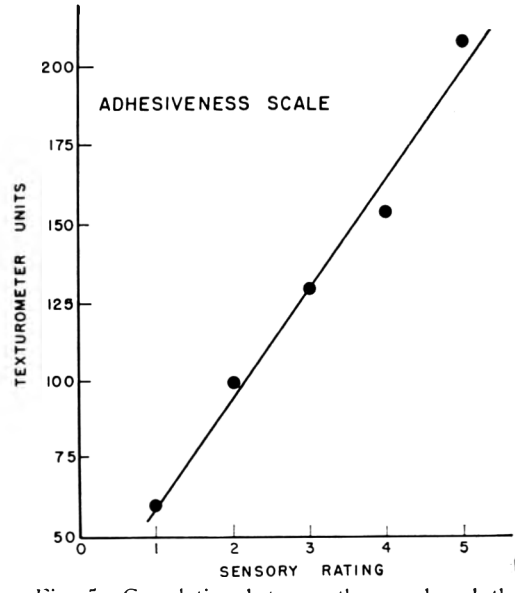

Fig. 5. Correlation between the panel and the texturometer on the adhesiveness scale.

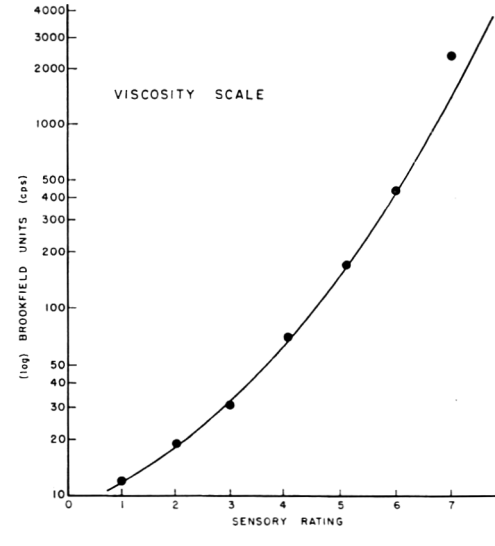

Fig. 6. Correlation between the panel and the Brookfield viscosimeter on the viscosity scale.

ner. The resulting set of numbers gives a texture "profile." Because they encompass the entire range of parameter intensities encountered in food products, the scales lend themselves to expansion at any desired point to allow for greater precision in a narrower range. Such expansion should be done by placing additional standard examples between the points defining the range of interest. The scales are composed of well defined, available, reasonably unchangeable standards. They are easy to use with highly trained or ordinary laboratory panels provided the definition of the basic concepts involved is well understood.

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Texture Profile Method

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(Manuscript received July 23, 1962)

SUMMARY

A texture profile method was developed that uses the A. D. Little flavor profile method as a model. A texture profile is defined as the organoleptic analysis of the texture complex of a food in terms of its mechanical, geometrical, fat, and moisture characteristics, the degree of each present, and the order in which they appear from first bite through complete mastication. The texture profile analysis requires a panel of judges with prior knowledge of the texture classification system, use of standard rating scales, and proper panel procedures with regard to the mechanics of testing and sample control.

INTRODUCTION

Development of a comprehensive sensory method for evaluating food textures is, by necessity, dependent upon a rational and well-defined system for classifying texture. The literature on sensory methods of texture evaluation contains fragmented information on definitions of texture, panel techniques, standardization of testing conditions, and correlation with instrumental measurements.

Deatherage and Gernatz (1952) reported poor correlation of sensory measurements of meat tenderness with the Warner-Bratzler shear machine. Apparently, the panel was not evaluating the same property as the instrument. Cover (1936) described a sensory method for evaluating the tenderness of two comparable samples of meat.

Boggs and Hanson (1949) and Raffensperger *et al.* (1956) pointed up the lack of organized knowledge in this field.

With few exceptions, there is a notable lack of information on the interrelations of several texture properties in a given food. Such an attempt was made by Cover (1959) to assess the influence of softness on the tenderness of rare steaks and, more recently, by Cover *et al.* (1962a,b,c) to explain the tenderness of beef in terms of six components. In addition, little has been done to assess the mechanics of panel evaluation so as to arrive at a measurement of a given textural characteristic. A recent paper by Harrington and Pearson (1961) discusses chew count as a measurement of tenderness of pork loins.

Work by Szczesniak (1963) and Szczesniak *et al.* (1963) on classifying textural characteristics and the development of rating scales contributed a logical and well-defined basis upon which to build a comprehensive sensory method for evaluating texture of a given food product. This classification of textural characteristics included three main types: mechanical properties, geometrical properties, and those relating to fat and moisture content. Since most foods are composed of varying degrees of these main groups of textural characteristics, it was necessary to design a sensory procedure whereby the entire texture of a food product could be assessed from first bite through complete mastication.

The flavor profile method described by Cairncross and Sjöström (1950) was used as a model in developing the texture profile technique.

EXPERIMENTAL

Mechanical characteristics of texture. Evaluation of the mechanical characteristics of texture is both qualitative and quantitative. These parameters are evaluated with standard rating scales developed by Szczesniak *et al.* (1963). The scales cover the entire range of intensity of the particular textural characteristic encountered in food products. They lend themselves to expansion in selected areas to allow for a more precise description of differences between closely related samples.

Each point on the scale is represented by a selected example, which makes it possible to assign a numerical rating to an "unknown" by comparing it to a known product.

Geometrical characteristics of texture. Evaluation of the geometrical characteristics of texture is qualitative and semi-quantitative. These characteristics are related to the size, shape, and arrange-

Table 1. Geometrical characteristics of texture.

A. Characteristics relating to particle size and shape	Reference	B. Characteristics relating to shape and orientation	Reference
Powdery	confectioner's sugar	flaky	boiled haddock
Chalky	raw potato, tooth powder	fibrous	base of asparagus shoot, breast of chicken
Grainy	Farina, Cream of Wheat	pulpy	orange sections
Gritty	pear stone cells, sand	cellular	raw apples, white cake
Coarse	cooked oatmeal	aerated	chiffon pie filling, milk shake
Lumpy	cottage cheese	puffy	puffed rice, cream puffs
Beady	tapioca pudding	crystalline	granulated sugar

ment of particles within a food. They are evaluated as to the type and amount present.

Table 1 lists these characteristics, with appropriate examples for reference. Characteristics relating to particle size and shape are perceived organoleptically as discrete particles relatively harder than the surrounding medium or the carrier. This group can be scaled in the same manner as the mechanical characteristics. For example, chalky, gritty, grainy, and coarse comprise a scale of increasing particle size.

Characteristics relating to shape and orientation represent highly organized structures of different geometrical arrangements within each product. A puffy texture is an organization of hard or firm outer shells filled with large, often uneven, air pockets. These are usually formed through application of heat and expansion of the material forming the shell (e.g. cream puffs, puffed rice). An aerated texture is a network of relatively small, even cells filled with air and surrounded by usually, but not always, soft cell walls. This type of a texture is usually formed by incorporating air through whipping or beating into a medium capable of film formation (e.g. marshmallows, meringue). Sometimes, as in case of cakes, the gaseous components are formed inside the structure and expanded in volume by means of applied heat.

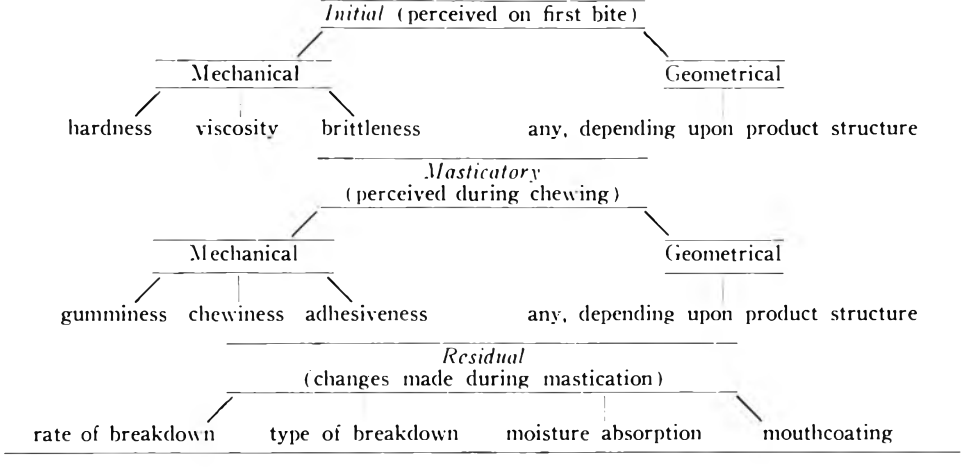
Order of appearance. A systematic means of recording all the texture characteristics of a given food was developed from the "order of appearance" principle of the flavor profile method described by Cairncross and Sjöström (1950).

Unlike flavor, where the order of appearance of notes cannot be anticipated, texture follows a definite pattern regarding the order in which characteristics are perceived. These can be subdivided into first-bite, masticatory, and residual phases.

The first-bite, or initial phase encompasses the mechanical characteristics of hardness, brittleness, and viscosity and any geometrical characteristics observed initially.

The second, or masticatory, phase encompasses the mechanical characteristics of gumminess, chewiness, and adhesiveness and any geometrical characteristics observed during chewing.

Table 2. Procedure for evaluating texture.



The third, or residual, phase encompasses changes induced in mechanical and geometrical characteristics throughout mastication.

Table 2 shows the procedure used in evaluating the different textural characteristics with respect to their appearance.

Moisture and fat content. Since the characteristics of moisture and fat content are multidimensional, it is impractical to set up scales that cover the entire range of these characteristics in food products. The characteristic of fat content can be evaluated not only for degree present but also in terms of other characteristics, such as melting time, geometrical properties, mouth coating, etc. Therefore a scale is constructed for the particular product in question.

Moisture content can be evaluated not only for the degree of moisture present but also for the rate at which it is released. A scale of moisture is constructed for a particular product type so that the reference points function in the same manner as the product under examination. In meats, the rate at which moisture is released appears to be more important than the amount present. Conversely, in cake the amount of moisture present is more important than the rate at which it is released.

Panel selection. A panel of 6–9 technical people are chosen on the basis of interest and availability. Previous experience in sensory evaluations is helpful, though not required.

The panel used in the work reported herein was composed of nine technical employees of the General Foods Technical Center who had been trained in the A. D. Little flavor profile method and who had, on the average, four years of experience in describing food products according to the flavor profile method.

Panel training. The first phase of panel training is a study of the classification of textural characteristics developed by Szczesniak (1963). The panel is then presented with the organoleptic definitions of the mechanical characteristics on texture, viz., hardness, brittleness, viscosity, gumminess, chewiness, and adhesiveness. Several weeks are devoted to studying each of these characteristics through repetitive evaluations of the selected examples that constitute the points on the standard rating scales.

The second phase of training consists of evaluating a wide selection of foods other than those that represent points on the standard rating scales. This phase provides a good opportunity for evaluation of the reliability of the panel.

The third phase is a study of geometrical characteristics. The panel is presented with a list of the geometrical characteristics and with examples of foods that demonstrate them (Table 1). The panel is required to evaluate one or more other foods containing these characteristics.

Up to this point, the panel's main concern is to acquire a thorough understanding of the various aspects of texture and to develop reliable skills in recognizing and identifying degrees of each characteristic.

The final phase of the program consists of drawing up a complete profile of several products, following the prescribed method as to procedures regarding mechanics and order of perception.

Panel technique. In approaching a complete texture analysis of a food, a panel spends at least one preliminary session in evaulating various

Table 3. Texture profiles of two dry flake cereals.

	Cereal *X*	Cereal *Y*
Initial		
A. Mechanical characteristics		
Brittleness (1–7 scale)	2.7	3.3
B. Geometrical characteristics	Hard clumps of grits	A few small grits
Masticatory	No obvious or important mechanical or geometrical characteristics	
Residual		
A. Rate and type of breakdown	Breakdown is very slow and uneven. The brittle portion breaks down quickly while the gritty clumps take longer and break down further into individual grits and mealy particles	Breakdown is moderately slow and uneven. The brittle phase breaks down quickly while the grits take longer and break down into small mealy particles
B. Moisture absorption	Absorbs saliva slowly and unevenly due to grits	Absorbs saliva quickly

samples of the food type. This helps orient them to the magnitude of difference that exists among the samples and allows for expanding scale points where necessary.

Next, the panel establishes standard procedures for evaluation as to size and temperature of the product in question. The appropriate reference samples are presented for each characteristic. Particular attention is paid to following the standard procedures regarding size, temperature, and mechanics of perceiving for each of the selected examples. Each panelist evaluates the test samples independently. A panel moderator records the end findings and leads discussions to resolve disagreements or misunderstandings. The session is repeated if discrepancies are too large.

APPLICATION AND ILLUSTRATIONS OF TEXTURE PROFILES

Tables 3, 4, 5, and 6 show texture profiles of cereal, rice, whipped topping, and chemically leavened biscuits.

The samples illustrate the different textural characteristics that are appropriate to each product-type, as well as differences between samples in each product-type.

With the two cereals, the appropriate mechanical parameter is brittleness. Cereal *Y* was more brittle than cereal *X*. No important mechanical or

Table 4. Texture profile of two rice samples.

	Rice *R*	Rice *Q*
Initial		
A. Mechanical characteristics		
Hardness (1–9 scale)	2.3	2.7
B. Geometrical characteristics	None	None
Masticatory		
A. Mechanical characteristics		
Chewiness (1–7 scale)	1.3 (12 sec.)	1.7 (15.0 sec.)
B. Geometrical characteristics	Two phase system—outside phase slimy and soft; inside phase gelatinous and rubbery.	Slightly mealy
Residual		
Rate and type of breakdown	Type of breakdown is uneven due to heterogeneous structure. Rate of breakdown is fairly rapid because of relatively low hardness and chewiness	Even and slow breakdown. Kernel texture is uniform, slightly mealy, and dry after breakdown.

Table 5. Texture profile of two whipped toppings.

	Topping *A*	Topping *B*
Initial		
A. Mechanical characteristics		
Viscosity (1–8 scale)	5.2	5.2
B. Geometrical characteristics	Moderate aeration	Very low, if any, aeration
Masticatory	None	None
Residual		
A. Rate and type of breakdown	Breaks down evenly into thin liquid	Breaks down slowly and unevenly into viscous liquid
B. Coating properties	Thin, cream-like mouth coating; very cool mouthfeel	Fatty and lingering mouth coating; slightly warm mouthfeel

Table 6. Texture profile of chemically leavened biscuits.

	Raw dough	Freshly baked	Frozen at −10°F for 72 hr, thawed	Held at room temperature for 72 hr
Initial				
A. Mechanical characteristics				
Hardness (1–9 scale)	1.0	3.7	4.1	7.0
Brittleness (1–7 scale)	0	0	0	4.5
Adhesiveness (1–5 scale)	1.5	0	0	0
Masticatory				
A. Mechanical characteristics				
Chewiness (1–7 scale)	1.3 (12 sec)	2.5 (21 sec)	2.5 (20 sec)	(2.5 (20 sec)
Gumminess (1–5 scale)	3.2	0	0	0
B. Geometrical characteristics	Soft lumps	Aerated	Aerated	Hardened and collapsed cell walls, uneven air pockets, hard grits
C. Degree of moisture	Moderately to very moist	Slightly to moderately moist	Slightly moist	Very dry
Residual				
Breakdown characteristics	Breaks down quickly and evenly into a liquid	Absorbs saliva quickly and becomes very slightly gummy	Absorbs saliva somewhat slowly and becomes slightly gummy	Fractures into smaller, hard pieces that break down into small, mealy particles; absorbs saliva slowly

geometrical characteristics were observed during mastication. Differences in rate of breakdown were found between the samples: cereal *X* broke down more slowly than cereal *Y* (Table 3).

The two rice samples differed in the degree of hardness. Rice *Q* was slightly harder than rice *R*. During mastication, rice *Q* was slightly more chewy than rice *R*. In addition, differences in geometrical characteristics were noted. Rice *R* was described as having a slimy and soft outer-phase and a gelatinous and rubbery inner-phase, whereas rice *Q* was homogeneous and slightly mealy. These differences in geometrical characteristics resulted in differences in the rate and type of breakdown (Table 4).

The whipped toppings were similar in viscosity, both rated at 5.2. Differences were found, however, in the geometrical characteristic of aeration. Sample *A* was moderately aerated whereas *B* had very low, if any, aeration. Rate and type of breakdown were different for each sample. Sample *A* broke down evenly into a thin liquid; sample *B* broke down unevenly and slowly into a viscous liquid. Mouth coating properties also differentiated the samples. Sample *A* was described as having a thin, cream-like mouth coating, whereas *B* had a fatty and lingering mouth coating. Sensations of coolness and warmth were noted in the samples. These differences were not related to variation in physical temperature, but rather to the coating properties (Table 5).

Table 6 illustrates the effect of processing and storage on qualitative and quantitative aspects of texture, with biscuits used as an example. On baking, the biscuit dough lost gumminess and adhesiveness, apparent on mastication, acquired firmness (as shown by the increased rating for hardness), increased in chewiness and decreased in moistness, and assumed a different set of geometrical characteristics and a different type of breakdown in the mouth. Freezing at −10°F for 72 hr resulted in about a 10% increase in firmness, reduced moistness, and slowed saliva absorption. As was to be expected, holding the biscuits at room temperature for 72 hr caused a drastic increase in hardness (almost 100%) and the appearance of a fairly high intensity of the parameter of brittleness. In addition, the stored sample showed a very low degree of moisture and totally different geometrical and breakdown characteristics. It is interesting to note that gumminess, an important characteristic of raw dough perceived at a high intensity during the masticatory stage, was almost totally destroyed by baking, being noticed in biscuits only during the residual stage and only at a very low intensity. It was totally destroyed by further firming up and drying out on storage at room temperature.

USEFULNESS OF THE TEXTURE PROFILE METHOD

The texture profile method is offered as a means of helping the food researcher obtain descriptive and quantitative sensory data on the textural characteristics of food products. The advantages of this method lie in: 1) its flexibility of application to any food product or textural characteristic, and 2) its objectivity through rigidly defined points of reference and nomenclature. The limitations of the method are related to the degree of proficiency of the panels applying the method.

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Objective Measurements of Food Texture[a]

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(Manuscript received September 5, 1962)

There are many reasons for the food scientist to be concerned with food texture and to want to describe it in terms of numbers. The principal reason is to meet consumer acceptability by proper quality control of present products and by assessing advantages and disadvantages of new manufacturing processes and new foodstuffs. The second reason is to develop theoretical generalizations and hypotheses, e.g., correlation between chemical composition or chemical modifications of components and the resulting texture (Scott Blair, 1960). Because of the problems involved in organoleptic evaluations of food attributes, food scientists are constantly searching for reliable and accurate objective measurements.

Objective methods of texture measurement may be divided, after Scott Blair (1958), into three main categories: fundamental, empirical, and imitative.

The devices that have been used in measuring different aspects of food texture are much too numerous to be considered in a short space, so this review is limited to a few selected examples of typical devices that come under the three classifications above. Knowledge on physical measurements of food texture, with special reference to fruit and vegetable products, was reviewed by Kramer and Twigg (1959).

FUNDAMENTAL TESTS

Fundamental tests measure fundamental rheological properties, such as viscosities and elastic moduli. Rheology is defined as "the science of deformation and flow of matter." Since matter starts to be deformed or to flow only when it is acted on by forces, rheology is mainly concerned with forces, deformation, and time, the last being introduced in measuring rates of changes of forces and deformations.

The fundamental tests relate the nature of the tested product to the two basic rheological prototypes: a dashpot for a Newtonian liquid and a metal spring for a Hookean solid. Since foodstuffs are very complex, one ends up with a model of dashpots and springs linked in series and/or in parallel. The former allows for partially recoverable deformations, and the latter accounts for delayed elastic effects. To complicate the picture further, one often finds it necessary to introduce elements representing the static frictions that must be overcome before a deformation takes place, and elements representing mechanisms, such as vibrations or temperature, that must be put in action in order to relax the pressure of stretched spring-like units.

Thus, from a rheological standpoint, a complex system may be described by means of numbers of viscosities (for springs), elastic moduli (for dashpots), and by the ratios of these, termed "relaxation times." Static frictional terms are occasionally used. Often it is not sufficient to obtain a few definite values of these constants, but rather one must measure continuous "spectra" under various conditions of time, temperature, and stress.

Examples of fundamental tests are vector tests, which consist of compressing or stretching pieces of material under controlled conditions and calculating viscosities, moduli, and relaxation times from deformation curves. Many materials (e.g., dough and butter) exhibit the property of work hardening, and it is important that the tests be performed with different stresses and that conditions of tests be carefully defined.

Since most foodstuffs do not have simple rheological properties that are independent of stress and strain conditions, and since rheological properties, once measured and defined, are not meaningful in a practical

[a] Presented at the Eastern Experiment Station Collaborators' Conference on Deciduous Fruits, October 17–18, 1961, at the Eastern Utilization Research and Development Laboratory, Philadelphia, Pa.

sense unless related to functional properties, fundamental tests serve the greatest value to the food technologist by providing bases for the development of more meaningful empirical tests.

EMPIRICAL TESTS

Empirical tests measure parameters, often poorly defined, that practical experience indicates to be related to textural quality. Used for empirical tests are penetrometers, compressors, consistometers, shear measures, and other devices.

Penetrometers

Perhaps the most common instruments are penetrometers. They are used to determine: the rigidity of gels, the force required to penetrate materials such as fruits, and the consistency of a material as measured by resistance to sinking or by total depth of penetration following impact.

An example of a penetrometer used for testing the rigidity of gels is the well-known Bloom gelometer (Bloom, 1925), a standard instrument for measuring the gelling power of edible gelatin. With this device, the rigidity of the gel is measured as the weight of lead shot required to push a standard plunger 4 mm deep into the gel. The Boucher jelly tester uses a somewhat similar principle, but a hollow plunger 13 mm in diameter is used instead of a pointed one, the depression made is 5 mm instead of 4 mm, and water is used to give the weight instead of lead shot.

Nacci and Schachat (1960) modified the Bloom gelometer with a transistor relay in order to increase sensitivity and precision by preventing excessive pitting on the contacts. The transistor relay in the electrical circuit reduced the current to 1/5000 of the amount that would normally flow across the contacts.

A well-known device for testing the maturity of fruits and some vegetables is a fruit pressure tester that determines firmness by reading the force necessary for the plunger to penetrate the sample to a given distance (Magness and Taylor 1925). Fruit pressure testers and their practical applications were reviewed in 1941 by M. H. Haller. The various pressure testers available vary in precision and accuracy, and

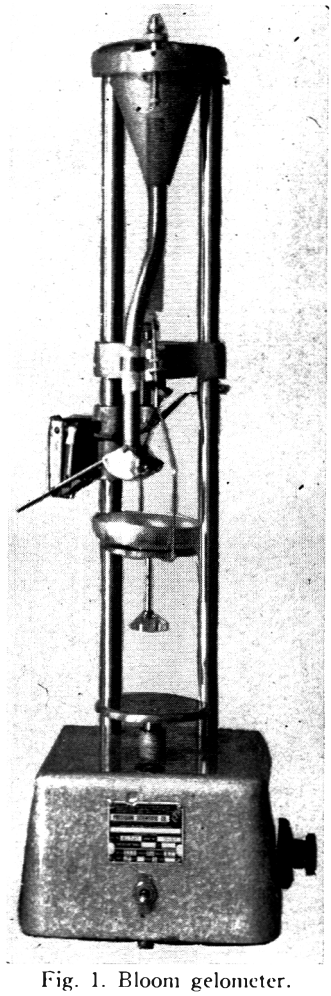

Fig. 1. Bloom gelometer.

usually require special calibration for use with different types and varieties of samples.

The ASTM grease penetrometer is a similar device but measures firmness in terms of the depth to which a metal cone sinks into the surface of the sample under defined conditions. The cone, of a standard size and shape, is attached to a shaft, and the two are released by a stop in front of the gauge. The gauge registers the distance the cone and shaft penetrate the sample, which is placed directly under it. The weight of the cone and shaft may be varied (Rich, 1942).

The consistency of mayonnaise and of similar products may be determined objectively with the "plumit," invented by Kilgore and first described in 1931. The measurement is very simple. The plumit is

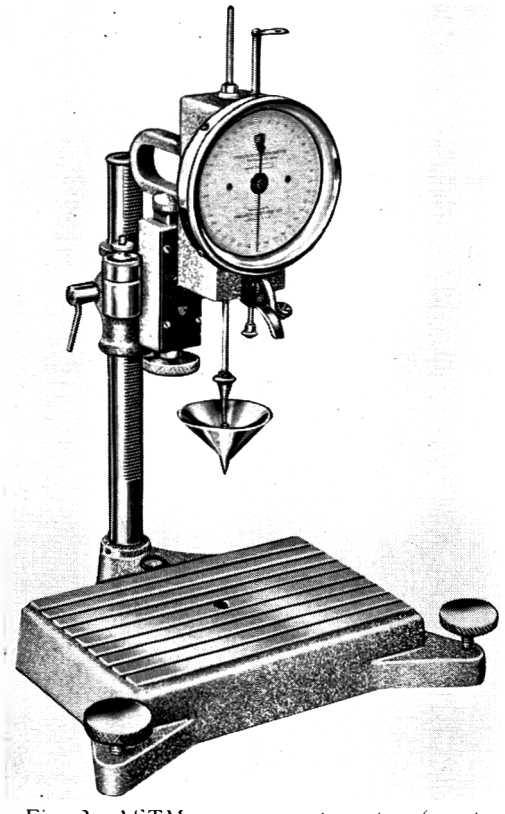

Fig. 2. ASTM grease penetrometer (courtesy of Precision Scientific Co.).

dropped from a fixed height into a jar of mayonnaise, and the depth of penetration is read off a scale on the plumit. Plumits are usually made of aluminum, and are 13 cm long and weigh 14.5 g. The weight may be adjusted with a lead shot in the point. Plumits of different sizes appropriate to the range of consistency exhibited by a particular product may be constructed.

Before the introduction of the "plumit," plummets of different shapes were used to measure the consistency of mayonnaise, recording either the force required for penetration or the time required for a given penetration.

Compressors

These instruments determine hardness or firmness of materials by measuring resistance to a compressing force. In this category is the Delaware jelly tester, a well-known instrument consisting of an inverted and clamped glass syringe. Air pressure is applied at the tip forcing out the plunger

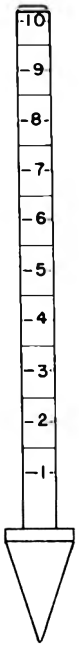

Fig. 3. Plumit.

which compresses the specimen cut in the shape of a cylinder. The flow of air is so adjusted that the pressure on the plunger increases almost linearly with time. The Delaware jelly tester has been used for measuring the firmness of many foods other than jellies. Among its numerous applications is the adaptation by Whittenberger (1951) to measuring the firmness of cooked apple tissues and to other products such as white potatoes, carrots, beets, and other fleshy plant tissues.

The Brinell hardness tester, used for metals and alloys, has been adopted to measuring the hardness of foods. An example is the instrument devised by Lovegren *et al.* (1958) for measuring the hardness of fats and waxes. A round steel ball with a diameter of 0.1250–0.500 inches is pressed with a force of 0.2–6 kg into the surface being tested. The hardness index is calculated from a formula relating weight on the ball, the diameter of the ball, and the diameter of the impression. The index is relatively independent of test conditions within certain ranges.

A similar device has been used for measuring firmness of cheese. Caffyn and Baron (1947), at the National Institute for Research in Dairying, at the University of

Reading, England, devised a ball compressor consisting of a metal hemisphere to which is applied a certain load by means of a series of lever arms. The deformation in the cheese is measured on a dial gauge before and after the removal of the load. Total deformation indicates firmness of the sample, and the difference between the two readings denotes the degree of the elastic recovery. The application of this device to the description of a number of commercial cheeses has been discussed by Cox and Baron (1955).

The Baker compressimeter for testing the firmness of bread (Am. Assoc. of Cereal Chemists 1947) is a standard Cereal Laboratory Method. The apparatus consists of a plunger that depresses a slice of bread by rotation of a small drum actuated slowly and uniformly by a motor and shaft beneath the bed of the apparatus. The amount of stress and the corresponding strain or depression of the plunger can be read off simultaneously from two scales.

An earlier apparatus for measuring bread softness was devised by Platt (1930). It is listed in the Cereal Laboratory Methods as the balance-type compressimeter since it consists of a large balance with a plunger

Fig. 4. Ball compressor (Baron, 1952). (Photograph supplied by Dr. G. W. Scott Blair; reproduced by permission of Dr. Baron.)

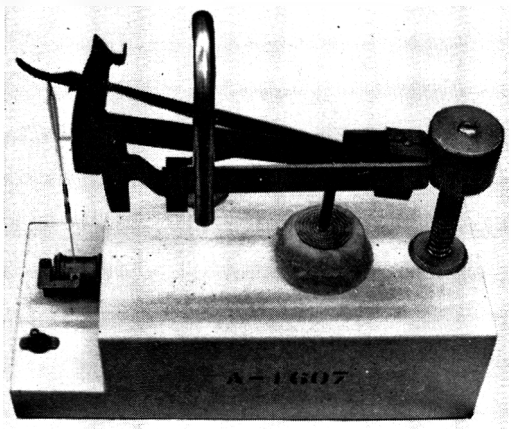

Fig. 5. Baker compressimeter (courtesy of Baker Process Co.).

fastened to the underside of one of the pans. The bread to be tested is placed on a platform immediately beneath the plunger, and the plunger is allowed to touch the upper surface of the bread. Weights are then applied for a specified time, and the amount of compression is measured with a pointer. Platt's apparatus differs from the Baker compressimeter in that it uses a fixed weight acting for a fixed time.

Considerable literature is available on the compression method for measuring bread softness. Detailed studies have been made of the effect of bread slice thickness, amount of stress applied, rate of deformation, etc. (Platt and Powers 1940).

A compression type of apparatus for measuring the force-deformation characteristics of gel systems, termed the gel characterization apparatus (GCA) was constructed at the General Foods Technical Center (MacAllister and Reichenwallner 1959). It consists of a transducer mounted for reciprocal movement at a constant rate and driven by an electric motor. Another synchronized motor moves the chart drive of the Brown recorder. The transducer consists of four strain gauges connected to form a bridge. Plungers of different types may be firmly attached to the transducer. When the plunger moves downward with the transducer and meets the resistance of the test surface, the bridge becomes unbalanced and activates the recording pen on the slide wire, which then moves to rebalance the bridge. The degree of this imbalance

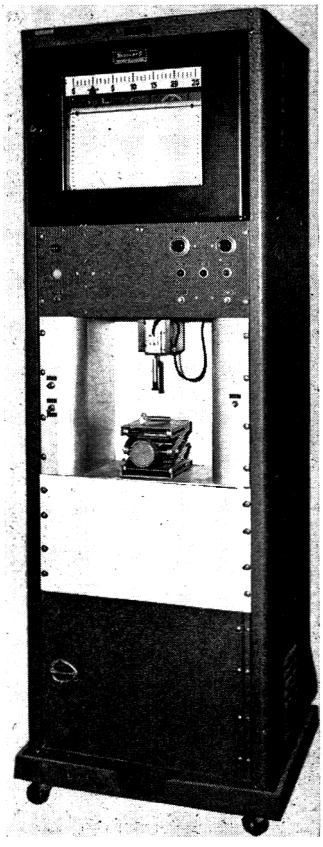

Fig. 6. The gel characterization apparatus (GCA).

can then be read from a calibrated chart in terms of grams of force. A series of time-delay relays and microswitches is used to make possible a completely automatic cycle of forward and reverse motions, thus tracing out a hysteresis loop. The depth of plunger travel reversal is controlled by an adjustable lower-limit microswitch. The instrument is designed for a maximum load of 200 g. It is calibrated by detaching the transducer from its mount, reversing it, and placing known weights on the plunger. The deflection on the chart should correspond to the applied weight.

The GCA is a very valuable instrument in measuring certain characteristics of starches, gelatin, pectin, alginate, etc. The obtained curves are characteristic of the gel type tested and provide a permanent record of the measurement. In addition to rigidity, values for gel strength and cohesiveness can be read off the curves.

Consistometers

Liquids and semisolids are tested with consistometers. Such consistency-testing instruments have been reviewed by Rich (1942).

Typical of this class of measuring device is the Bloom consistometer, which indicates the relative pressure necessary to force the product through an opening in the end of a plunger (Bloom 1938). As modified by Clardy *et al.* (1952) the instrument consists of a barrel, a displacement gauge, and a plunger. The plunger is made of a supporting yoke with a small cylinder at one end. The inside of the cylinder has the shape of a frustum of a cone so that deformation is caused as the sample passes through it. A spring in the barrel is connected to the displacement gauge. The instrument may be applied by hand or may be motor-driven.

The Bostwick consistometer is listed in the laboratory methods of the National Canners' Association as an official test for the consistency of catsup. The instrument measures the maximum distance a given amount of catsup travels at 20°C down a

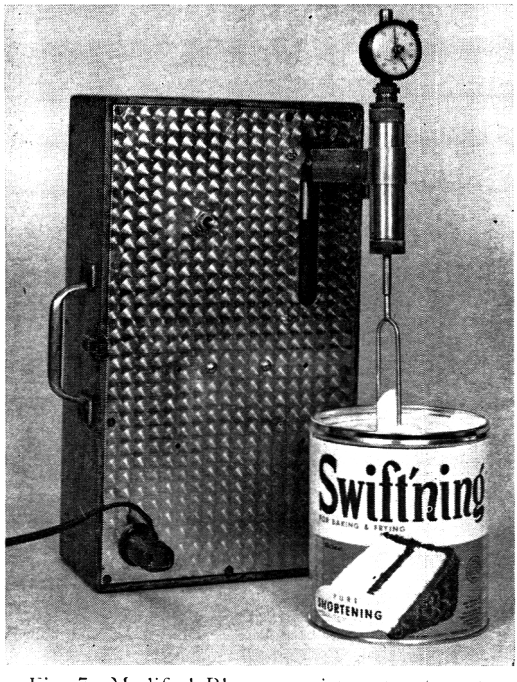

Fig. 7. Modified Bloom consistometer (courtesy of W. D. Pohle, Swift and Co.).

trough when released from a container (Townsend, 1956a).

Of the large number of viscosimeters used in the food industry, probably typical of this class are the MacMichael and Brookfield viscosimeters. The MacMichael viscosimeter consists of a cup and an inner cylinder suspended from a wire. The test material is placed in the cup, and the inner cylinder is immersed in it. The cup is rotated, and the torsional force in the wire required to hold the inner cylinder stationary is taken as the measure of the consistency of the test material. The Brookfield viscosimeter uses the reverse situation. The container holding the test material is held stationary and a spindle is rotated inside it. Such spindles may have a variety of shapes and sizes, depending on the characteristics of the test material. Since most food substances are non-Newtonian in character, the rotating speed under which the measurements are made should be specified in reporting the viscosity.

Shearing devices

These instruments are used to measure the textural characteristic of "tenderness" in meats, fish, vegetables, etc., by recording the force needed to shear the test material.

The Warner-Bratzler apparatus consists of a 1-mm-thick blade equipped with a hole in which is placed a core of meat. The blade is led through a narrow slit between two shear bars. The force may be applied either to the blade or to the shear bars. The force builds up to a maximum, which is taken as the measure of toughness. The fracture is sudden and complete (Bratzler, 1932).

Reports in the literature do not agree as to the correlation with tenderness values judged organoleptically, and a number of modifications in the instrument have been suggested. Hurwich and Tischer (1954) concluded that the slope of the shear-force-vs.-time curve is a better criterion of tenderness than the maximum shear force. They recommended that the instrument be redesigned to lower the inherent experimental error.

A pea tenderometer developed by the American Can Company (Martin *et al.*, 1938) directly determines the relative tenderness of peas and other products by measuring the force required to shear them through a standard grid. The apparatus consists of two grids through which the product is sheared, motive power for moving one grid with respect to the other at a constant rate, and a pendulum mechanism for measuring the force exerted in the shearing operation. The commercial model is completely automatic in cleaning itself and stopping after each measurement. The shortcomings of the instrument are difficulties in calibration and the fact that only an average value is given for products like peas.

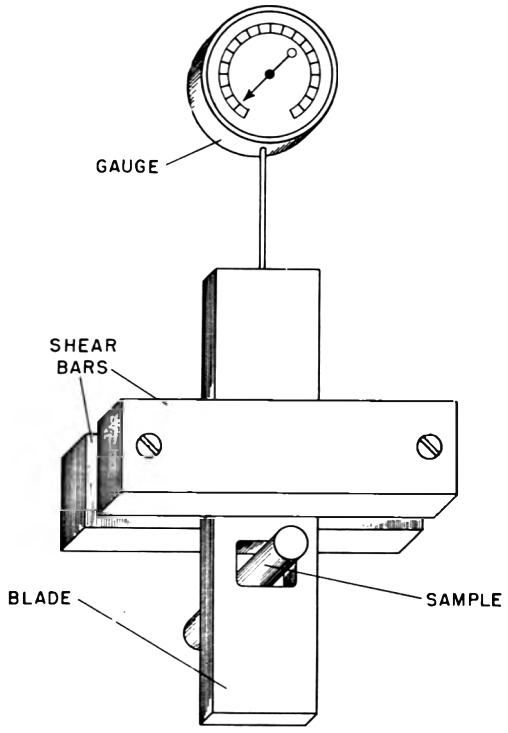

Fig. 8. Warner-Bratzler apparatus (Sale, 1960). (Reproduced by permission of the Society of Chemical Industry.)

Similar in principle to the pea tenderometer is the shear press developed and used extensively in Prof. Kramer's laboratory at the University of Maryland. A new model, which culminated a five year research program sponsored by the government and several food processors has recently been developed and is available commercially (Kramer and Backinger, 1959). The instrument consists of shear bars that pass through a box having a corresponding

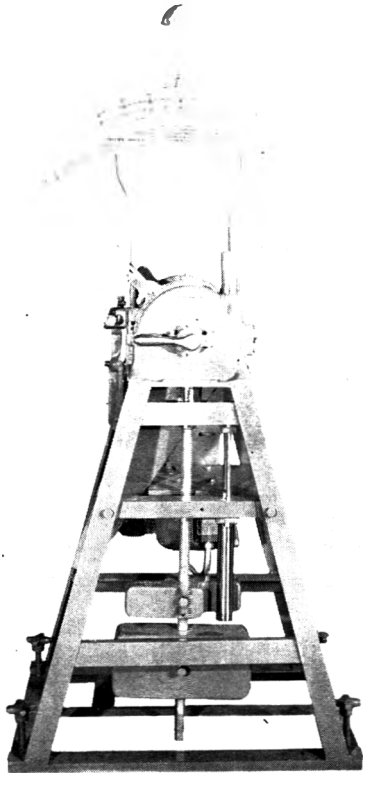

Fig. 9. Pea tenderometer (courtesy of FMC Corp.).

number of slots. The sample is laid across the slots in the box, the shear bars are driven through it, and the force used is recorded. The new unit is equipped with a proving ring dynamometer to which the test cells are attached directly, thus eliminating frictional error since the resistance of the test material to shearing or compression is transferred directly to the measuring system.

The apparatus has been made more versatile by equipping it with three standard cells. One is similar in design to the pea tenderometer and simulates the compression and shearing action of the teeth. Another cell simulates cutting action and is suitable for the measurement of the fibrousness of products. The third test cell simulates the sensory reaction to juiciness and is similar in design to the succulometer (see below). The instrument is adaptable to textural measurements of a number of different products, including meats, sea food, vegetables, and fruits.

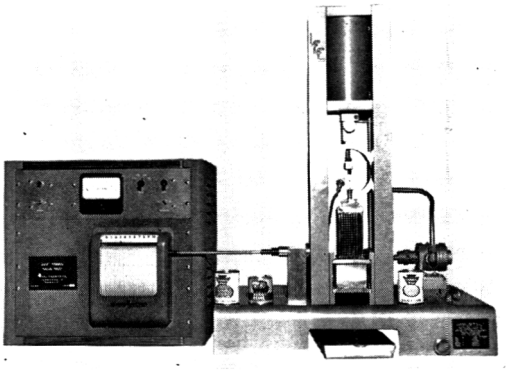

Fig. 10. Shear press (courtesy of Food Science Corp.).

The advantage of the shear press over the Warner-Bratzler apparatus is that it reduces sampling error by taking a larger sample. Both methods are dependent on the sharpness or dullness of the blades.

Miscellaneous

Other instruments are available that cannot be placed in the above categories but should be mentioned:

The succulometer measures juiciness of the food as the volume of the liquid expressed from a known weight of material at a given pressure at a given time. Such an instrument was first described by Kramer and Smith in 1946. A good agreement was found with moisture, alcohol-insoluble solids, and maturity ratings. Turning the handle of a screw-rod displaces a heavy oil, which pushes a piston and plunger against the sample, building up enough pressure to force out the juice. Other workers in the field have used the Carver press, which is very similar in principle.

The food mincer has been used in characterizing the texture of meat by measuring its resistance to grinding (Miyada and Tappel, 1956). It consists of an electric mixer equipped with a grinder and fitted with an ammeter. The work required to grind a given amount of material to a given particle size is taken as a measure of toughness. The method has recently fallen into disrepute because of poor correlations with organoleptic evaluation.

The fiberometer, similar in principle to the shear press, is used with fibrous products like asparagus. The instrument, devel-

oped by Wilder (in 1947, for the National Canners' Association) consists of a supporting block of a parabolic shape with vertical slots 0.039–0.42 inches wide through which the cutting wire is passed to press at right angles against the asparagus. The standard cutting device weighs 3 lb. The length of asparagus that can be cut with that weight pressing on the wires is taken as a measure of fibrousness. The shorter the length, the tougher and more fibrous the sample (Townsend, 1956b).

IMITATIVE TESTS

Imitative methods of measurement imitate the conditions to which the material is subjected in practice.

Butter spreaders belong to the category of imitative devices. The literature describes several machines that imitate the action of spreading butter on bread. Most recent is probably the one designed by Prentice (1956) at the National Institute for Research in Dairying, at the University of Reading, England. It is actually a screw extruder in which a cube of butter is extruded and sheared by a knife edge at standard loads and speeds. The amount of shear is measured to indicate spreadability. Unfortunately, the correlation with organoleptic scores is only fair. One possible reason is that the spreading in the instrument is done on an artifically roughened surface, which is different from the bread surface.

Similarly to the butter spreader, the Brabender farinograph measures the handling properties of a material, in this case dough, rather than its mouthfeel, and gives a picture of the gluten properties of a flour. It uses the dynamometer as the measuring device, and registers the torque of a given quantity of dough under a continued kneading and stretching abuse until the dough is fully broken down. Stability and fermentation tolerance can be determined.

Another device used for studying the textural properties of dough is the alveograph, in which a sheet of dough is subjected to air pressure, forming a large bubble. The internal pressure and volume of the bubble can be recorded until it bursts. Taken as a measure of the strength

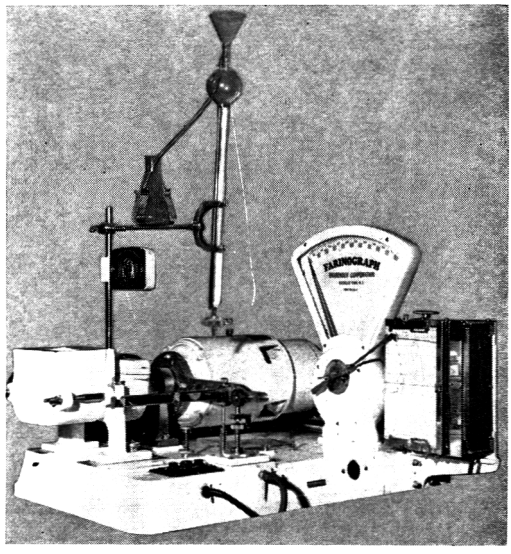

Fig. 11. Brabender farinograph.

of the flour is the area under the curve representing the work done in forming and bursting the bubble.

The Brabender amylograph measures the viscosity behavior of pastes when subjected to heating and cooling. It is used with flours, starches and other heat-thickening materials and was originally designed for the purpose of estimating the α-amylose activity of flours. Although it imitates the effect of cooking or baking, the amylograph is similar in principle to a viscosimeter. It consists of a cup surrounded by an air bath for temperature control. The cup, which holds the sample, is revolved at a constant speed. The viscosity is measured by the torque impressed on the measuring unit which consists of a disc with several short rods extending into the sample. The temperature is raised at a constant rate. The viscosity may also be measured during the cooling cycle.

The Volodkevich bite tenderometer attempts to imitate the action of teeth on the food. First described in 1938, it has been used and modified by a number of workers. It records the force of biting on a piece of food as a function of the resulting deformation and determines the total energy utilized for this deformation. Two wedges with rounded points substitute for teeth. The lower wedge is fixed on a frame, and the

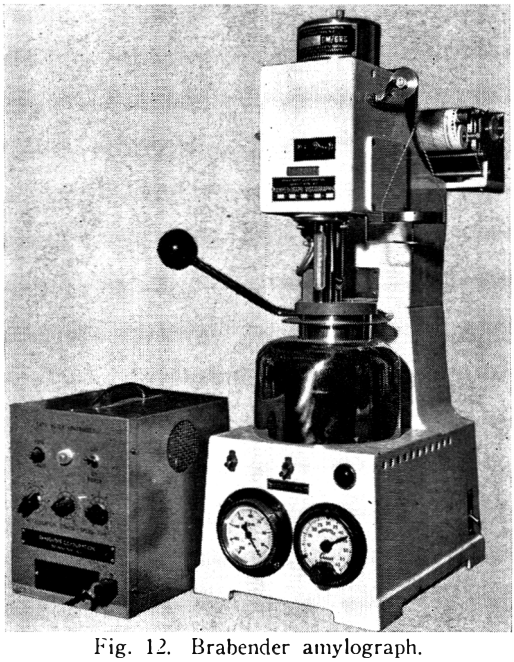

Fig. 12. Brabender amylograph.

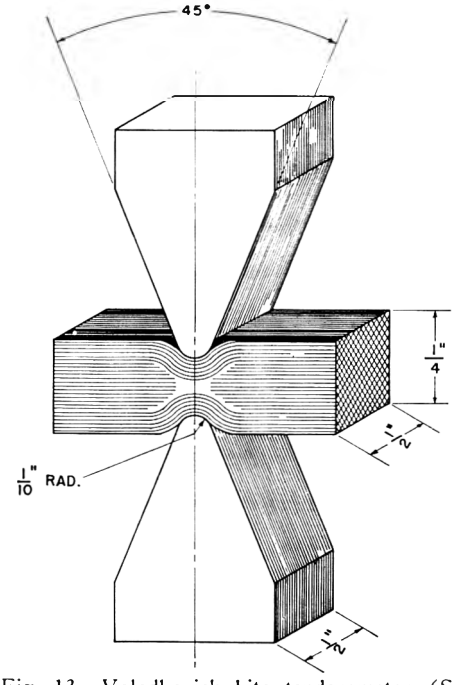

Fig. 13. Volodkevich bite tenderometer (Sale, 1960). (Reproduced by permission of the Society of Chemical Industry.)

upper one can be moved down with a lever, thus squeezing a sample put between the wedges. Sale (1960) modified this apparatus in his laboratory by using wedges rounded to a radius of curvature that is easy to reproduce and does not easily wear out of shape. The force on the wedges is gradually increased and recorded, together with their penetration until the wedges meet. The force is applied by drawing a steel ball along a beam that carries one wedge. The force is measured mechanically on a recording drum coupled to the apparatus so that the horizontal deflection of the recording pen is proportional to the force of crushing, and the vertical movement of the drum is proportional to the distance between the crushing surfaces. The resulting plot can be correlated with the structural properties of the tested food. Later refinement in the original apparatus enabled separate evaluations of crushing and shearing forces.

Probably the best adaptation of Volodkevich's apparatus is the denture tenderometer, designed to simulate the denture surfaces and motions of mastication in the mouth (Proctor *et al.*, 1955, 1956a,b). Mastication consists of the process of biting and chewing the food between the teeth by moving the lower jaw with respect to the upper jaw. The tongue, cheeks, and lips help to control and direct the food between the teeth; saliva helps soften and disintegrate the food, and acts as a lubricant besides carrying digestive enzymes. The denture tenderometer, designed at the Massachusetts Institute of Technology, utilizes a complete set of human dentures in a mechanical chewing arrangement, with provisions for variation of the motions. A drive motor moves the upper jaw of the articulator. The impulse sent to the ampli-

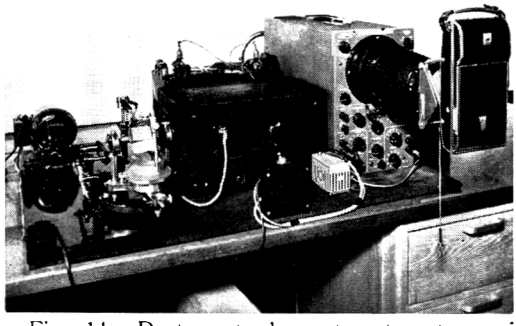

Fig. 14. Denture tenderometer (courtesy of Prof. S. Goldblith, Massachusetts Institute of Technology.)

fier by the strain gauge fitted on the arm connected to the upper jaw is fed into a cathode-ray oscilloscope. Traces on the face of the cathode-ray tube are photographed with a Polaroid Land camera. Plastic dentures are fastened securely to the articulator with simulated cheeks, lips, and tongue built up from a resilient plastic material to keep the food between the teeth during the measurement. In contrast to actual chewing, the upper jaw of the apparatus moves relative to the lower jaw. This, however, is said not to detract from the validity of measurement. The apparatus has been used successfully in measuring tenderness and some other textural parameters of a variety of foods including fruits, vegetables, meats, eggs, and candies. Its electronic modification was adapted to the tenderness measurement of individual peas, thus giving information on the distribution of maturity of peas in a lot (Davison *et al.*, 1959).

The MIT denture tenderometer offers the great advantage of measuring a spectrum of textural parameters rather than one isolated characteristic. It served as the prototype for the texturometer currently used at the Technical Center of General Foods Corporation (Friedman *et al.*, 1962). There, dentures are replaced with a plunger, the sensing element is moved from the articulator arm to the sample area in order to eliminate gravity effects, and the oscilloscope is replaced with a ¼-second strip-chart recorder. The last allows for easy recording of any desired number of consecutive chews, and the curves obtained represent a permanent record of the textural spectrum of the test material. Textural parameters (Szczesniak, 1962) of hardness, cohesiveness, elasticity, brittleness, and adhesiveness can be read off the recorded curves and the parameters of chewiness and gumminess can be calculated. With proper modifications, the instrument may also be used to measure viscosity. Instrument values appear to be well correlated (Szczesniak *et al.*, 1962) with organoleptic evaluations by a trained texture-profile panel (Brandt *et al.*, 1962).

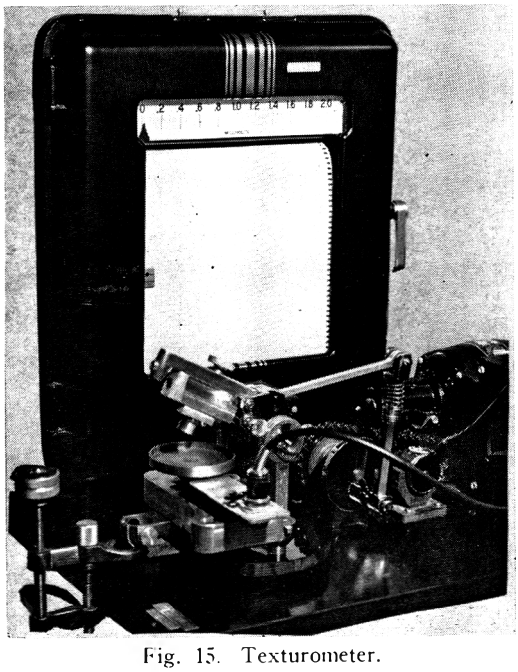

Fig. 15. Texturometer.

The large variety of instruments that have been devised for objective measurements of food texture indicates the food scientist's determination to find adequate means of describing texture in terms of reproducible numbers. This search still continues, since a perfect instrument has not been constructed yet. The latest trend from empirical to imitative tests holds the promise of providing a better understanding of the textural properties of foods as actually perceived by human senses. Continued efforts in this area will ultimately provide the food industry with an objective tool for describing textural qualities accurately and reliably, correlating with the so-far irreplaceable judge of food quality, the human senses.

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Use of the Balanced Lattice Design in Determining Consumer Preferences for Ham Containing 16 Different Combinations of Salt and Sugar[a]

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(Manuscript received September 17, 1962)

SUMMARY

There were no significant differences in consumer preferences between different levels of salt (from 1.5 to 3%) in hams, but there was a significant difference in preference between levels of sugar. Hams having 2% sugar were preferred significantly over hams containing no sugar, 1% sugar, and 3% sugar. There were no significant preferences in the interactions between salt and sugar levels. Preferences for neither salt nor sugar were linear. It appears that the balanced lattice design is an efficient model that may be used to guide the presentation of a large number of treatments to members of large-scale consumer panels.

PURPOSE

Tests in the Michigan State University Food Science Department with a seven-member trained taste panel (Pearson *et al.*, 1962; Goembel, 1962) showed a clear-cut preference for ground hams cured with approximately 1.1–2% of sugar compared to hams containing no sugar. These tests were later repeated with a consumer panel using hams cured with salt alone and hams cured with salt plus 2% sugar. The consumer panel also significantly preferred the hams containing sugar. The present study was designed to determine the response of "typical" consumers to ham cured with various levels of salt and sugar and to try to determine the most preferred level of salt and sugar.

THE PANEL

The Michigan State University Consumer Preference Panel was used for this study. This panel was designed to determine preferences of consumers with annual incomes ranging from $4,000 to $10,000, of ages 30 to 45, and with 12–13 years of formal education. The panel was chosen without regard to ability to differentiate qualities of products.

This panel was initiated in 1956 to establish consumer preferences among grades, varieties, sizes, color, and processing techniques for agricultural products. Since its initiation, the panel has met 4–5 times a year at Wayne State University, in Detroit, Michigan. Consumers for the panel were selected at random from listings in the Detroit telephone directory.

Consumers ranked several different types of products at each panel meeting. Immediately prior to the panel session the groups of consumers were given instructions concerning the different series of items to be ranked. Each panelist was also assigned a number at this time. The products were displayed on tables in a large room, and 10–20 consumers at a time proceeded independently to rank the samples within the room. When an individual consumer completed ranking of the products, the forms were checked to make sure that he had ranked all of the products within each series.

The consumers were not told the purpose of any specific test. In the case of the various levels of salt-and-sugar-cured ham, the consumers were only told that there were four samples of ham to be ranked according to their preference.

The panel members were asked to rank the samples in order of preference but were not asked to explain the reasons for their preferences. The respondents could, however, make voluntary written comments.

EXPERIMENTAL PROCEDURE

Paired hams were removed from carcasses of pigs weighing between 180 and 220 lb live weight. The arteries on the hams were dissected out and tied with string before the carcasses were cut in

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order to be sure that a brine could be pumped through the arteries. A total of 64 hams were used for this study. The hams were then randomized into 16 different treatments as follows:

Treatment	Salt	Sugar	Treatment	Salt	Sugar
1	1.5%	0%	9	2.5%	0%
2	1.5%	1%	10	2.5%	1%
3	1.5%	2%	11	2.5%	2%
4	1.5%	3%	12	2.5%	3%
5	2.0%	0%	13	3.0%	0%
6	2.0%	1%	14	3.0%	1%
7	2.0%	2%	15	3.0%	2%
8	2.0%	3%	16	3.0%	3%

Presentation to the panel made use of a 4 × 4 balanced lattice design (Cochran and Cox, 1950) so that each of the 16 different treatments was compared to each of the other treatments an equal number of times. In this design there were 20 possible combinations of the 16 treatments, compared four at a time.

The balanced lattice design was chosen because preferences for the 16 types of ham had to be compared. This design is most efficient in this respect since it allows one to compare preferences for ham containing 16 different levels of salt and sugar with a minimum of panel members. The balanced lattice design was also chosen because it has the advantage of being an organized method of presenting the 16 types of ham to the large-scale panel.

Each of the 160 panel members received a sample of four treatments at each of the two tables from which ham was being served. The samples were tested independently. A randomized design was drawn up so that the respondent's previously assigned number was used to determine the combination of samples he was to taste. The design was such that no participant tasted the same four samples at both tables. This was done by using the 20 possible combinations of treatments in a randomized 4 × 4 balanced lattice design in a consecutive manner at the first table (combination 1 was given to No. 1 panelist, combination 2 was given to No. 2 panelist, ... , and combination 20 was given to the No. 20 panelist). At the second table, combination 20 was given to the No. 1 panelist, combination 19 to the No. 2 panelist, ..., and combination 1 was given to the No. 20 panelist. This design was repeated 8 times for the 160 consumers.

The samples were presented to consumers four at a time on coded paper plates. The samples were coded by symbols to prevent the possible influence of ranking association by use of letters or numbers. The four symbols used at the first table were %, ( ), *, and &. The symbols used at the second table were &, ( ), %, and #. The treatments were rotated so that each treatment was placed by each symbol an equal number of times. The position that each symbol occupied on the paper plate was also rotated, so that each symbol appeared in each of the four positions on the plate an equal number of times.

The panelists were asked to rank the four samples in the order of their preference, using 1 for the sample most preferred, 2 for the sample second-most preferred, 3 for the sample third-most preferred, and 4 for the sample least preferred.

STATISTICAL ANALYSIS OF RESULTS

Statistical analysis of the results included analysis of variance and the Duncan (1955) Studentized Range test, together with comparisons with previous work on the triangle-test method of comparing samples.

An analysis of variance computation revealed that the total sums of squares equals 129,706 — the correction term of 128,000, which is 1706.

The treatment sums of squares = $(200)^2 + (187)^2 + (179)^2 + (212)^2 + (206)^2 + (214)^2 + (171)^2 + (209)^2 + (210)^2 + (200)^2 + (197)^2 + (202)^2 + (210)^2 + (201)^2 + (195)^2 + (207)^2 = 642{,}196/5 = 128{,}439.2$, which, minus the correction term of 128,000, equals 439.2. Where 200 is the sum of the rankings for treatment, 187 is the sum of the rankings for treatment 2, . . . and 207 is the sum of the rankings for treatment 16.

The sums of squares for the sugar treatments = $(826^2 + 802^2 + 742^2 + 830^2)/20 = 128{,}247.2$, from which the correction term of 128,000 is subtracted, giving 247.2 as the sum of squares, where 826 is the sum of rankings for all treatments containing 0% sugar, 802 is the sum for all treatments containing 1% sugar, 742 is the sum of the rankings for all treatments containing 2% sugar, and 830 is the sum for all treatments containing 3% sugar.

The sum of squares for salt = $(778^2 + 800^2 + 809^2 + 813^2)/20 = 128{,}036.7$, from which the correction of term of 128,000 is subtracted, giving 36.7 as the salt sums of squares. The 778 is the sum of all rankings for the treatment containing 1.5% salt, 800 is the sum of all rankings for all treatments containing 2.0% salt, 809 is the sum of all rankings for all treatments containing 2.5% salt, and 813 is the sum of all rankings for all treatments containing 3.0% salt.

The analysis of variance is summarized in Table 1.

The hypothesis tested by the analysis of variance is that the treatments (different percentages of salt, sugar, and any interaction between them)

Table 1. Analysis of variance of a 4 × 4 balanced lattice design to determine consumer preferences among hams prepared with four levels of sugar and four levels of salt.

Source of variation	Degrees of freedom	Sums of squares	Mean square	F	Needed F	
					.05	.01
Total	79	1706				
Treatments	15	439.2	29.29	1.48	1.83	2.34
Sugar	3	247.2	82.40	4.16**	2.75	4.10
Salt	3	36.7	12.23	.62	2.75	4.10
Sugar × salt	9	155.3	17.25	.87	2.02	2.70
Error	64	1266.8	19.79			

** Significant at the 1% level.

have no effect on consumer preferences for ham. Although the hypothesis cannot be rejected for salt level and the interaction of salt and sugar levels, there was a significant difference in the preferences for different levels of sugar. The difference was significant at the 1% level. The hypothesis that the percent of sugar has no effect on consumer preferences for ham was rejected. The odds were greater than 99 to 1 that the agreement in preferences between sugar levels was not due to chance alone. Tests for linearity of the salt and sugar treatments were nonsignificant.

The analysis of variance may be regarded as a preliminary test since it shows only that the two means with the widest range differ significantly. In this case, the 2% sugar level was significantly preferred over the 3% sugar level. To draw more precise conclusions, it must be determined whether there are other significant differences among the means of the sugar treatment.

The Duncan Studentized Range test was used to determine the significance for the preferences of ham cured among the four different sugar levels. The sum of the rankings for ham cured with the four percentages of sugar were: 826 for 0% sugar, 802 for 1% sugar, 742 for 2% sugar, and 830 for 3% sugar. The greater the preference the smaller the rank score. The design was replicated 20 times, so the respective means were: 41.3, 40.1, 37.1, and 41.5. Differences were tested for significance at both the 1 and 5% levels.

RESULTS

The results show that the panelists preferred ham containing 2% sugar over any other level tested. The result is significant at the 5% level. Ham containing 2% sugar was significantly preferred (at the 1% level) over ham containing 0 and 3% sugar, but was not different from ham containing 1% sugar.

These findings are supported by results obtained from previous work in this laboratory using the triangle method of comparing samples as outlined by Roessler *et al.* (1948 and 1955). In that test there was a significant difference between hams cured with salt alone and hams cured with salt plus 2% sugar. The preference for ham containing 2% sugar was significant at the 1% level. That is, presentation to the panel was arranged so that each person received three samples, two the same and one different. They were then asked to identify the different samples. After they identified the different sample, they were then asked to indicate which sample they preferred—the like or unlike sample. Results from this earlier work showed that 69 of 125 panel members could discern the difference between the paired and different samples. Thus, the panel showed the ability to discern differences in treatment. The difference was significant at the 1% level. Of the 69 panelists able to distinguish differences, 47 preferred the hams containing sugar. This difference in preference was statistically significant at the 1% level.

In this study, only 55% of the panelists were able to recognize the paired and unpaired samples. Of those members of the panel able to distinguish between samples, approximately two-thirds preferred the hams containing 2% sugar. Even though there was a statistically significant difference in preference favoring the hams containing

Table 2. Consumer preferences among sugar differences in ham at the 1% level of significance.

Treatment:	2% sugar	1% sugar	0% sugar	3% sugar
Means:	37.1	40.1	41.3	41.5

Note: Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different. The smaller the mean the higher the preference.

Table 3. Consumer preferences among sugar differences in ham at the 5% level of significance.

Treatment:	2% sugar	1% sugar	0% sugar	3% sugar
Means:	37.1	40.1	41.3	41.5

sugar, some 45 people out of every 100 could not tell the difference between samples. Furthermore, of the 55 people in every 100 who were able to discern differences, 18 preferred the hams containing no sugar. Thus, some 18% of the panel preferred the hams containing no sugar and an additional 45% had no preference, indicating that some 63% of the panel would be as well or better satisfied with hams containing no added sugar. On the other hand, some 73% of the panel were as well or better satisfied with the hams containing 2% sugar. The difference in preferences was surprisingly small when examined in this manner. As pointed out by Mrak *et al.* (1959) and Baker *et al.* (1961), some consumers are very sensitive to differences and others are very insensitive. This ham study showed that some 45% could not detect differences due to sugar or its absence and were quite insensitive to this treatment. Although the statistical treatment favored the hams containing sugar, a more insensitive panel might not have discerned any difference. On the other hand, a more sensitive group might have shown an even more pronounced preference for the hams containing 2% sugar.

DISCUSSION

The similar results obtained with both methods of testing indicate that the balanced lattice design can adequately reflect differences in preferences. The advantage of this method of presentation is that it allows one to compare preferences for a large number of treatments from a minimum number of panel members. This is especially important if one is comparing a large number of treatments. In such cases the use of paired comparisons or triangle tests would require a large number of respondents. In this example, use of the paired comparison method would require 120 respondents if each of the 16 different kinds of ham were to be compared with each other once and if each respondent made only one comparison. Therefore, it would have taken 1,920 panelists to compare each of the 16 different kinds of ham with each other 16 times. By using the 4 × 4 balanced lattice design, only 20 panel members were required to compare each of the 16 kinds of ham with each other one time. By each panel member making two comparisons of four samples each, 160 panel members compared each of the 16 different kinds of ham with each other 16 times, and significant differences in preferences were obtained.

Statistical significance may not always parallel the relative economic importance of differences in preference. Nevertheless, results show that a larger segment of the panel preferred the ham containing 2% sugar. The fact that some consumers preferred the other levels of sugar or none points out the variability in individual taste preferences.

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Effects of Frozen Storage on Chicken Muscle Proteins [a]

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(Manuscript received November 16, 1962)

SUMMARY

Quantitative examination of chicken muscle proteins showed that protein extractability in both breast and leg muscle decreased during frozen storage because of loss of solubility of actomyosin fraction. This decrease accompanied a decrease in the sulfhydryl-group content of muscles and loss in myosin-adenosinetriphosphatase activity. The stroma-protein fraction remained unaffected, and the sarcoplasmic-protein fraction decreased only after long storage. In the non-protein-nitrogen fraction, the amount of free amino acids and other protein-breakdown products increased as a result of proteolysis. The rate of these changes depended directly on storage temperature and time. It is suggested that chicken muscles in frozen storage undergo proteolysis and that the myofibrillar-protein fraction is denatured.

INTRODUCTION

Chicken meat has been shown to lose flavor and juiciness and become "dry" in texture during frozen storage (Gutschmidt, 1959; Partmann, 1959; Stewart *et al.*, 1945; Stewart and Lowe, 1948). The rate at which these changes occurred was markedly dependent on storage temperature, although other factors, such as rate of freezing and post-storage conditions (Harshaw *et al.*, 1941; Kondrup and Boldt, 1960; Lowe, 1948; Poole *et al.*, 1959; Stewart *et al.*, 1943), were also important. The effect of storage conditions on the quality of poultry meat has been studied extensively, but there are few reports on the biochemical changes resulting from frozen storage. In the present investigation the proteins of breast and leg muscle were examined for biochemical changes during storage at −18, −10, and −4°C. The object was to study the nature and extent of biochemical damage to the proteins during 100 weeks of storage. A preliminary report has been given (Khan, 1962a).

EXPERIMENTAL

Breast and leg muscles were analyzed for total extractable, myofibrillar (actomyosin and myosin), sarcoplasmic, and stroma proteins, and non-protein-nitrogenous materials. The changes in these fractions during frozen storage were studied quantitatively. Also determined were the myosin-adenosine-triphosphatase activity, protein-breakdown products, and sulfhydryl-group content of muscles.

Materials and procedure. Tests were made with meat from 10-week-old chickens from a single flock, slaughtered in accordance with good commercial practice, cooled, and held 24 hr in air at 0°C. Birds were cut into halves, and each half was packed in a Cry-O-Vac bag (Stewart and Lowe, 1948) and frozen in an air blast (300–500 fpm) at −30°C. To obviate the effect of bird-to-bird variability, comparisons were made between left and right halves of one bird, one half stored at −80°C as control sample and the other half at the test temperature (−18 or −10°C) for the same length of time. Some experiments were also made at −4°C.

Extraction and fractionation. Meat was thawed for 2 hr in running water at 18–22°C. Preparation of sample and extraction and fractionation technique found suitable for the routine analysis of chicken muscle proteins have been described (Khan, 1962b). In brief, breast and leg muscles were extracted with KCl-borate buffer at pH 7.5 and at ionic strength ($\Gamma/2$) 1.0, and fractionated into myofibrillar (actomyosin, myosin), sarcoplasmic, and stroma proteins and non-protein-nitrogenous materials. The non-dialyzable-nitrogen fraction soluble at $\Gamma/2 = 0.50$ and insoluble at $\Gamma/2 = 0.08$ is reported as myofibrillar-protein fraction, and that soluble at $\Gamma/2 = 0.50$ and insoluble at $\Gamma/2 = 0.25$ as actomyosin fraction.

Myosin-adenosinetriphosphatase (ATPase) activity. The myosin-ATPase activity was determined in the actomyosin fraction. This fraction was washed with KCl-borate buffer, pH 7.5, $\Gamma/2 = 0.25$, and dissolved in KCl-borate buffer,

[a] Contribution from the Division of Applied Biology, National Research Council, Ottawa 2, Canada. Issued as N.R.C. No. 7491.

$\Gamma/2 = 1.0$. The final concentrations of the reactants were: ATP (disodium salt, Pabst), $2.5 \times 10^{-3}M$; calcium chloride, $5 \times 10^{-3}M$; potassium chloride, $10^{-2}M$; sodium barbitone, $15 \times 10^{-3}M$; actomyosin fraction, 0.15–0.20 mg of protein nitrogen in the test solution (volume 4 ml). The reaction was allowed to proceed at pH 7.8–8.4 for 10 min at 35°C, then stopped by the addition of trichloroacetic acid, and the reaction mixture centrifuged. A suitable aliquot of the supernatant was taken for the determination of free phosphate (Allen, 1940). The activity is expressed as μg of phosphorus released/mg of soluble nitrogen/min.

Sulfhydryl-group content. Sulfhydryl-group content of muscles was estimated by a method similar to that of Coleby *et al.* (1961). Minced sample (5 g) was weighed accurately, homogenized with sodium chloride (3 g) and metaphosphoric acid (2.25%, 10 ml), and made to 20 ml with metaphosphoric acid solution. The mixture was shaken, left for 10 min, and filtered (Whatman No. 40 paper). These operations were performed at 0°C. One to four ml of filtrate (a solution containing 2.25% metaphosphoric acid and 1.5% sodium chloride was used to make 4 ml volume, in case the filtrate sample was less than 4 ml) was placed in a cuvette located in a photoelectric colorimeter (Coleman Junior) and allowed to react with 4% sodium nitroprusside (1 ml) and 1.5*M* sodium carbonate–0.033*M* sodium cyanide (2 ml). The mixture was stirred and absorption at 500 mμ measured within 30 sec. Results are reported as equivalents of glutathione.

Proteolysis. Protein-breakdown products were estimated in the non-protein-nitrogen fraction colorimetrically, both by Folin-Ciocalteu reagent (Folin and Ciocalteu, 1927, Bradley and Bailey, 1940) and by ninhydrin method (Rosen, 1957), and were expressed as L-tyrosine equivalents.

RESULTS

Protein solubility in potassium chloride-borate buffer (pH 7.5 and ionic strength 1.0) decreased with storage time and temperature in both breast and leg muscle (Fig. 1). Although the results showed a considerable variability between birds, in most experiments the protein extractability differed noticeably between the two halves of one bird stored at two different temperatures for the same length of time. At −80°C, protein solubility did not change appreciably in breast muscle but decreased slightly in leg muscles (8% in 95 weeks). The results of quantitative fractionation of breast and leg muscle (Table 1) show that the loss of protein extractability appears as a decrease in the actomyosin fraction content of the extract. The changes in the amount of myosin fraction were not significant. The amount

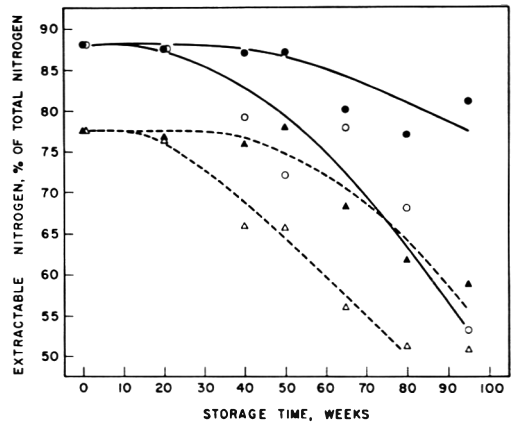

Fig. 1. Solubility of chicken muscle proteins in KCl-borate buffer (pH 7.5, $\Gamma/2 = 1.0$). ●, breast muscles stored at −18°C; ○, breast muscles stored at −10°C; ▲, leg muscles stored at −18°C; △, leg muscles stored at −10°C.

of sarcoplasmic fraction decreased after prolonged storage—for example, in 50 weeks at −4°C in breast muscle and in 95 weeks at −10°C in both breast and leg muscles. The amount of stroma nitrogen (5% and 19% of the total nitrogen in breast and leg muscle, respectively) remained unaffected.

The myosin-ATPase activity of the actomyosin fraction decreased in both breast and leg muscles with storage time and was related to storage temperature (Fig. 2). These results show that during frozen storage the actomyosin fraction loses its ATPase activity before losing its solubility characteristics. The ATPase activity of the control samples varied from 140 to 225 units in breast muscles, and from 140 to 180 units in leg muscles.

The sulfhydryl-group content of chicken breast and leg muscles decreased with storage temperature and time (Fig. 3). The sulfhydryl-group content (expressed as mg of glutathione/g of meat) of the control samples varied from 0.162 to 0.196 in breast muscles, and from 0.292 to 0.316 in leg muscles.

Folin-Ciocalteu-reagent-positive materials (tyrosine complexes, phenols, tryptophane, sulfhydryl compounds including H_2S and other reducing agents) (Bradley and Bailey, 1940) and ninhydrin-positive materials increased with storage time and with increase of storage temperature as compared with the control sample stored at −80°C (Figs. 4, 5). As a result of bird-to-bird variability, in control samples the amount of Folin-Ciocalteu-reagent-positive materials (expressed as mg of tyrosine/100 g of meat) varied from 26.3 to 31.3 in breast muscles and from 17.5 to 26.2

Table 1. Effect of frozen storage at different temperatures on chicken breast and leg muscle protein fractions.

Kind of muscle	Storage time (weeks)	Storage temp. (°C)	Extractable nitrogen (% of total nitrogen)	g Nitrogen/100 g muscle			
				Myofibrillar	Actomyosin	Sarcoplasmic	Non-protein
Breast	50	−80	83.0	1.34	1.28	1.20	0.56
		−18	83.0	1.33	1.29	1.24	0.58
		−10	71.4	0.85	0.83	1.10	0.60
		− 4	56.2	0.59	0.56	0.96	0.68
Leg		−80	72.4	1.42	0.93	0.60	0.43
		−18	65.5	1.10	0.73	0.58	0.43
		−10	52.7	0.72	0.23	0.57	0.45
		− 4	44.6	0.32	0.11	0.60	0.48
Breast	80	−80	85.6	1.51	1.35	1.01	0.52
		−18	76.8	1.12	0.90	0.91	0.53
		−10	67.9	0.71	0.68	0.96	0.56
Leg		−80	67.3	1.02	0.60	0.69	0.33
		−18	58.1	0.69	0.46	0.71	0.35
		−10	50.9	0.57	0.35	0.68	0.36
Breast	95	−80	85.2	1.45	1.37	1.11	0.52
		−18	80.0	1.30	0.92	1.01	0.55
		−10	52.9	0.65	0.43	0.78	0.58
Leg		−80	64.4	0.92	0.63	0.63	0.33
		−18	58.4	0.82	0.50	0.62	0.37
		−10	50.8	0.78	0.47	0.49	0.40

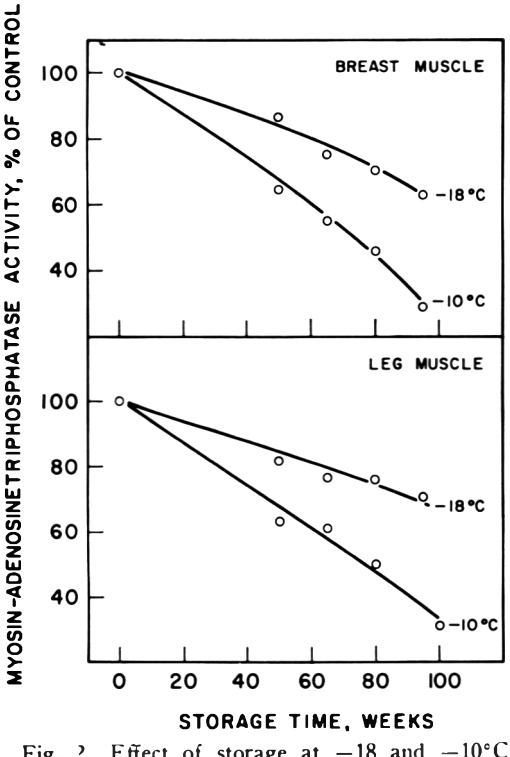

Fig. 2. Effect of storage at −18 and −10°C on myosin-adenosinetriphosphatase activity of actomyosin fraction.

in leg muscles, and the ninhydrin-positive materials (expressed as g of tyrosine/100 g of meat) varied from 0.37 to 0.94 in breast muscles and from 0.40 to 1.04 in leg muscles. The results show that during frozen storage the increase in the non-protein-nitrogen fraction was mainly due to the accumulation of ninhydrin-positive nitrogen. However, the ninhydrin-positive nitrogen (calculated on the basis of nitrogen present as tyrosine) constituted only about 5 to 10% of the total non-protein nitrogen in breast muscle and about 10 to 20% of the total non-protein nitrogen in leg muscle.

DISCUSSION

The results indicate that during frozen storage the chicken muscle proteins become less extractable, and that the rate of loss of protein extractability increases with storage temperature and time. Similar results have been reported for cod muscle proteins (Connell, 1960; Love, 1962), and it appears that the phenomenon of "denaturation" with the loss of solubility is common to fish and chicken muscles. Some workers have tried to correlate protein "denaturation" with toughness development in fish muscle (Love, 1962) and with change in water-holding capacity of beef muscle (Deatherage and

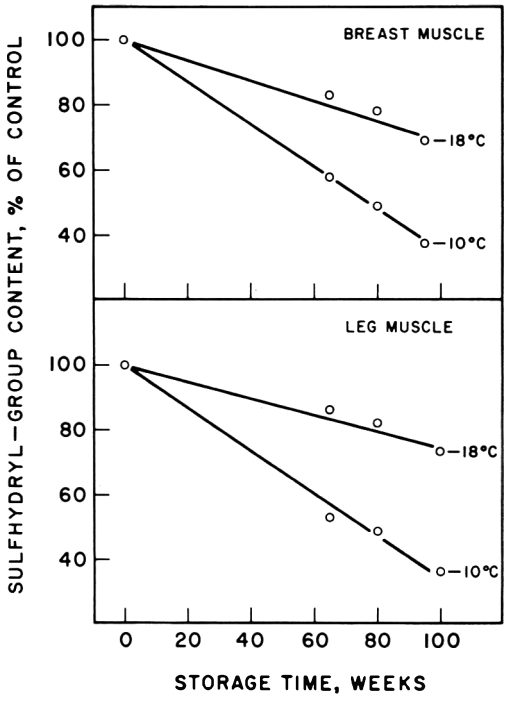

Fig. 3. Effect of storage at −18 and −10°C on sulfhydryl-group content.

Hamm, 1960), but similar correlations have not been attempted for chicken muscles. The results derived from the present work show that the loss of extractability could be accounted for by a decrease in the actomyosin fraction. The concomitant loss of sulfhydryl-group content and solubility of chicken muscle proteins suggests that the destruction of sulfhdryl group can be used as an index of protein damage during frozen storage.

It has been postulated (Blum, 1960; Greville and Tapley, 1960; Perry, 1961) that myosin contains two types of sulfhydryl groups, that one type is concerned in the ATPase activity and the other type in the combination with actin. The loss of myosin-ATPase activity without increase in the myosin fraction suggests that the sulfhydryl groups of the myosin concerned in the ATPase are affected during frozen storage more extensively than those concerned in the formation of actomyosin. Since a relation between ATPase activity and water-holding capacity of muscles has been shown in beef (Hunt and Matheson, 1958; Lawrie *et al.*, 1961), it appears that a similar relation holds for chicken muscles. Denaturation of the actomyosin with loss of ATPase activity has also been shown during freeze drying of beef and fish muscles (Hunt and Matheson, 1958).

Muscle proteases have been reported to have pH optima of 4 and 7 and temperature optima at 37°C (Bandack-Yuri and Rose, 1961; Slinwinski *et al.*, 1959), whereas the pH of the muscle varies from 5 to 7 (Bate-Smith, 1948). It was expected, therefore, that the activity of these enzymes in our experiments should be retarded by both storage temperature and pH. However, proteolysis occurred in both breast and leg muscle at −18, −10, and −4°C. Beef contains a protease active at freezing temperatures (Ball, 1938), and it is possible that a similar enzyme system is present in chicken muscles. These results, however, do not elucidate the nature of proteolytic changes and their contribution to the changes studied.

These results indicate that chicken muscle proteins undergo both denaturation and proteolysis during frozen storage. The decrease in ATPase activity and protein solu-

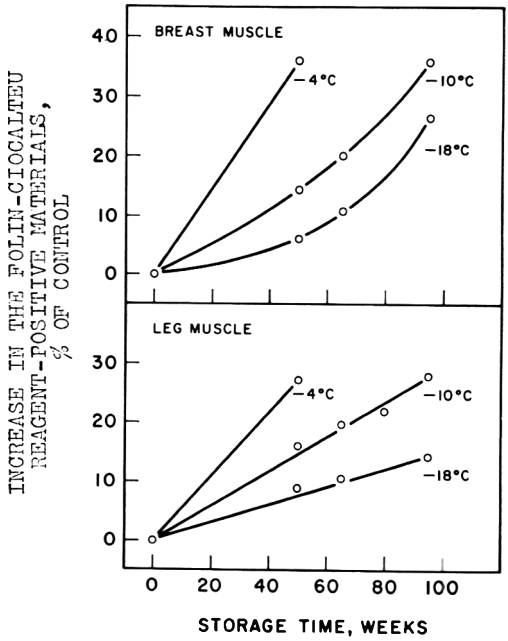

Fig. 4. Accumulation of Folin-Ciocalteu-reagent-positive materials in chicken muscle during storage at −4, −10, and −18°C.

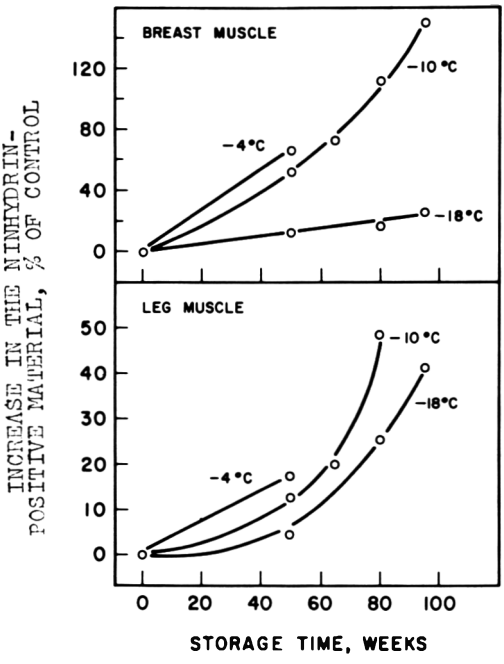

Fig. 5. Accumulation of ninhydrin-positive materials in chicken muscle during storage at −4, −10, and −18°C.

bility appears to be caused by a stepwise denaturation of actomyosin. Since muscles studied in these tests were frozen and thawed under identical conditions and no bacterial multiplication was noted during storage at these temperatures, it is plausible to conclude that the biochemical changes discussed in this paper are governed by temperature and time of storage.

ACKNOWLEDGMENT

The authors acknowledge in particular the technical assistance of Mr. G. W. Daechsel.

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Determination of Chlorophylls, Chlorophyllides, Pheophytins, and Pheophorbides in Plant Material [a]

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(Manuscript received January 7, 1963)

SUMMARY

Equations have been developed for calculation of the total quantity of pigments consisting of chlorophyll *a* and its derivatives (chlorophyllide *a*, pheophytin *a*, and pheophorbide *a*) and of chlorophyll *b* and its derivatives (chlorophyllide *b*, pheophytin *b*, and pheophorbide *b*) in a mixture of these pigments in diethyl ether. Calculations are based on conversion of the chlorophylls and chlorophyllides into the pheophytins and pheophorbides, respectively, by the addition of hydrochloric acid and reading absorbances at two wavelengths. After removal of chlorophyllides *a* and *b* and pheophorbides *a* and *b* from the diethyl ether solution of the pigment mixture by 0.01*N* KOH, the quantity of each of the eight components listed above is estimated by indicated experimental and mathematical procedures.

The chlorophylls are magnesium complex salts of the phorbins and exist as esters of phytyl alcohol. Chlorophyllase, an enzyme commonly found in green plant tissue, can hydrolyze phytyl alcohol from the chlorophylls, forming chlorophyllides. The chlorophylls and the chlorophyllides in the presence of even dilute acid readily undergo loss of the magnesium, respectively forming pheophytins and pheophorbides. The chlorophyllides are visually similar to the chlorophylls, and the pheophorbides are visually similar to the pheophytins. Willstätter and Stoll (1928) report that chlorophyllase will also split phytyl alcohol from the pheophytins, forming pheophorbides.

Comar and Zscheile (1942) described a method for the estimation of chlorophylls *a* and *b* from the spectral curves of these pigments in diethyl ether. Vernon (1960), by similar procedures, estimated the concentration of each of four components, the chlorophylls *a* and *b* and the pheophytins *a* and *b*, in mixtures of these pigments in 80% acetone. Jones *et al.* (1962) developed procedures for determination of the pheophorbides *a* and *b* as well as the chlorophylls *a* and *b* and the pheophytins *a* and *b* in diethyl ether. A need has been recognized for a procedure permitting quantitative estimation of the concentration of chlorophyllides.

Recent studies on chlorophyll changes during the brining of cucumbers, demonstrating that pheophorbides are the principal derivatives formed (Jones *et al.*, 1961, 1962), suggest that the brining treatment favors chlorophyllase activity following denaturation of chloroplastin of green tissue by salt similar to that induced by the action of hot water on plant tissue as reported by Weast and Mackinney (1940). If such were the case, then chlorophyllides should be formed in measurable quantities in green tissue brined under conditions of acidity low enough to avoid replacement of the magnesium atom of the chlorophyll molecule with hydrogen atoms. The studies described herein were made to test this conclusion.

MATERIALS AND METHODS

The plant material used, small whole pickling cucumbers, selected because of the rather extensive study of pigment changes in this tissue by the authors, was brined according to the method outlined for Lot A material by Jones *et al.* (1962). Pigment analyses were made essentially as described by Jones *et al.* (1962) with modification indicated schematically in Fig. 1.

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[a] Contribution from the Dept. of Food Science, N. C. Agricultural Experiment Station, Raleigh, N. C. Published with the approval of the Director of Research as Paper 1551 of the Journal Series.

[b] Dept. of Chemistry.

[c] Dept. of Food Science.

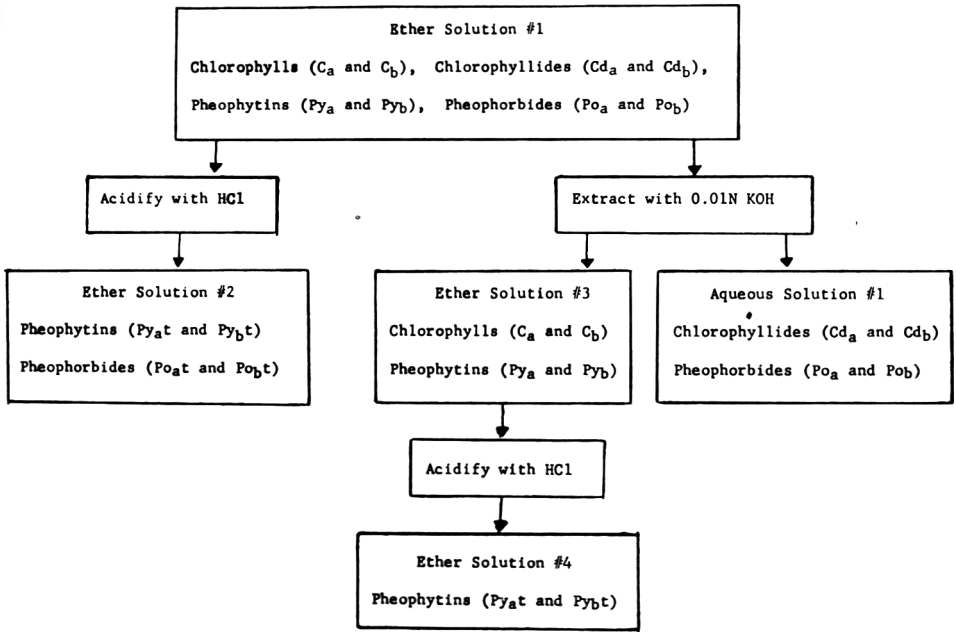

Fig. 1. Scheme for pigment separation. Prepared sample extracted with acetone: extract transferred to diethyl ether.

The plant tissue was extracted by blending a suitable weight in such volume of acetone that the equilibrium concentration was about 80%. The pigments were then transferred to diethyl ether (Mallinckrodt Ether Anhydrous, Analytical Reagent ACS), designated as ether Solution no. 1, and absorbance was measured at 660 and 642.5 mμ, the respective absorption maxima of chlorophyll *a* and chlorophyll *b*. Ether Solution no. 2 was prepared by the addition of one drop of concentrated hydrochloric acid to 25 or 50 ml of ether Solution 1 to convert chlorophylls and chlorophyllides to pheophytins and pheophorbides, respectively. Granular anhydrous Na_2SO_4 (Mallinckrodt Analytical ACS) was also added, to clarify and dry the solution. Ether Solution 2 was allowed to stand for 2 hr in the dark at room temperature. Its absorbance was then measured at 666.5 and 653 mμ, the respective absorption maxima of pheophytin *a* and pheophytin *b*.

A suitable aliquot of ether Solution 1 was also extracted with 0.01*N* KOH until all the chlorophyllides and pheophorbides were removed from the ether solution. This extract, designated as aqueous Solution No. 1, was discarded. The chlorophylls and pheophytins remained in the ether solution (ether Solution no. 3), which was subsequently washed five times with 5% Na_2SO_4 solution, dried with granular anhydrous Na_2SO_4, and made to a suitable volume. The absorbance of ether Solution 3 was measured at 660 and 642.5 mμ. Ether Solution 4 was prepared by the addition of one drop of concentrated hydrochloric acid to 25 ml of ether Solution 3 to convert chlorophylls to pheophytins. Granular anhydrous Na_2SO_4 was also added, to clarify and dry the solution. Ether Solution 4 was allowed to stand for 2 hr in the dark at room temperature. Its absorbance was measured at 666.5 and 653 mμ.

All absorbances were determined with a Beckman DK-2 spectrophotometer. The maximum absorption of ether Solution 2 in the red region of the spectrum was assumed to be 666.5 mμ. Corrections at other wavelengths were obtained from a wavelength calibration curve based on the 666.5-mμ adjustment. This procedure was adopted to correct for instrument variation in wavelength setting, as suggested in Official Methods of Analysis (AOAC, 1960) for selection of the 660-mμ peak point for pure chlorophylls in diethyl ether.

CALCULATIONS

Equations were developed for calculating the concentration of each pigment in a mixture of chlorophylls, chlorophyllides, pheophytins, and pheophorbides from their absorbances in diethyl ether solutions at certain wavelengths. A brief discussion of the symbols used and of the origin of the formulas is in order. The pigment fractions chlorophylls *a* and *b*, chlorophyllides *a* and *b*, pheophytins *a* and *b*, and pheophorbides *a* and *b*, are respectively designated by the symbols C_a, C_b, Cd_a, Cd_b, Py_a, Py_b, Po_a, and Po_b. In the equations that follow for the chlorophylls and their deriva-

tives, the concentration of each pigment was calculated in micromoles per liter of diethyl ether solution read, because $1\mu M$ of chlorophyll is equivalent to $1\mu M$ of chlorophyllide, pheophytin, or pheophorbide.

Following the reasoning of Jones *et al.* (1962), as a working hypothesis, the assumptions are made that the coefficients for the two pheophytins do not differ materially from their respective pheophorbides, and that the same applies also to chlorophylls *a* and *b* and their chlorophyllides. The error in such assumptions is systematic, and probably does not exceed 5%. Table 1 summarizes a survey of published coefficient values for chlorophylls *a* and *b* and their derivatives.

The following millimolar absorption coefficients of Zscheile and Comar (1941) were used in deriving Equations 9 and 10:

Chlorophyll *a*, 91.1 at 660 mμ and 14.6 at 642.5 mμ

Chlorophyll *b*, 4.08 at 660 mμ and 52.2 at 642.5 mμ

Pheophytin *a*, 36.6 at 660 mμ and 5.05 at 642.5 mμ

Pheophytin *b*, 17.7 at 660 mμ and 11.7 at 642.5 mμ

The same coefficients were used in deriving Eqs. 7 and 8, based on the assumption discussed previously.

The following millimolar absorption coefficients of Holt and Jacobs (1954) were used in deriving Eqs. 3 and 4:

Ethylpheophorbide *a*, 51.4 at 666.5 mμ and 16.1 at 653 mμ

Ethylpheophorbide *b*, 5.75 at 666.5 mμ and 33.2 at 653 mμ

The same coefficients were used in deriving Eqs. 1 and 2, based on the assumption discussed previously.

The sum total of chlorophylls, pheophytins, chlorophyllides, and pheophorbides may be estimated by Eqs. 1 and 2 from absorbances of ether Solution 2, which contains pheophytins and pheophorbides only, the chlorophylls and the chlorophyllides having been respectively converted to pheophytins and pheophorbides by the addition of hydrochloric acid, as indicated earlier.

Table 1. Absorption maxima and millimolar absorption coefficients of the chlorophylls and their derivatives.

Chlorophyll or derivative	Solvent	Wave length, mμ, maximum	Millimolar absorption coefficient
Chlorophyll *a*	Diethyl ether	662.	90.2 [a]
Chlorophyll *a*	Diethyl ether	661.	91.1 [b]
Chlorophyll *a*	Diethyl ether	660.	91.1 [c]
Ethylchlorophyllide *a*	Diethyl ether	660.	89.3 [d]
Methylchlorophillide *a*	Dioxane	660.	100.0 [e]
Pheophytin *a*	Diethyl ether	667.	55.5 [a]
Pheophytin *a*	Diethyl ether	667.	56.6 [b]
Pheophytin *a*	Diethyl ether	666.5	52.4 [c]
Ethylpheophorbide *a*	Diethyl ether	666.7	51.4 [d]
Pheophorbide *a*	1% Pyridine in diethyl ether	662.5	48.9 [f]
Methylpheophorbide *a*	Dioxane	666.0	52.8 [g]
Chlorophyll *b*	Diethyl ether	644.	56.3 [a]
Chlorophyll *b*	Diethyl ether	642.5	58.5 [b]
Chlorophyll *b*	Diethyl ether	642.5	52.2 [c]
Ethylchlorophyllide *b*	Diethyl ether	640.5	53.3 [d]
Pheophytin *b*	Diethyl ether	655.	37.3 [a]
Pheophytin *b*	Diethyl ether	655.	37.0 [b]
Pheophytin *b*	Diethyl ether	653.	33.2 [c]
Ethylpheophorbide *b*	Diethyl ether	653.	33.2 [d]
Pheophorbide *b*	5% Pyridine in diethyl ether	650.9	31.8 [f]
Methylpheophorbide *b*	Dioxane	652.5	30.6 [h]

[a] Smith and Benitez, 1955
[b] Davidson, 1954
[c] Zscheile and Comar, 1941
[d] Holt and Jacobs, 1954
[e] Stern and Wenderlein, 1936
[f] Hagenbach *et al.*, 1936
[g] Stern and Wenderlein, 1935a
[h] Stern and Wenderlein, 1935b

$Py_a + Po_at = 20.57.A_{660.5} - 3.56.A_{653}$ (ether Solution 2) [1]

$Py_b + Po_bt = 31.85.A_{653} - 9.98.A_{660.5}$ (ether Solution 2) [2]

where

$Py_at = C_a + Py_a$; $Po_at = Cd_a + Po_a$;
$Py_bt = C_b + Py_b$; and $Po_bt = Cd_b + Po_b$

The sum total of chlorophylls and pheophytins may be estimated by Eqs. 3 and 4 from absorbances of ether Solution 4, which contains pheophytins only, the chlorophylls having been converted to pheophytins by the addition of hydrochloric acid.

$Py_at = 20.57.A_{660.5} - 3.56.A_{653}$ (ether Solution 4) [3]

$Py_bt = 31.85.A_{653} - 9.98.A_{660.5}$ (ether Solution 4) [4]

The sum total of chlorophyllides and pheophorbides is obtained by difference, as follows:

Po_at = value Eq. 1 − value Eq. 3 [5]

Po_bt = value Eq. 2 − value Eq. 4 [6]

The total quantity of the chlorophylls and the chlorophyllides may be estimated by Eqs. 7 and 8 as follows:

$Cd_a + C_a = 17.33.A_{660} + 5.83.A_{642.5} - 0.664\ (Py_at + Po_at) - 0.375\ (Py_bt + Po_bt)$ [7]

$Cd_b + C_b = 23.32.A_{642.5} - 4.086.A_{660} + 0.0318\ (Py_at + Po_at) - 0.2005\ (Py_bt + Po_bt)$ [8]

The values for A_{660} and $A_{642.5}$ are taken from the spectral curve for ether Solution 1. The values for $Py_at + Po_at$ and $Py_bt + Po_bt$ are those calculated for ether Solution 2 by Eqs. 1 and 2.

The quantities of chlorophylls *a* and *b* in a mixture of the chlorophylls and their derivatives may be estimated by Eqs. 9 and 10 by substituting absorbances of ether Solution 3 and inserting values obtained for Py_at and Py_bt in Eqs. 3 and 4, respectively.

$C_a = 17.33.A_{660} + 5.83.A_{642.5} - 0.664Py_at - 0.375Py_bt$ [9]

$C_b = 23.32.A_{642.5} - 4.086.A_{660} + 0.0318Py_at - 0.2005Py_bt$ [10]

The quantities of chlorophyllides *a* and *b* are estimated by differences in values from Eqs. 7 and 9 and from 8 and 10, respectively.

Cd_a = value Eq. 7 − value Eq. 9 [11]

Cd_b = value Eq. 8 − value Eq. 10 [12]

Similarly, the values for Py_a, Py_b, Po_a, and Po_b, are estimated by differences as indicated below:

Py_a = value Eq. 3 − value Eq. 9 [13]

Py_b = value Eq. 4 − value Eq. 10 [14]

Po_a = value Eq. 5 − value Eq. 11 [15]

Po_b = value Eq. 6 − value Eq. 12 [16]

RESULTS AND DISCUSSION

Table 2 shows the concentrations of chlorophylls or of various chlorophyll derivatives during the brining period, and standard errors of individual determinations.

These data indicate that the equations developed are applicable for estimation of the chlorophylls, chlorophyllides, pheophytins, and pheophorbides of plant tissue in the

Table 2. Pigment values expressed as micromoles per 100 grams in fresh and brined cucumbers, as related to duration of brining treatment. Values are means of triplicated samples.

	Chlorophylls and chlorophyll derivatives								
Time days in brine	Chlorophyll a C_a	Chlorophyll b C_b	Chlorophyllide a Cd_a	Chlorophyllide b Cd_b	Pheophytin a Py_a	Pheophytin b Py_b	Pheophorbide a Po_a	Pheophorbide b Po_b	pH of brine
0	5.80	2.91	0.00	0.00	0.00	0.00	0.00	0.00	5.9
1	3.58	2.51	0.62	0.15	1.18	−0.05	0.39	0.05	5.8
2	1.84	1.74	0.15	0.51	1.19	0.18	2.74	0.41	5.9
3	1.21	1.34	−0.17	0.51	0.64	−0.06	3.82	0.87	5.9
4	0.91	1.12	0.21	0.67	0.77	0.01	3.70	0.85	5.3
5	0.59	0.86	0.27	0.76	0.87	0.14	4.18	1.02	5.1
6	0.34	0.58	0.26	0.46	0.90	0.26	4.06	1.33	4.6
7	0.09	0.25	0.17	0.38	1.25	0.57	4.86	1.84	4.1
8	0.00	0.00	0.00	0.00	1.19	0.63	5.20	2.29	3.9
9	0.00	0.00	0.00	0.00	1.15	0.58	5.42	2.56	3.8
16	0.00	0.00	0.00	0.00	1.43	0.76	5.19	2.31	3.6
42	0.00	0.00	0.00	0.00	1.36	0.64	4.95	2.41	3.6
SE =	0.14	0.10	0.10	0.10	0.10	0.10	0.41	0.17	

absence of interfering chlorophyll derivatives, such as metal complexes and allomerized compounds. The purity of the pigments was checked by the phase test (Willstätter and Stoll, 1928), hydrochloric acid number (Willstätter and Stoll, 1928), column chromatography, and metal analyses. The phase test for allomerized products was negative. Allomerization is prevented in acid media (Willstätter and Stoll, 1928). Plant tissues are commonly slightly to strongly acid. Pigment extracts were analyzed for magnesium, copper, zinc, and iron. Results were negative for all metals except magnesium, which was present until the chlorophylls and the chlorophyllides were completely converted.

Fig. 2 presents the pigment changes that took place during the brining process. To clarify the method of calculation of the points on the different curves the following example is given for the estimation of pheophorbides from data in Table 2 for the seventh day.

$$\frac{Po_a + Po_b}{C_a + C_b + Cd_a + Cd_b + Py_a + Py_b + Po_a + Po_b} \times 100 = \% \text{ pheophorbides}$$

or

$$\frac{4.86 + 1.84}{0.09 + 0.25 + 0.17 + 0.38 + 1.25 + 0.57 + 4.86 + 1.84} \times 100 = 71.2\%$$

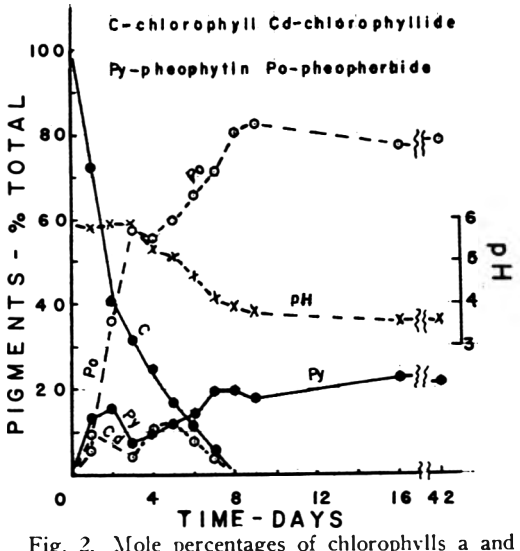

Fig. 2. Mole percentages of chlorophylls a and b, chlorophyllides a and b, pheophytins a and b, and pheophorbides a and b in brined cucumbers.

Conversion of chlorophylls to chlorophyllides and to pheophytins and conversion of chlorophyllides to pheophorbides began immediately after the brining operation was started. The chlorophyllides, though never present in high concentration, were found in measurable quantities for the first seven days, during which time chlorophylls were also present. Acidity of the brine in which the cucumbers were preserved, as indicated by the pH curve in Fig. 2, increased rapidly from the third through the seventh days because of a naturally occurring acid fermentation. The resulting acidity was responsible for conversion of chlorophylls and chlorophyllides to their magnesium-free counterparts.

The rapid rate of pheophorbide formation is evidence of high chlorophyllase activity in the cucumber tissue under the condition of the experiment and, furthermore, is evidence of the ease with which the magnesium of the chlorophylls and the chlorophyllides may be replaced by hydrogen in a brine medium near the pH value of 6.0.

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Influence of Blanching or Brining Treatments on the Formation of Chlorophyllides, Pheophytins, and Pheophorbides in Green Plant Tissue [a]

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(Manuscript received January 7, 1963)

SUMMARY

Green vegetable tissue was analyzed for chlorophyll and chlorophyll derivatives before and after subjecting the tissue to differential blanching treatments representative of those used in commercial food processing, and to a brining treatment. The plant material studied was okra, snapbeans, turnip greens, and pickling cucumbers. Blanching at 180°F promoted the rapid formation of chlorophyllides and pheophorbides in certain tissue in addition to pheophytins, which were formed in all blanching studies.

The conversion of chlorophyll to pheophytin during the processing of green plant tissue by canning, dehydration, freezing, and brining has been reported by many investigators. The rate and extent of such change has been studied by Mackinney and Weast (1940), Dietrich (1958), Dietrich *et al.* (1957, 1959), Sweeney and Martin (1958, 1961), and others. Weast and Mackinney (1940) demonstrated that when some green plant tissue was subjected to the action of hot water, chlorophyllase activity was marked. Jones *et al.* (1961) reported that during the brining of cucumbers for pickling the chlorophylls are converted to the pheophorbides as well as to the pheophytins.

Jones *et al.* (1962) and White *et al.* (1963) outlined methods for quantitatively estimating pheophorbides and chlorophyllides, end products of chlorophyllase activity. These methods provide a measure of chlorophyllase action. It has seemed desirable to investigate the use of these procedures for studying the influence of blanching techniques on chlorophyll pigment change in green tissue of several vegetables. The effect of blanching and brining treatments on the nature and speed of chlorophyll change in cucumber tissue was also studied.

MATERIALS AND METHODS

The plant materials used were snapbeans, okra, and turnip greens from the retail market, and small pickling cucumbers recently harvested. Cucumbers were included because of demonstrated chlorophyllase activity previously cited. Pigment analyses were made according to the method of White *et al.* (1963).

Fifty-gram quantities of snapbeans and okra and 10-g quantities of turnip greens were blanched in duplicate and analyzed for pigment content. Blanching treatments of vegetable tissue, approximating those used in commercial canning and freezing preservation, were conducted by submerging prepared, weighed portions of tissue for 4 min in water at either 180 or 212°F, followed by immediate cooling for 4 min in running cold water. Duplicated lots of 10 small whole cucumbers and of slices ¼-inch thick from 10 small cucumbers were blanched by the procedure used for the other vegetables and were analyzed for pigment content as described by White *et al.* (1963).

Unblanched cucumbers were brined as outlined for Lot A samples by Jones *et al.* (1962). Pigment analyses were conducted in triplicate. Lots of cucumbers, as whole fruit and as slices, receiving the differential blanching treatments, were brined separately for 9 days in approximately 700-g quantities in 7.5% NaCl solution. Pigment analyses were made in duplicate.

RESULTS AND DISCUSSION

Table 1 gives data from differential blanching treatments with okra, turnip greens, and snapbeans. Chlorophyllides and pheophorbides were formed in appreciable quantities in okra and turnip greens as a result of the 180°F treatment. The greater

[a] Contribution from the Dept. of Food Science, N. C. Agricultural Experiment Station, Raleigh, N. C. Published with the approval of the Director of Research as Paper 1550 of the Journal Series.

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Table 1. Pigment values expressed as % of total on a mole basis, in unblanched and blanched lots of okra, turnip greens, and snapbeans.

Vegetables	Chlorophylls and chlorophyll derivatives							
	Chlorophyll *a* C_a	Chlorophyll *b* C_b	Chlorophyllide *a* Cd_a	Chlorophyllide *b* Cd_b	Pheophytin *a* Py_a	Pheophytin *b* Py_b	Pheophorbide *a* Po_a	Pheophorbide *b* Po_b
Okra								
Unblanched	53.3	27.2	0.0	0.0	15.1	4.4	0.0	0.0
Blanched, 180°F	22.1	14.4	26.5	12.1	13.4	0.5	7.5	3.5
Blanched, 212°F	48.2	25.8	1.3	1.2	18.3	4.2	0.4	0.5
Turnip greens								
Unblanched	62.7	28.1	0.0	0.0	8.6	0.7	0.0	0.0
Blanched, 180°F	51.2	24.8	4.3	0.7	13.0	−0.2	0.3	5.9
Blanched, 212°F	60.5	27.6	0.0	0.0	12.1	−0.2	0.0	0.0
Snapbeans								
Unblanched	49.4	25.2	0.0	0.0	17.7	7.7	0.0	0.0
Blanched, 180°F	40.3	24.8	0.0	0.0	27.8	7.1	0.0	0.0
Blanched, 212°F	36.7	23.9	0.0	0.0	29.1	10.2	0.0	0.0

conversion of chlorophyll to magnesium-free derivatives in okra than in turnip greens may have been due to greater chlorophyllase concentration in okra tissue or to slower heat inactivation of chlorophyllase in the thick tissue of okra pods than in the thin leaf tissue of turnip greens. Mackinney and Weast (1940) reported chlorophyllase activity at a maximum of 75°C, a temperature somewhat lower than that used in this study.

The 212°F blanching treatments apparently inactivated chlorophyllase in the okra and turnip greens tissue, as evidenced by formation of little or no chlorophyllide or pheophorbide. From this study it appears that the snapbeans were devoid of chlorophyllase. This observation is in agreement with findings of Mackinney and Weast (1940).

Table 2 gives data on pigment changes induced in whole and sliced cucumbers by blanching and brining treatments. The blanching treatments at 180°F were responsible for formation of relatively large quantities of chlorophyllides and appreciable quantities of pheophorbides, as shown by the values for the unbrined lots blanched at 180°F. Blanching cucumbers at 212°F favored some chlorophyllase activity, as shown by presence of small amounts of chlorophyllides and pheophorbides in such

Table 2. Pigment values expressed as % of total on a mole basis, in unbrined and brined cucumber tissue following blanching treatments.

Treatments	Chlorophylls and chlorophyll derivatives							
	Chlorophyll *a* C_a	Chlorophyll *b* C_b	Chlorophyllide *a* Cd_a	Chlorophyllide *b* Cd_b	Pheophytin *a* Py_a	Pheophytin *b* Py_b	Pheophorbide *a* Po_a	Pheophorbide *b* Po_b
Unbrined								
Unblanched, whole	50.7	30.0	0.0	0.0	14.8	4.5	0.0	0.0
Blanched, whole, 180°F	14.9	10.1	26.4	16.7	8.0	3.7	21.0	−0.7
Blanched, sliced, 180°F	15.6	11.1	23.2	14.6	9.1	1.5	18.9	5.9
Blanched, whole, 212°F	34.2	23.6	5.8	3.3	21.5	0.5	4.7	6.5
Blanched, sliced, 212°F	36.7	25.7	3.6	1.3	26.3	1.1	−1.4	6.7
Brined								
Unblanched, whole	0.0	0.0	0.0	0.0	11.8	6.0	55.8	26.4
Blanched, whole, 180°F	0.0	0.0	0.0	0.0	16.6	5.4	54.8	23.4
Blanched, sliced, 180°F	0.0	0.0	0.0	0.0	16.4	5.6	52.1	25.9
Blanched, whole, 212°F	0.0	0.0	0.0	0.0	46.3	24.3	19.1	10.2
Blanched, sliced, 212°F	0.0	0.0	0.0	0.0	51.4	25.6	16.0	7.0

blanched unbrined lots. The accumulation of 23–29% pheophorbides in the brined lots blanched at 212°F showed that inactivation of chlorophyllase was not complete. Evidence is presented that blanching treatments are in many cases responsible for the formation of the chlorophyllides and pheophorbides as well as pheophytins. Pheophorbides may in fact represent a greater portion of the converted chlorophyll than pheophytins in certain plant tissue receiving blanching treatment.

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Reaction of Sugar Alcohols with the Anthrone Reagent

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SUMMARY

Under appropriate conditions of acid concentration, heating time and temperature of heating, the sugar alcohols react with the anthrone reagent in a quantitative manner. Suitable conditions for reproducible quantitative results are: 0.15% anthrone in concentrated sulfuric acid and a heating time of 60 min at 99°C (in a boiling-water bath). Color measurement should be made 30 minutes after cooling the reaction tubes in an ice water bath, since the intensity of the colors increased with time. Of the various sugar alcohols tested, the order of reactivity is: Glycerol > sorbitol > mannitol > dulcitol > erythritol > arabitol > ribitol > xylitol. Absorption maxima occurred at 720 mμ for mannitol, sorbitol, and dulcitol, and at 740 mμ for erythritol. Glycerol and the other sugar alcohols exhibited no sharp absorption maxima.

Since its introduction for the determination of carbohydrates (Dreywood, 1946), anthrone in sulfuric acid has been used to determine various sugars (Gimberteau, 1960; Helbert and Brown, 1955; Roe, 1955), polysaccharides (Helbert and Brown, 1957; McCready *et al.*, 1950; Roe, 1954–1955; Scott and Melvin, 1953; Seifter *et al.*, 1950), uronic acids (Helbert and Brown, 1956), and rutin (Sakagami and Shiraishi, 1960). Although the sugar alcohols are very prevalent (Gortner and Gortner, 1949; Merck and Co., 1960) and may be present in many of the materials analyzed for sugars and other carbohydrates, little attention has been paid to the reaction of this class of compounds with the anthrone reagent. Morris (1948) claimed that the sugar alcohols produced no color with the anthrone reagent, and Koehler (1952) reported a negative reaction with sorbitol. However, Schutz (1938) reported on the detection and determination of glycerol with this reagent. Many food and pharmaceutical products (Merck and Co., 1960; Atlas Chemical Industries, 1962) and dietetic foods (Genest and Chapman, 1962), contain glycerol, sorbitol, mannitol, and possibly dulcitol. The sugar alcohols are also gaining academic importance (Kinoshita *et al.*, 1962), and the Spans and Tweens, widely used emulsifiers and stabilizers, contain sorbitan, the dehydrated form of sorbitol.

While investigating optimum conditions for the determination of food gums (hydrocolloids) with the anthrone reagent (Graham, 1962) it was noted that several of the sugar alcohols produced color if the mixtures were heated for 30 min or more. Since information is scant on the interaction of the sugar alcohols with the anthrone reagent, a thorough investigation was deemed profitable. Preliminary experiments indicated that, for production of appreciable color and for quantitative determination, the critical factors were the concentration of acid in the anthrone reagent, the time and temperature of heating, and the time of measurement of the resulting color after its development. This article reports on these conditions. Glycerol, ethylene glycol, and methyl alcohol were included, in keeping with the classification of Carr and Krantz (1945).

EXPERIMENTAL

Reagents. Table 1 lists the sugar alcohols and related compounds used. They were all obtained from the Nutritional Biochemicals Corporation, Cleveland, Ohio. *Sulfuric acid* was reagent grade, 95–98%, sp. gravity 1.8407–1.8437. *Anthrone reagent* was, unless otherwise stated, a solution containing 0.15 g of anthrone per 100 ml of concentrated sulfuric acid. The reagent was prepared daily and never used if older than 24 hr. It was precooled for at least 4 hr before addition to the tubes containing the sugar alcohols.

Equipment. The equipment used was volumetric pipettes, Coleman Universal spectrophotometer, model 14, 40-ml ground-glass-stoppered test tubes, and a constant-temperature water bath or oil bath.

General procedure. One ml containing 1–10 mg

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Table 1. Characteristics of the reaction of sugar alcohols with concentrated H_2SO_4 and with the anthrone reagent (0.15% anthrone in concentrated H_2SO_4; mixtures heated for 60 minutes in a boiling bath).

Sugar alcohol (or other compound added)	Sugar alcohol or other compound heated in:			
	Concentrated H_2SO_4		Anthrone reagent	
	Color produced [a]	Max.	Color produced	Max.
Sorbitol	Y	410	R–B	720(500–520) [b]
Mannitol	Y	410	R–B	720(500–520) [b]
Dulcitol	Y–B	420	R–B	720(500–520) [b]
Erythritol	Y	410	R–B	740(500–520) [b]
D-arabitol	Y	410	Y–G	(500–520) [b]
Xylitol	Y	410	Y–G	(500–520) [b]
Ribitol	Y	410	B–G	(500–520) [b]
Glycerol	Y	410	O	(500–520) [b]
Inositol	NC	None	Y–G	None
Methyl alcohol	NC	None	R	(500–520) [b]
Ethylene glycol	NC	None	R	(500–520) [b]

[a] B = brown; O = orange; R = red; Y = yellow; NC = no color.
[b] Slowly descending arm or sensitive area of color measurement.

of each sugar alcohol was placed into Pyrex ground-glass-stoppered test tubes, and the tubes were placed in the freezer for 6–12 hr. Nine ml of the cold anthrone reagent was added with the tubes immersed in an ice-water bath. The tubes were then heated 1 hr in a boiling-water bath, cooled 15 min in an ice-water bath, and then allowed to stand 30 min at room temperature. The intensity of the colors developed was then measured against a reagent blank, at the appropriate wavelength of maximum absorption with the spectrophotometer.

Establishment of wavelength of maximum absorption of the colors developed.

The wavelength of maximum absorption of the colors developed was ascertained by treating the hydrocolloid-anthrone mixtures according to the general procedure. The colors developed were measured against sulfuric acid over the wavelength range of 350–800 mμ. A plot of the optical density as a function of wavelength over the wavelength range employed established the wavelength of maximum absorption.

Since several factors are critical for the development of color in the interaction of the sugar alcohols with the anthrone reagent, these were investigated. All measurements were made at 500 mμ, and color development was achieved as described under the general procedure. To assess the influence of temperature of heating on color development, 1–10 mg of the particular sugar alcohol was used. Information on the variation of color intensity with heating time was gained by heating mixtures of sugar alcohols and the anthrone reagent at 99°C (boiling-water bath) for varying periods. The influence of the concentration of sulfuric acid was assessed by heating mixtures of the sugar alcohols and anthrone reagent containing various levels of sulfuric acid for 60 min in a boiling-water bath. The influence of the concentration of anthrone was determined by varying the anthrone concentration between 0–0.5% (w/v). The influence of aging was determined by measuring the colors developed after time elapses of 0–96 hr. The results are summarized in Table 2.

Quantitative response of the sugar alcohols with the anthrone reagent.

After the influence of the several variables was established, the quantitative nature of the interaction was investigated. For this, increasing quantities of the sugar alcohols were heated with the anthrone reagent according to the general procedure, and the intensities of the colors developed were measured against a reagent blank at 500 mμ. The results are summarized in Fig. 2.

DISCUSSION

Sorbitol and mannitol are usually determined after their oxidation in alkaline solution with periodate to produce formaldehyde, which is then measured by reaction with chromotophic acid to give a red or wine color with an absorption maximum at 570 mμ (Snell *et al.*, 1961). The development of a quantitative procedure using the anthrone reagent offers the possibility of using a single reagent for the determination of these alcohols and carbohydrates when they are separated from mixtures. Methods have

Table 2. Influence of variables on color development in the interaction of the sugar alcohols with anthrone reagent.

Variable	Range investigated	Limits for reproducibility		Value or condition selected
		Maximum	Minimum	
Temperature of heating (°C)	60–99	.....	95	99
Time of heating (min)	0–90	.....	60	60
Concentration of sulfuric acid in anthrone reagent (% w/v)	70 to concentrated	.....	95	concentrated
Concentration of anthrone in concentrated sulfuric acid (% w/v)	0.1–0.5	0.5	0.10	0.15
Aging time (hr)	0–96	1.0	0.5	0.5

been proposed for the separation of these alcohols (Genest and Chapman, 1962; Jones and Wall, 1960; Moore *et al.*, 1960). Since the absorption maximum for the sugars and most other polysaccharides is at 620 mμ, interference by small amounts of sugars would be minimal, and becomes even less because extended heating of the sugar- or carbohydrate-anthrone mixture destroys the color (Helbert and Brown, 1955; Graham, 1962). Uronic acids and their polymers (Helbert and Brown, 1956, 1957) and alginates (Graham, 1962) will interfere since they produce a red color that absorbs maximally at around 540 mμ and this color is intensified by prolonged heating.

Fig. 2 shows that glycerol, sorbitol, mannitol, and dulcitol are the most reactive of the sugar alcohols with the anthrone reagent. The red color developed by methyl alcohol, glycerol, and erythritol is understandable in view of the red color obtained with acrolein and formaldehyde by Newkom and Hui (1959) and by acetone, pyruvic acid, pyruvic aldehyde, and lactic acid as reported by Shetlar (1952).

As with the sugars, chain length seems important insofar as color production is concerned since the hexitols, mannitol, and sorbitol were highly reactive whereas the penitols were much less reactive. The higher reactivity of erythritol than of the pentitols indicates that configuration may also play an important role in reactivity (Sattler and Zerban, 1948).

The spans (which contain sorbitan, the dehydrated form of sorbitol) reacted with the anthrone reagent. Spans 20 and 60 produced the typical sorbitol 720-mμ peak, but Tween 80 did not, because of interfering substances in the commercial sample. The temperature at which the reaction mixture is heated has a great influence on the final optical density of the color developed. The color produced is very little at 70°C, somewhat more at 85°C, and considerable at 99°C (boiling-water bath). Up to a period of 60 min, no definite time of heating was established for the maximum production of color. However, as the time of heating increased, color density also increased, and for convenience, a heating time of 60 min at 99°C was selected.

Beyond a concentration of 0.1%, the level of anthrone in the reagent has little influence on the optical density of the color developed. Although no definite optimum was established between the levels of 0.05–1.0% used, for convenience, a level of 0.15% was chosen. The anthrone-sugar alcohol color increases in intensity with age, a phenomenon resembling that noted for the uronic acids (Helbert and Brown, 1956). In the anthrone-concentrated sulfuric acid reagent, sorbitol, mannitol, and dulcitol exhibited a distinct absorption maximum at 720 mμ. For erythritol this absorption maximum was at 740 mμ. Glycerol and the other sugar alcohols showed no distinct absorption maximum over the wavelength range of 350–800 mμ. Between 450 and 510 mμ, glycerol, sorbitol, mannitol, and dulcitol exhibited a slow descending arm in their absorption spectra. On the other hand, the spectrum of the reagent blank descends more drastically within this range (Fig. 1).

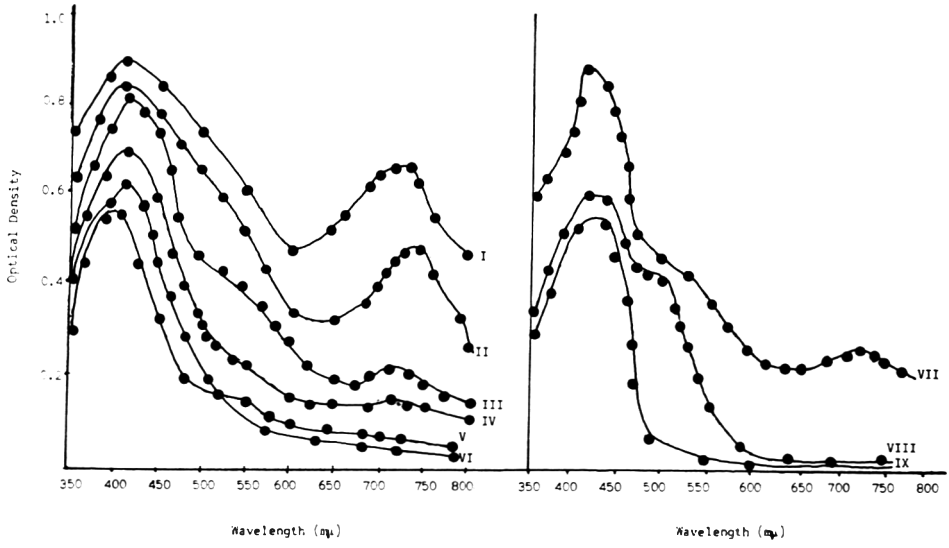

Fig. 1. Absorption spectra of the colors produced by sugar alcohols when heated with the anthrone reagent. I = dulcitol heated 60 min in anthrone in conc. H_2SO_4; II = erythritol heated 60 min in anthrone in conc. H_2SO_4; III = Span 20 heated 60 min in anthrone in conc. H_2SO_4; IV = sorbitol heated 60 min in anthrone in conc. H_2SO_4; V = glycerol heated 60 min in anthrone in conc. H_2SO_4; VI = ribitol heated 60 min in anthrone in conc. H_2SO_4; VII = mannitol heated 60 min in anthrone in conc. H_2SO_4; VIII = mannitol heated 60 min in anthrone in 70% H_2SO_4; IX = reagent blank.

Absorption measurements (against a reagent blank) were much higher at 500 mμ than at 720 mμ (or 740 mμ for erythritol). Therefore, these measurements (500 mμ) were used in establishing the standard curves shown in Fig. 2.

The acid concentration of the anthrone reagent profoundly influences the intensity of the color developed. As the acid concentration increased, the optical density of the resulting color also increased. Acid concentration also influences the absorption spec-

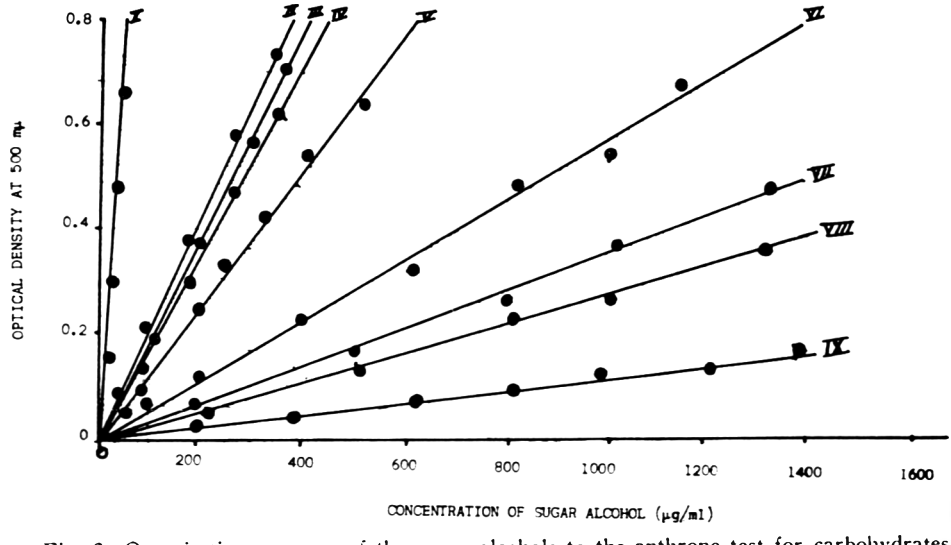

Fig. 2. Quantitative response of the sugar alcohols to the anthrone test for carbohydrates. I = glycerol; II = dulcitol; III = sorbitol; IV = mannitol; V = erythritol; VI = ribitol; VII = D-arabitol; VIII = xylitol; IX = inositol.

trum. The peak at 720 mμ (740 for erythritol) is quite apparent in anthrone-concentrated sulfuric acid, but when the acid concentration was reduced to 85% or less the absorption peak was either diminished or completely eliminated.

Several of the sugar alcohols, if allowed to react with H_2SO_4 in the absence of anthrone, yielded some color. However, when anthrone was added, the chromogenic response was enhanced in most cases, resulting in increased sensitivity. The characteristics of the colors produced in both cases are summarized in Table 1.

In the reaction of carbohydrates with the anthrone reagent, color production has been attributed to the formation of furfural or some derivative thereof. For the formation of a furfural derivative it has been postulated that there must be a possibility of dehydration in the 2,3 position (Sattler and Zerban, 1950). Although sorbitol has been classified as non-reactive, because of its failure to give the typical green color (Koehler, 1952), the strong absorption of the alcohols used here in the region of 500–520 mμ and peaks in the region of 720–740 mμ indicates that interaction does occur between the sugar alcohols and the anthrone reagent provided conditions are optimum. Negative results obtained by previous workers can perhaps be attributed to failure to obtain the classical green color or, alternately, to the treatment of the sugar alcohols under conditions that are optimum for the sugars and other carbohydrates. The drastic influence of acid concentration, heating time, and temperature of heating points to the possibility of negative results if less severe conditions of treatment were employed. The substance(s) responsible for color formation cannot yet be described unequivocally. Quite likely, some degradation product(s) might have contributed to the final picture of the absorption spectra. The strong absorption in the region of 500–520 mμ is somewhat comparable to the anthrone-uronic acid interaction, where absorption maximum occurs at 540–550 mμ.

ACKNOWLEDGMENTS

The author thanks Misses Carolyn Sue Kemper, Patricia A. Smith, and Yede Marie Baker for technical assistance. This work is an outgrowth of studies on "The Reactions and Determination of Food Gums" supported by grant EF-00173 from the National Institutes of Health.

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Spectrometry in Organic Analysis. Application to Small Samples

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(Manuscript received December 7, 1962)

SUMMARY

Gas chromatography offers the food chemist an unparalleled technique for separation and isolation of the components of complex volatile mixtures. Unfortunately, the problem of compound identification still remains. Often, the available quantity of an isolated pure compound may not exceed one or two milligrams. Conventional organic analytical techniques are seldom adequate for identification of such small samples. An empirical examination of infrared, ultraviolet, mass, and nuclear magnetic resonance (NMR) spectra permits the solution of a gratifying number of identification problems.

The application of mass spectrometry to the identification of organic compounds is discussed briefly. Two sets of spectra are translated into organic structures as examples of the methodology.

Gas chromatography has not been an unmixed blessing to the organic analytical chemist in industry. On the one hand, it is a fantastically effective tool for isolation; on the other hand, it affords practically no help in identification, and has the further characteristic of being most effective with small samples. Thus, the analytical journals, especially those concerned with food technology, contain numerous papers whose climax consists of the presentation of a gas chromatogram. Simple molecules such as acetone or acetaldehyde, whose presence can be predicted, may be "identified" by comparison of retention volumes. In most cases, however, identification of these compounds contributes little or nothing to the characterization of flavor. Having graphically demonstrated the complexity of his sample, and the probable presence of a few simple compounds, the investigator comes to an abrupt halt. What does he do with a milligram of a pure, unknown compound that he has been able to isolate from the effluent of the chromatograph? Certainly the classical training, valuable though it is for teaching organic chemistry *per se*, is inadequate for the task at hand.

In an earlier paper (Silverstein and Bassler, 1962), we pointed out the deficiencies of academic training in qualitative organic analysis, and we presented our experience in developing and teaching at San Jose State College and the University of San Francisco a one-unit course entitled "Spectrometric Identification of Organic Compounds." A book (Silverstein and Bassler, 1963) bearing the same title has evolved from the material gathered for this course and from work carried out in these laboratories.

The organic chemist in industry is no stranger to instrumental analysis, but relatively few chemists seem to have recognized the potential of a *combination* of four techniques: mass, infrared, nuclear magnetic resonance (NMR), and ultraviolet spectrometry. He is quite familiar with infrared and ultraviolet spectrometry, and generally has this rugged, simple, and inexpensive instrumentation available. He also recognizes the utility of nuclear magnetic resonance spectrometry, and is acquainted—at least through the literature—with empirical interpretation of NMR spectra. The mass spectrometer, however, has been largely ignored by the organic chemist concerned with compound identification; certainly the cost and complexity of a high-resolution instrument have been deterrents. And yet, as we shall show, it is undoubtedly the most powerful tool of the four we use.

In a large number of cases, a completely unknown compound can be identified from mass, infrared, and ultraviolet spectra obtained on a tenth of a milligram or less of

a sample. If a milligram sample is available, the unique data afforded by an NMR spectrum can be obtained. These data extend the range of identification manyfold. All of the sample may be recovered except the tenth of a milligram or less lost in the mass spectrometer.

We shall assume that the reader has adequate familiarity with infrared, ultraviolet, and NMR spectrometry, and we shall present only a brief discussion of the application of mass spectrometry to compound identification. A list is appended of general references to mass, infrared, ultraviolet, and NMR spectrometry. Two sets of spectra—a set consisting of the four kinds of spectra under discussion—will then be interpreted. Each spectrum of a set is examined for indications of specific structural characteristics. Confirmation is then sought in the other spectra. Since complementary information is available from each spectrum, a detailed analysis of a spectrum is seldom necessary. Even a modest level of sophistication in each of the four areas of spectrometry will permit solution of a gratifying number of identification problems. This methodology can obviously be extended from identification of rather simple compounds, about which little or no information is available, to elucidation of structural details of complex molecules about which quite a bit is known.

MASS SPECTROMETRY

A small sample of the compound (several milligrams to less than 0.1 milligram) is introduced into the inlet system of the mass spectrometer, where it volatilizes at very low pressure. A portion of the gaseous sample is metered into the ionizing chamber, where it is bombarded by a stream of electrons. The positive ion fragments resulting from the bombardment are separated according to their mass/charge ratio. The damage inflicted upon the molecules is recorded as a spectrum of the positive ion fragment masses and their relative abundance.

An electron beam of 9 to 15 ev will produce a molecular radical ion, so-called parent ion, by removal of a single electron from the molecule. Recognition of the parent ion is extremely important since its mass is the molecular weight of the compound. This is an exact molecular weight, not the approximate molecular weight obtained by procedures most frequently employed by the organic chemist. In the spectrum of a pure compound, the parent ion peak will usually be the peak with the highest m/e value, with the exception of peaks arising from parent ions containing heavier isotopes.

As the potential of the bombarding electron stream is increased beyond that necessary for producing a parent ion, additional ion fragments result. The most abundant peak in the spectrum is designated the "base" peak and is assigned a value of 100%. The abundances of all other peaks are expressed as percentages of the "base" peak.

In a separate table, headed "isotope abundances," the parent peak is assigned a value of 100% and the abundances of the isotope peaks are reported in percent relative to the parent peak. The isotope peaks are designated $P + 1$ (parent-plus-one), $P + 2$ (parent-plus-two), etc.

The isotope peaks result from parent ions containing isotopes heavier than the isotopes of lowest mass. For example, the $P + 1$ peak reflects the ability of the mass spectrometer to distinguish between a molecule containing only C^{12} atoms and one containing a C^{13} atom. The contribution of an S^{34} atom will be reflected in the abundance of the $P + 2$ peak.

In our discussion of the determination of structure we have limited ourselves to compounds containing C, H, O, N, S, Cl, and Br. Table 1 lists the principal isotopes of these elements and their abundances relative to the isotope of lowest mass.

An examination of the table will make it apparent that the $P + 1$ peak of a compound containing a single carbon atom should be about 1.1% of the parent peak. This value becomes about 2.2% when the compound contains 2 carbon atoms, etc. The $P + 2$ peak of a compound containing a single S atom should be about 4.4%, with a contribution of 0.78% to the $P + 1$ peak arising from the S^{33} isotope. The presence of a single chlorine or bromine atom will be evident from the large value of the $P + 2$ peak. The presence of additional atoms of chlorine or bromine will result in peaks at $P + 4$, $P + 6$, etc., depending on the number of chlorine or bromine atoms present.

Table 1. Principal stable isotopes and relative abundances.

Isotopes	Percent of isotope of lowest mass
C^{13}	1.1
H^{2}	0.015
O^{18}	0.2
N^{15}	0.37
S^{33}	0.78
S^{34}	4.4
Cl^{37}	32.5
Br^{81}	98.0

The first step in our procedure for determining the structure of a compound is to utilize the isotope abundance data to establish an empirical formula. Possible combinations of C, H, N, and O that could account for masses up through 250 have been tabulated by Beynon (1960). His table also lists the calculated $P + 1$ and $P + 2$ values for the various allowable combinations of these atoms. Since the Beynon table does not include S, Cl, or Br atoms, the weights of these atoms must be subtracted from the molecular weight before using the table to establish possible empirical formulas of the remaining portion of the molecule. The final selection of a single empirical formula, from several allowable formulas, may have to be made on the basis of data from the other spectra.

The "nitrogen rule" often allows us to reduce the number of possible empirical formulas. The "nitrogen rule" states that an odd-numbered molecular weight permits only an odd number of nitrogen atoms, and an even-numbered molecular weight permits only an even number of nitrogen atoms (including zero).

We are not always successful in establishing an empirical formula since the parent peak may be so small that the $P + 1$ and $P + 2$ values are undetectable. In this event, we must be satisfied with the molecular weight. In some cases, the parent peak may be absent; we then rely upon the fragmentation pattern and the other spectra to establish the molecular weight. If this approach fails, we may have to resort to the preparation of a derivative, or to some other method of obtaining the molecular weight.

The fragmentation pattern depicts the arrangement of atoms in the molecule. Some general rules have been formulated to aid in the prediction of prominent fragmentation peaks. These rules can be summarized briefly as follows.

1) Cleavage is favored at branched carbon atoms.

2) Aromatic compounds generally give a larger parent peak than do aliphatic compounds.

3) Double bonds favor allylic cleavage.

4) Saturated rings lose side chains at the α-carbon; special case of branching.

5) In alkyl-substituted aromatic compounds, cleavage is most probable at the bond beta to the ring.

6) A heteroatom will induce cleavage at the bond beta to it.

In addition, a fair amount of work has been published indicating the general fragmentation patterns to be expected for various classes of compounds. For example, primary alcohols exhibit a prominent peak (frequently the base peak) at m/e = 31 arising from the CH_2OH^+ fragment. The interpretation of fragmentation patterns is frequently complicated by the appearance of peaks due to rearrangements. These rearrangements usually involve cleavage accompanied by the transfer of hydrogen atoms.

Application of established rules, and reference to fragmentation patterns for compound classes, form the basis for interpretation of mass spectral data.

EXAMPLES

The first set of spectra appear as Fig. 1. The first step in the translation of the spectra into a molecular structure is to establish an empirical formula. The parent peak in the mass spectrum is 206; thus the molecular weight is 206. We list possibilities under that molecular weight. Consideration of the $P + 2$ peak brings us up short. Obviously, we are no longer dealing with compounds containing only C, H, O, and N. The $P + 2$ peak is too small for a chlorine or a bromine atom and too large for a sulfur atom. But it will accommodate two sulfur atoms very nicely.

We subtract the mass of two sulfur atoms from 206 and get 142, which is the weight of the rest of the molecule. We now compile the list of possibilities from Beynon's table under 142, using the $P + 1$ peak (and, of course, the fact that 142 is an even number) to narrow the possible empirical formulas. The $P + 1$ peak becomes 12.5 minus 2×0.78, which gives 10.9; this removes the contribution of the two S^{33} atoms.

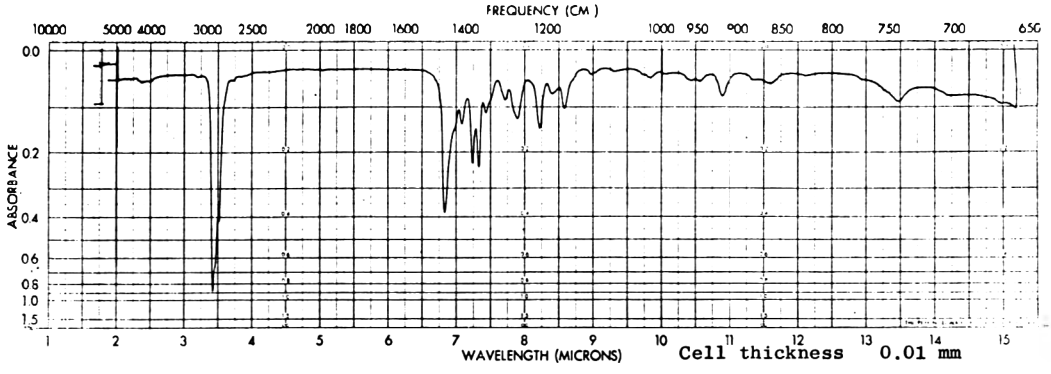
Formula	$P + 1$
$C_{10}H_{10}O$	10.94
$C_{10}H_{22}$	11.16
$C_{11}H_{10}$	12.05

The infrared and the NMR spectra convey a strong impression that we are dealing with an aliphatic compound. The ultraviolet spectrum is not especially informative. The infrared gives no evidence for the presence of an oxygen atom; in fact, it is rather featureless save for the strong aliphatic C—H stretching bands at 2915–2841 cm^{-1} (3.43–3.52 μ), the CH_2 and CH_3 bending vibration at 1464 cm^{-1} (6.83 μ), and the twin peaks at 1381 and 1364 cm^{-1} (7.24 and 7.33 μ), which we may often associate with chain branching.

Although $C_{11}H_{10}$ is a possible empirical formula, we can find no support for unsaturated character. We find no evidence for the presence of oxygen, so we write $C_{10}H_{22}S_2$ as our empirical formula.

The base peak, mass 43, in the mass spectrum allows us to write $CH_3CH_2CH_2$ or CH_3CHCH_3. We choose the latter for several reasons. A base peak is more likely to result from cleavage at a branch. The large doublet in the NMR spectrum

INFRARED SPECTRUM



MASS SPECTRAL DATA (RELATIVE INTENSITIES)

m/e	% of base peak	m/e	% of base peak
27	21.	70	6.
29	20.	71	61.
39	10.	72	4.
41	28.	79	5.
42	4.	87	4.
43	100.	101	5.
44	4.	102	4.
45	6.	103	3.
47	5.	136	11.
57	3.	206	25.90
59	4.	207	3.24
69	7.	208	2.48

Isotope Abundances

m/e	% of P
206 (P)	100.
207 (P+1)	12.5
208 (P+2)	9.6

ULTRAVIOLET DATA

λ EtOH max.	log ϵ max.
248	2.55

NMR SPECTRUM

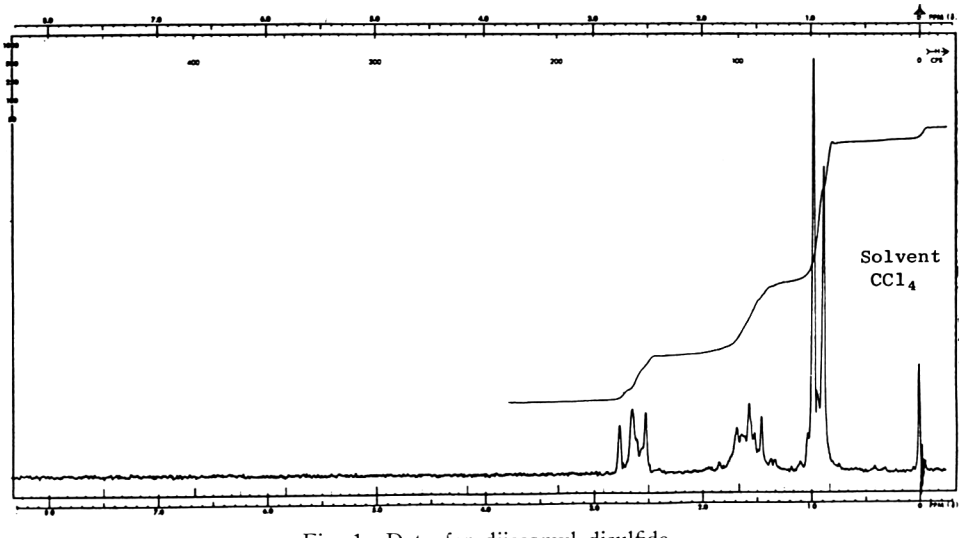
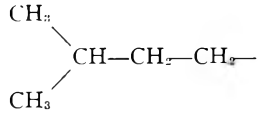


Fig. 1. Data for diisoamyl disulfide.

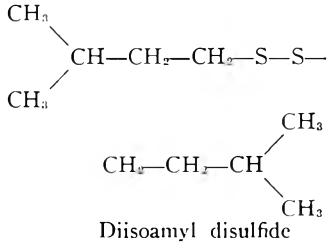
is familiar as the methyl protons of an isopropyl group. We also associated a pair of peaks in the infrared spectrum with chain branching.

We note a slightly distorted triplet in the NMR spectrum centered on δ 2.65, τ 7.35. This represents two protons (possibly CH_2) if we assign six protons to the large methyl doublet. A sulfur atom adjacent to the methylene group would account for its downfield shift.

If we examine the multiplet in the NMR spectrum centered at about δ 1.55, τ 8.45, we can pick out a triplet whose component peaks have the same spacing as those of the triplet further downfield. However, the integration shows that the multiplet contains three protons. It cannot be a methyl group because that would have produced a quartet rather than a triplet at the downfield position. It must then be another methylene and contain the CH group whose proton is responsible for producing the large doublet upfield. The extraneous peaks in and around the triplet at δ 1.55, τ 8.45, must then belong to the CH proton. We now have enough information to write



Since this is exactly one-half of the required weight of the alkyl portion, we may exercise a modicum of chemical sense and write the full structure



Diisoamyl disulfide

The fragmentation pattern, although complex, bears this structure out. The large peak at 71 represents cleavage α to the sulfur with retention of the charge on the alkyl fragment. The peak at 136 results from the same cleavage, with shift of a hydrogen atom to the sulfur-containing fragment, which retains the charge.

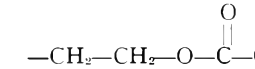
We have not rigorously proved that the two sulfur atoms are contiguous, although the ultraviolet spectrum supports the disulfide structure. But it would be difficult to write another structure to fit the spectra. We could carry out reductive cleavage and obtain conclusive spectral data on the resulting mercaptan.

Consideration of an additional set of spectra, Fig. 2, will serve to point up other aspects of the procedure. The parent ion peak (mass 131) of this compound was very small. The $P + 1$ and $P + 2$ peaks were too small for accurate measurement of intensity, and we cannot arrive at an empirical formula.

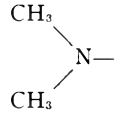
The molecular weight calls for an odd number of nitrogen atoms; let us begin with a single nitrogen atom. In the absence of evidence for primary or secondary amines, nitriles, amides, nitro compounds, or heteroaromatic compounds, we shall assume we may be dealing with a tertiary amine. There is no evidence for unsaturation on aromaticity in any of the spectra.

The infrared spectrum shows a strong carbonyl band at 1748 cm^{-1} (5.72 μ) and a typical broad strong C—O—C band at about 1235 cm^{-1} (8.10 μ). This combination is evidence for the presence of an acetate group. As supporting evidence there is a prominent mass 43 ($CH_3—C=O$) in the mass spectrum, and a singlet at δ 1.95, τ 8.05, in the NMR spectrum, which we may attribute to the CH_3 of the acetate group.

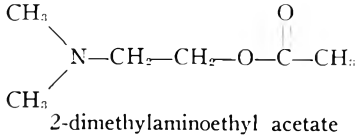
The NMR spectrum shows two triplets of equal areas with the same spacings. We are justified in writing $—CH_2—CH_2—$ and in placing the more deshielded methylene group on the oxygen of the acetate group; thus,



We have postulated the presence of a tertiary amine group. The molecular weight allows for C_2H_6N. The singlet at δ 2.20, τ 7.80, in the NMR, with double the area of the acetate CH_3 group, permits us to write

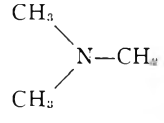


We can now write the complete structure

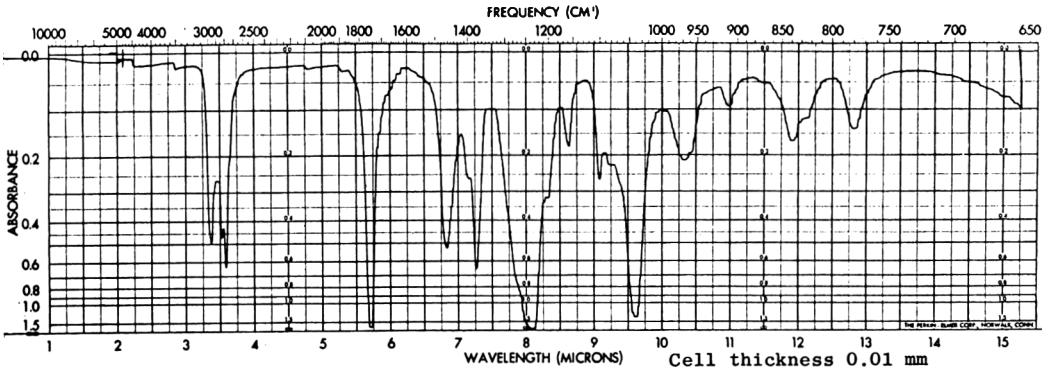


2-dimethylaminoethyl acetate

There are other possible lines of observation and reasoning we could have followed. The base peak, mass 58, is a characteristic amine fragmentation peak, which results from cleavage of the bond beta to the nitrogen atom. This, together with consideration of the other spectra, would have led us directly to the fragment



INFRARED SPECTRUM



MASS SPECTRAL DATA (RELATIVE INTENSITIES)

m/e	% of base peak	m/e	% of base peak
26	3.	55	3.
27	6.	56	7.
28	9.	57	3.
30	6.	58	100.
41	4.	59	4.
42	23.	71	11.
43	17.	131 (P)	0.346
44	7.		

Isotope Abundance

P+1) Too small
P+2) to measure

ULTRAVIOLET DATA

Transparent above 210 mμ

NMR SPECTRUM

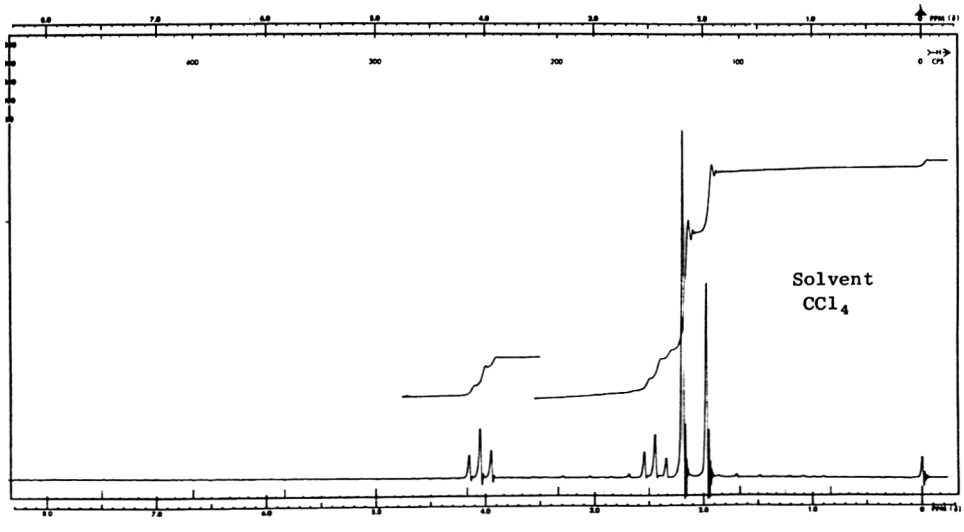


Fig. 2. Data for 2-dimethylaminoethyl acetate.

ACKNOWLEDGMENT

The authors are indebted to the Perkin-Elmer Corporation and to Stanford Research Institute for financial support, to Varian Associates for the NMR spectra, and to their colleagues at SRI for the other spectra and for numerous helpful discussions.

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After-Cooking Discoloration of Potatoes. Iron Content in Relation to Blackening Tendency of Tissue[a]

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(Manuscript received January 7, 1963)

SUMMARY

Data are presented on the iron contents of stem and bud end, whole and deproteinized, extracts of 41 samples of potatoes representing various degrees of discoloration. The stem end generally contained more iron than the bud end. Statistical analysis of all samples treated as one group revealed a highly significant correlation (1% level) between increasing iron content and increasing degree of after-cooking discoloration. Of the two types of iron studied, "free iron" and "protein iron," the protein iron gave the higher degree of correlation with blackening.

The highly significant correlation between iron values and degree of discoloration was lost in about half the cases when subgroups of the samples were formed according to location grown, crop year, and variety.

The data revealed that the percentage of total iron associated with the protein is higher in the stem end of the potato. Also, the stem-end protein contained considerably more iron than the bud-end protein. The difference in iron content between stem-end protein and bud-end protein showed a highly significant correlation (1% level) with tendency to blacken.

INTRODUCTION AND LITERATURE REVIEW

The after-cooking discoloration problem in potatoes, and its importance, have been reported (Yanovsky, 1955; Hunter *et al.*, 1957; Heisler *et al.*, 1962). It is now generally accepted that the discoloration is due to the formation of a dark-colored complex of ferric iron and an orthodihydric phenol, probably chlorogenic acid, since this is the major compound of this class in the potato (Juul, 1949; Kiermeier and Rickerl, 1955a). Many investigators have attempted to reduce or eliminate after-cooking blackening with metal-chelating agents (Hawkins *et al.*, 1959; Hunsader and Hanning, 1958; Greig and Smith, 1955, 1960; Smith and Davis, 1962). The iron-chlorogenic acid theory of blackening is supported by the success of some of those experiments. Published analytical data on iron content, however, are contradictory. Robison (1941), working with an acidic extract, found that iron content and after-cooking discoloration were correlated in tubers drawn from the same sample but not in tubers drawn from different samples. Muneta (1959) analyzed an aqueous extract of cooked potato, and found correlation between iron content and blackening. Other workers (Tottingham, 1939; Juul, 1949) found little or no correlation between these two factors. These apparent contradictions are explained by the presence of modifying factors such as pH, citric acid, and phosphoric acid. Hughes and Swain (1962b) studied the effect of citric, orthophosphoric, and malic acids, and of pH on the color of various phenol-iron complexes and concluded that citric acid was the most important of these factors in reducing the intensity of color of the chlorogenic acid–iron complex. They also correlated blackening with the ratio of citric acid to chlorogenic acid: the

[a] Presented at the 23rd annual meeting of the Institute of Food Technologists, Detroit, Michigan, May 26–29, 1963.

lower the citric acid content the more intense the blackening. This result is supported by potassium data of Heisler *et al.* (1962) in which a correlation was established between low potassium content and tendency to blacken, since it is reasonable to assume that potassium content would be directly proportional to citrate content.

Thus, it can be seen that the iron-chlorogenic acid theory is fairly well established. in an attempt to establish iron further as a prime factor in the blackening mechanism, we have determined the iron content of the stem and bud ends of a large number of samples representing a wide range of discoloration, and made a statistical study of the data. An important consideration in the following treatment of the data is the separation of the total iron into "free iron" and "protein iron."

METHODS

Potatoes. The 41 samples of potatoes used were sent to us as blackening samples from various parts of the country over a three-year period. Most were received from Wisconsin (19) and Michigan (15), with the remainder coming from Pennsylvania (3), Maine (3), and Long Island (1).

Experimental details for obtaining the sample and determining the degree of discoloration were as presented previously (Heisler *et al.*, 1962). For convenience, a brief outline is given here.

Potato sampling. Longitudinal plugs were taken from the stem- and bud-end sections of thoroughly washed and scrubbed potatoes with a no. 15 cork borer. The plugs were cut to a length of approximately ½ in. and then cut in half lengthwise. One half was used for the iron determination, and the other for measurement of degree of discoloration by a reflectance test. The half-cylinders used for the iron determination were adjusted in length so that 26 pieces totaled 100.00 g. Those used for the reflectance measurement were adjusted to total 65.00 g.

Determination of discoloration. Taken as the measure of discoloration was the reflectance obtained from a smooth surface (under glass) of cooked mashed potato. The reflectance attachment to the Beckman model B spectrophotometer (no endorsement implied) was used with $MgCO_3$ as standard. In this study, "degree of discoloration" was arbitrarily taken as $(R_B - R_S)/R_S$, where R_B is the reflectance of mash from the bud end, and R_S represents that for the stem end. The more discoloration in the potato, the greater the difference between R_B and R_S and the lower the value of R_S. Thus, these factors reinforce each other to amplify the value for degree of discoloration, making the system more sensitive in differentiating samples.

Preparation of extracts. The 100.00-g sample of potato tissue (26 half plugs from stem or bud end) was ground for 2 min in 300 ml of iron-free water in a Waring blender (no endorsement implied). The slurry was filtered through Whatman no. 12 paper, the filtrate collected, and the volume measured. Half of it was bottled and set aside. This is referred to herein as "whole extract." The other half was immersed 5 min in a boiling-water bath to coagulate the protein, and then filtered, while hot, through Whatman no. 12 paper. Loss of vapor during heating and filtering was kept to a minimum by stoppering the flask with a ground-glass stopper and by covering the funnel with a watch glass. The filtrate, containing about 1% solids, was labeled "deproteinized extract." The iron contents of both the whole and deproteinized extract were determined.

The resulting samples of whole and deproteinized extracts, though prepared by the same procedure, varied slightly in solids content because of differences inherent in the various lots of potatoes. The exact solids content of each extract was determined by loss of weight after drying. Iron was determined as the quantity present in the whole or deproteinized extract, but it can be related, percentagewise, to the fresh potato weight or to the solids content of the juice.

Although it is recognized that the above procedure is not an exhaustive extraction it was believed sufficient for the purpose of determining differences in the iron content of various samples and between the stem and bud ends of the same sample.

Iron determination. The method of Collins and Diehl (1959) for determining iron in urine was used. This method uses the very sensitive iron reagent, 4,7-diphenyl-1,10-phenanthroline (bathophenanthroline), a wet digestion with perchloric acid, and an extraction of the colored complex with nitrobenzene. As little as 2 ml of the 1% solids extract can be used. The conditions for the wet digestion had to be changed somewhat from those outlined by Collins and Diehl to accommodate the potato sample.

Two to 6 ml of the whole or deproteinized extract, the amount depending on the concentration of iron expected, was transferred to a 30-ml Kjeldahl flask. The volume was made up to 6 ml with iron-free water, and 1.25 ml concentrated HNO_3 and 0.50 ml of 70% perchloric acid were added. The mixture was digested at low

Table 1. Iron and protein values of potatoes and their relation to after-cooking discoloration.

Sample	Variety	$\frac{R_{11}-R_8}{R_8}$	Iron in micrograms/ml of extract [b]									d Iron assoc. with Protein (%)			e Protein (% in extract [b])			f Iron based on Protein (%)		
			a Total iron			b Free iron			c Protein iron											
			Stem	Bud	Stem-Bud	Stem	Bud	Stem-Bud	Stem	Bud	Stem-Bud	Stem	Bud	Stem-Bud	Stem	Bud	Stem-Bud	Stem	Bud	Stem-Bud
59–23	Wisc. Antigo	0.697	1.91	1.19	0.72	1.04	1.01	0.03	0.87	0.17	0.70	45.5	14.3	31.2	0.18	0.23	−0.05	.048	.007	.041
59–24	Wisc. Red Lasoda	0.695	2.83	1.01	1.82	1.15	0.71	0.44	1.68	0.30	1.38	59.4	30.7	28.7	0.15	0.18	−0.03	.112	.017	.085
59–19	Wisc. Katahdin	0.623	2.43	1.26	1.17	0.75	0.68	0.07	1.60	0.57	1.03	68.1	46.1	22.0	0.13	0.15	−0.02	.123	.041	.082
59–20	Wisc. Kennebec	0.572	2.84	1.09	1.75	1.01	0.56	0.45	1.83	0.53	1.30	64.4	49.1	15.3	0.21	0.24	−0.03	.087	.023	.064
59–25	Wisc. Red Lasoda	0.522	1.51	0.81	0.70	1.09	0.55	0.54	0.42	0.26	0.16	27.8	32.1	−4.3	0.15	0.18	−0.03	.028	.014	.014
59–15	Wisc. Early Gem	0.519	1.66	1.21	0.45	0.41	0.34	0.07	1.25	0.87	0.38	75.3	71.4	3.9	0.14	0.19	−0.05	.089	.046	.043
61– 1	Mich. Ontario	0.516	0.33	0.32	0.01	0.05	0.13	−0.08	0.28	0.19	0.09	84.8	60.0	24.8	0.13	0.11	0.02	.022	.017	.005
61– 3	Mich. Huron	0.503	1.27	0.51	0.76	0.80	0.34	0.46	0.47	0.17	0.30	37.1	33.1	4.0	0.13	0.15	−0.02	.036	.011	.025
59–22	Wisc. Antigo	0.497	1.58	1.12	0.46	0.84	0.86	−0.02	0.74	0.30	0.44	46.8	24.1	22.7	0.15	0.19	−0.04	.049	.014	.035
59–18	Wisc. Katahdin	0.475	3.03	1.49	1.54	1.28	0.84	0.44	1.71	0.63	1.08	57.2	42.9	14.3	0.22	0.22	0	.078	.031	.047
60– 1	Pa. Merrimack	0.469	1.10	0.66	0.44	0.19	0.17	0.02	0.91	0.48	0.43	81.8	69.2	12.6	0.19	0.23	−0.04	.047	.020	.027
60– 2	Pa. Merrimack	0.449	0.95	0.54	0.41	0.20	0.22	−0.02	0.75	0.32	0.43	78.6	59.2	19.4	0.22	0.28	−0.06	.034	.011	.023
61– 2	Mich. Ontario	0.434	0.47	0.39	0.08	0.12	0.21	−0.09	0.35	0.18	0.17	74.2	45.7	28.5	0.10	0.10	0	.035	.018	.017
60– 3	Pa. Merrimack	0.421	1.43	0.74	0.69	0.15	0.19	−0.04	1.28	0.55	0.73	89.7	74.3	15.4	0.25	0.28	−0.03	.051	.020	.031
60– 5	Wisc. Antigo	0.405	0.97	0.60	0.37	0.24	0.29	−0.05	0.73	0.31	0.42	75.5	52.0	23.5	0.12	0.12	0	.061	.025	.036
61– 5	Wisc. Kennebec	0.396	2.23	0.69	1.54	1.84	0.28	1.56	0.39	0.42	−0.03	17.6	60.0	−42.4	0.23	0.15	0.08	.017	.028	−.011
60– 4	Me. Katahdin	0.332	0.99	0.49	0.50	0.45	0.33	0.12	0.54	0.16	0.38	54.8	32.3	22.5	0.21	0.18	0.03	.027	.009	.018
60– 7	Wisc. Katahdin	0.332	0.72	0.59	0.13	0.38	0.27	0.11	0.34	0.32	0.02	47.0	54.3	−7.3	0.12	0.10	0.02	.027	.031	−.004
59–16	Wisc. Early Gem	0.322	1.41	0.73	0.68	0.52	0.23	0.29	0.89	0.50	0.39	63.1	68.5	−5.4	0.16	0.16	0	.056	.031	.025
59–13	Mich. Huron	0.306	1.19	1.04	0.15	0.76	0.56	0.20	0.43	0.48	−0.05	36.1	45.6	−9.5	0.20	0.24	-0.04	.022	.020	.002
61– 6	Wisc. Katahdin	0.269	1.08	0.49	0.59	0.83	0.24	0.59	0.24	0.25	−0.01	22.6	51.0	−28.4	0.06	0.04	0.02	.040	.063	−.023

Table 1. Iron and protein values of potatoes and their relation to after-cooking discoloration *(concluded)*.

Sample	Variety	$\frac{R_B-R_S}{R_S}$ [a]	Iron in micrograms/ml of extract [b]: a Total iron			b Free iron			c Protein iron			d Iron assoc. with Protein (%)			e Protein (% in extract [b])			f Iron based on Protein (%)		
			Stem	Bud	Stem-Bud	Stem	Bud	Stem-Bud	Stem	Bud	Stem-Bud	Stem	Bud	Stem-Bud	Stem	Bud	Stem-Bud	Stem	Bud	Stem-Bud
59–17	Wisc. Ontario	0.259	1.22	1.26	−0.04	0.61	0.72	−0.11	0.61	0.53	0.08	50.0	42.1	7.9	0.10	0.15	−0.05	.061	.035	.026
61– 7	Me. Kennebec	0.256	0.83	0.46	0.37	0.20	0.14	0.06	0.63	0.32	0.31	75.8	68.7	7.1	0.22	0.16	0.06	.028	.020	.008
60– 6	Wisc. Red Lasoda	0.239	1.13	0.42	0.71	0.50	0.25	0.25	0.63	0.17	0.46	56.0	41.4	14.6	0.15	0.09	0.06	.043	.019	.024
60–16	Mich. Huron	0.235	0.99	0.69	0.30	0.22	0.32	−0.10	0.77	0.37	0.40	77.9	53.4	24.5	0.20	0.19	0.01	.038	.020	.018
60–11	Mich. Ontario	0.204	0.75	0.62	0.13	0.09	0.22	−0.13	0.66	0.40	0.26	88.0	64.7	23.3	0.15	0.06	0.09	.045	.065	−.020
60–12	Mich. Ontario	0.183	0.67	0.78	−0.11	0.20	0.27	0.07	0.48	0.52	−0.04	70.8	66.1	4.7	0.10	0.08	0.02	.049	.063	−.014
61– 4	L. I. Katahdin	0.162	0.59	0.47	0.12	0.25	0.34	−0.09	0.34	0.13	0.21	58.2	26.8	31.4	0.14	0.15	−0.01	.025	.008	.017
59–14	Mich. Cherokee	0.150	1.10	1.00	0.10	0.53	0.56	−0.03	0.57	0.44	0.13	51.8	43.4	8.4	0.25	0.33	−0.08	.023	.013	.010
60– 9	Wisc. Kennebec	0.134	1.53	0.56	0.97	0.85	0.24	0.61	0.67	0.32	0.35	44.1	57.9	−13.8	0.16	0.11	−0.05	.043	.029	.014
60–10	Wisc. Ontario	0.128	0.89	0.80	0.09	0.33	0.35	−0.02	0.56	0.45	0.11	62.7	56.6	6.1	0.07	0.09	−0.02	.076	.048	.028
60–13	Mich. Ontario	0.124	0.90	0.68	0.22	0.23	0.29	−0.06	0.67	0.39	0.28	74.2	56.8	7.4	0.12	0.10	0.02	.057	.040	.017
60–19	Mich. Ontario	0.095	0.87	0.65	0.22	0.18	0.28	−0.10	0.69	0.37	0.32	79.8	56.7	23.1	0.11	0.09	0.02	.062	.040	.022
59–12	Mich. Manota	0.069	0.74	0.75	−0.01	0.30	0.49	−0.19	0.44	0.26	0.18	59.5	44.7	14.8	0.16	0.21	−0.05	.028	.012	.016
60– 8	Wisc. Early Gem	0.052	0.81	0.53	0.28	0.25	0.13	0.12	0.56	0.40	0.16	69.7	76.1	−6.6	0.06	0.11	−0.05	.088	.036	.052
61– 8	Me. Katahdin	0.052	0.77	0.52	0.25	0.26	0.19	0.07	0.51	0.33	0.18	69.3	63.3	6.0	0.05	0.05	0	.064	.041	.023
60–14	Mich. ?	0.015	0.77	0.44	0.33	0.38	0.26	0.12	0.39	0.18	0.21	50.7	41.8	8.9	0.13	0.10	0.03	.031	.018	.013
60–17	Mich. Cherokee	0.010	1.36	0.89	0.47	0.24	0.43	−0.19	1.13	0.47	0.66	82.6	52.2	30.4	0.20	0.22	−0.02	.056	.021	.035
60–18	Mich. Russet Rural	0	0.74	0.56	0.18	0.16	0.21	−0.05	0.58	0.36	0.22	78.5	63.5	15.0	0.11	0.16	−0.05	.054	.023	.031
59–21	Wisc. Kennebec	0	1.44	0.82	0.62	0.33	0.38	−0.05	1.11	0.43	0.68	77.1	52.4	24.7	0.20	0.24	−0.04	.056	.018	.038
60–15	Mich. Russet Rural	0	0.47	0.55	−0.08	0.17	0.24	−0.07	0.30	0.31	−0.01	64.7	55.9	8.8	0.10	0.09	0.01	.031	.034	−.003

[a] "Degree of discoloration" where R_B is reflectance of cooked bud tissue and R_S reflectance of stem tissue.
[b] Extract contains approximately 1% total solids.

heat until the appearance of white (perchloric) fumes; then it was heated for 10 more min and cooled. The sides of the flask were washed with iron-free water, then heat was applied (to boiling) to dissolve the precipitate and remove Cl_2. Contents of the flask were then transferred while still hot to a 125-ml separatory funnel, using about 8 ml of iron-free wash water. Two ml of 10% $NH_2OH \cdot HCl$ (aqueous solution, iron-free) and 5 ml of bathophenanthroline (0.001*M* in 50% ethanol, iron-free) were added. A piece of congo-red indicator paper was placed in the funnel, and NH_4OH (iron-free) was added dropwise until the paper turned red. Five ml of buffer (4*M* NaAc-4*M* HAc) and 4 ml nitrobenzene were pipetted into the funnel and shaken vigorously. The nitrobenzene layer (lower) was collected in a 10-ml volumetric flask. Extraction was continued with 2 more portions (2 ml each) of nitrobenzene, and volume was made to mark with absolute ethanol. The colored solution was transferred to a spectrophotometer tube and read at 538 mμ. Iron content was obtained from a standard curve made using 2–6 ml of a standard ferrous iron solution (1.00 μg/ml).

RESULTS AND DISCUSSION

Forty-one samples, representing a wide range of after-cooking discoloration, were analyzed for iron content. The stem- and bud-end samples of whole and deproteinized extracts were studied. Table 1 lists the samples in order of decreasing degree of discoloration, giving the iron content of the whole extract (total Fe) and the deproteinized extract (free Fe), and, by difference, the protein Fe. The stem end generally contains more iron than the bud end. For example, the stem end is higher in 37 of the 41 samples of whole extract, and in 36 of the samples when considering the protein iron. With the deproteinized extract, however, there is no clear-cut differentiation of stem and bud ends in iron content. Since the blackening occurs only at the stem end, the above facts indicate that the protein iron is more likely involved in the after-cooking blackening than the free iron.

Another interesting aspect of the data is that the range of values is greater for the stem end than for the bud end, i.e., there is more variation in the stem-end iron content between samples. This fact is illustrated in Table 2.

Since only the stem end blackens, the bud end of each sample can be considered a control and the iron value for the difference between the two should be related to the tendency to blacken. For this reason this value is included in Table 1.

Table 2. Range of iron values (μg/ml).

	Stem	Bud
Total Fe	0.33–3.03	0.32–1.49
Free Fe	0.05–1.84	0.13–1.01
Protein Fe	0.24–1.83	0.13–0.87

A statistical study was made of the iron data in Table 1 by the linear regression method, and an analysis of variance test determined that the relation of degree of discoloration to iron content was highly significant (1% level) in 7 of the 9 cases tested. Table 3 summarizes the results.

Table 3. Summary of statistical study.

	"F" values		
	Stem	Bud	Stem minus bud
Total Fe	20.5**	7.4**	17.8**
Free Fe	13.9**	10.5**	3.9
Protein Fe	9.6**	0.04	12.7**
Fe assoc. with protein	1.4	1.7	<1
Protein	3.0	1.6	2.0
Fe content of protein	2.6	2.3	7.7**

** Significant at 1% level.

Again, considering the fact that the stem end blackens and the bud end does not, correlation of tendency to blacken should be obtained with stem-end and stem-end minus bud-end iron values. This situation exists for the protein iron. It is not surprising, however, that this ideal relation is not obtained in every case, since, as stated in the introduction, other factors are involved in the blackening mechanism.

The significant correlation between iron values and degree of discoloration was lost in some cases when subgroups of the samples were formed according to location grown, crop year, and variety. Table 4 summarizes the results of this statistical analysis of the subgroups. This same situation was encountered when studying the potassium data (Heisler *et al.*, 1962) and raised

Table 4. Statistical analysis of subgroups.

Subgroups	Correlation of stem end total Fe values with discoloration	
	Not significant	Significant
Crop year		
1959		X
1960	X	
1961	X	
Location Grown		
Wisconsin		X
Michigan	X	
Variety		
Antigo		X[a]
Red LaSoda		X[a]
Early Gem		X[a]
Ontario	X	
Katahdin		X
Kennebec	X	
Huron		X[a]
Merrimac	X[a]	

[a] By observation (not enough samples to warrant a statistical analysis).

a question regarding the validity of the significance found between discoloration and iron content for the data considered as a whole. However, when the complexity of the blackening mechanism is considered, it is not surprising that significant correlation is not obtained in every case. Hughes and Swain (1962a) stated that the relative importance of any one factor probably varies even from potato to potato.

An interesting fact brought out by the data was the relatively large percentage of iron associated with the protein. Immediately the question arose: Is the iron precipitated as a part of the protein molecule or simply removed by occlusion along with the protein? Levitt and Todd (1952) reported that as much as one-third of the total iron was associated with the protein, and their data indicated that the iron was chemically bound to the protein. Dialysis experiments at our laboratory also point to a chemical combination of the iron and the protein. The percentage of total iron associated with the protein is listed in Table 1. It is readily seen that the percentage of iron associated with the protein is considerably higher in the stem end than in the bud end. This is true in 33 of the 41 samples. In an attempt to find correlation between degree of blackening and the percentage of iron associated with the protein, a linear regression was fitted to the data. An analysis of variance test, however, determined that this relationship was not significant (see Table 3).

Since such a large percentage of the iron is associated with the protein it was thought advisable to study the protein values. A rough estimation of the amount of protein present was obtained by taking the difference in the soluble solids content of the extract before and after precipitation of the protein. The protein values obtained in this manner are listed in Table 1. There was no significant difference in the protein contents of the stem and bud ends, and a statistical study of the data showed no significant correlation with blackening (see Table 3). Going a step farther, the percentage of iron based on protein was calculated (Table 1). These data demonstrate that the stem-end protein contains a considerably higher percentage of iron than the bud-end protein. This is true in 35 of the 41 samples. A statistical study of the data showed no significant correlation with tendency to blacken when considering the stem- or bud-end values. However, a highly significant correlation (1% level) was obtained with the stem-end minus bud-end difference values (see Table 3).

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Alteration of the Flavor of Processed Vegetables by Enzyme Preparations

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(Manuscript received November 31, 1962)

SUMMARY

The role of enzymes as agents for the restoration of fresh food flavor in processed foods is embodied in the "flavorese" concept of Hewitt *et al.* (1956). The experimental validity of this concept has been tested by adding a vegetable enzyme fraction to food prepared from the same or phylogenetically related vegetable. The flavor of the enzyme-treated processed food approached that of the fresh vegetable but was not identical with it. In general, addition of enzyme tended to over-emphasize certain notes of the natural flavor. Odor changes were more readily detected than changes in taste. Both the qualitative and quantitative aspects of the flavor changes were affected by the processing of the vegetables, by the method and source of enzyme preparation, and by the conditions under which the alterations in flavor occurred. These results are discussed in light of the enzymology underlying the flavor changes.

INTRODUCTION

According to Hewitt *et al.* (1956), heating steps in food processing cause loss of natural flavor *via* evaporation of volatile flavor components. These steps also destroy the enzymes (flavorese) of the food source, which normally convert non-volatile flavor precursors to volatile flavor substances. It is assumed that the flavor precursors survive processing. The flavors of a wide variety of heat-processed foods are reported to be improved and restored to those of the raw commodities by addition of appropriate enzyme preparations (Konigsbacher *et al.*, 1959). The enzyme should be prepared from the same commodity as the processed food or a related commodity.

Weurman (1961) found that the odor and gas-liquid chromatographic pattern of the volatiles of processed raspberry were altered by the addition of an "enzyme" preparation from raspberries. One or more of the characteristic peaks of the altered chromatogram also appeared by addition of enzymes from sources other than raspberries. However, this treatment with foreign enzymes did not restore full raspberry flavor. Hultin and Proctor (1962) described production of fresh banana aroma from heat-processed banana purée treated with a protein preparation from fresh bananas. They report that the rate of flavor production could be increased by addition of pyruvic acid, valine or oleic acid, which they termed "banana aroma precursors."

The present paper reports experiments to test some aspects of the "flavorese" concept as applied to processed vegetables. The experiments were designed to ascertain: whether the addition of enzyme preparations does indeed alter flavor; to what extent these developed flavors resemble those of the natural unprocessed vegetables; the effects of sources and methods of enzyme preparations upon the flavor alterations; the enzymological basis for the deviation of the altered flavor from that of the fresh vegetables; and the effect on flavor of the survival of enzyme but not of precursor.

MATERIALS AND METHODS

Sources of precursors. The following processed vegetables, employed as sources of precursors (substrates), were obtained at local markets: cabbage, blanched, dehydrated; carrots, blanched, dehydrated; peas, puréed, canned (baby food);

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[a] A laboratory of the Western Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

Reference to a company or product does not imply recommendation to the exclusion of others.

tomato juice, canned; pole string beans, canned; broccoli, blanched, frozen; onions, canned. The following vegetables, used as sources of precursors, were prepared in the laboratory: cabbage, unblanched, dehydrated; horseradish, steam blanched and dehydrated at 150°F; horseradish, unblanched, dehydrated; horseradish, unblanched, treated with sulfur dioxide, and dehydrated. Tomato substrate was prepared according to a procedure similar to that of Hewitt *et al.* (1960). Four hundred g of fresh tomatoes were heated 10 min in an autoclave without pressure, cooled, and then blended with 10 ml of water. The resulting slurry was filtered by suction on a Buchner funnel through 8 layers of cheesecloth. To the filtrate was added 100 mg of charcoal (Norite A) plus 2 g of Celite, and the slurry was refiltered to yield tomato substrate solution with very little odor and a slightly acid taste. Onion precursor was a homogenate of onions boiled by means of ultrahigh frequency radiation (Schwimmer and Weston, 1961). Sinigrin was a commercial sample (Light and Company, London) recrystallized twice from 90% ethanol to yield sinigrin hydrate (mp = 124–126°C).

Preparation of enzymes. Mustard enzyme was prepared by a previously published method (Schwimmer, 1961). Briefly, defatted mustard powder was extracted with water, and the fraction of the resulting extract precipitating between 30 and 70% acetone was dialyzed and lyophilized.

The onion enzyme and the enzyme from *Albizzia lophanta* were the same as used in previous studies (Schwimmer *et al.*, 1960; Schwimmer and Kjaer, 1960).

Cabbage mitochondrial fractions were prepared by blending 320 g of cabbage leaf with 125 ml of cold 0.5*M* sucrose for 2 min in a chilled blender operated at 80 V. The homogenate was centrifuged at 3000 × G for 5 min at 5°C. The supernatant was recentrifuged at 18,000 × G for 20 min. The residue from the recentrifugation was resuspended in 100 ml of cold 0.5*M* sucrose and recentrifuged to yield 5–10 ml of washed mitochondria. The supernatant and washings were dialyzed against cold distilled water for 48 hr and then lyophilized.

The enzyme preparations from string beans, peas, broccoli, carrots, and tomatoes were prepared according to the procedures of Hewitt *et al.* (1960). The procedure, except for peas, consisted of comminution of the fresh vegetable in a blender to yield extracts from which the protein fractions were precipitated with cold acetone. The precipitates, after washing with acetone and drying *in vacuo*, were used as sources of flavor-generating enzymes. Pea enzyme preparation was essentially the residue from successive treatments with cold acetone and *n*-butanol. Yields of the enzyme preparations are summarized in Table 1.

Table 1. Enzyme preparations for flavor regeneration.

Sources of enzyme	Yield per 100 g of source
Yellow mustard seed powder	2.0 g
Cabbage mitochondria	1.5–3.0 ml
Cabbage supernatant	0.23 g
Carrots	1.1 g
String beans	2.3 g
Peas	7 g
Broccoli	0.83 g
Tomatoes	0.24 g
Onion	0.20 g

Flavor regeneration. Small groups of 5–10 or more trained individuals with extensive experience in organoleptic appraisal of processed vegetables served as judges of flavor alterations. For each test, each individual separately judged three samples in the following order. The first sample consisted of processed vegetable to which water was added. The second sample was processed vegetable to which was added a water suspension of enzyme preparation. The third sample was a water suspension of the enzyme preparation. The first two samples each containing processed vegetable did not differ in appearance. In most cases duplicate tests were conducted each at a different time. The judges were asked if they could detect differences in flavor (odor and/or taste), whether the differences were distinct, and to describe the distinct differences. At the levels of enzyme used, the "enzyme-only" controls did not contribute to the flavors.

In some instances, a fourth sample consisting of processed vegetable plus suspension of enzyme previously heated in a water bath for 10 min was evaluated. In no instance did the regenerated flavor of this fourth sample differ from that of the first sample, that is, the precursor material without enzyme. Hence, the flavor changes were not associated with heat-stable substances present in the enzyme preparations.

Table 2 summarizes the amounts of material used and the conditions of the experiments. The results are summarized in Table 3.

RESULTS AND DISCUSSION

Cabbage. Several experiments were conducted in which dehydrated cabbage was incubated with enzymes from various sources. The greatest alteration in flavor was observed when blanched dehydrated

Table 2. Conditions for flavor regeneration.

Precursor			Enzyme							
Source	Process	Amount	Source	Amount (mg)	H_2O (ml)	Ratio[a]	Time (min)	Temp.	Flavor[b] change
Cabbage	Blanched & dehydrated	5 g	Mustard	10	35	90	10–15	Ambient	+++
Cabbage	Blanched & dehydrated	5 g	Cabbage mitochondria	400	35	2–4	30	Ambient	++
Cabbage	Blanched & dehydrated	5 g	Cabbage supernatant	40	35	3	30	Ambient	++
Cabbage	Blanched & dehydrated	5 g	Onion	10	35	10	2–10	Ambient	±
(Sinigrin)	.....	10 mg	Unblanched dehydrated cabbage	5000	25	.....	60	Ambient	+
Horse-radish	Blanched & dehydrated	5 g	Mustard	10	30	85	30–120	95°F	+
Horse-radish	Unblanched, dehydrated	5 g	Mustard	10	30	85	5–120	Ambient	−
Horse-radish	Dehydrated plus SO_2	5 g	Mustard	10	30	85	5–120	Ambient	+++
Broccoli	Blanched frozen	50 g	Broccoli	25	0	33	60–120	Ambient	±
Peas	Canned puréed	50 g	Peas	200	5	18	110	95°F	+++
Beans	Canned sliced	75 g	Beans	50	25	35	90	98°F	+++
Carrots	Blanched, dehydrated	5 g	Carrots	50	25	20	90	98°F	++
Tomatoes	Heated fresh		Tomatoes						±
Tomatoes	Canned juice	50 ml	Tomatoes	25	0	5	60–120	86°F	++
Tomatoes	Canned whole		Tomatoes						++
Onions	Boiled	50 g	Onions	10	0	10	30–60	Ambient	+

[a] Ratio: equivalent amount of fresh vegetable used as precursor divided by equivalent weight of fresh vegetable corresponding to amount of enzyme used.
[b] (+) represents a distinct alteration in flavor as judged by a majority of panel members. For more detailed description of changes see "Results."

cabbage was rehydrated with a solution of mustard enzyme. All panel members agreed that the flavor of the enzyme-rehydrated product was closer to that of fresh cabbage than was the flavor of the water-rehydrated control. The flavor of the enzyme-treated cabbage was described as: mustard, horse-radish, lachrymatory, or pungent.

The flavor of the enzyme-rehydrated product was undoubtedly dominated by *iso*thiocyanate derivatives produced as a result of the action of the myrosinase on sinigrin and other thiohydroximyl glucosides present in cabbage (Kjaer, 1958; Gmelin and Virtanen, 1961; Mackay and Hewitt, 1959). In this relatively simple system consisting of cabbage and mustard enzyme (myrosinase), the nature of the flavor could conceivably be influenced by the conditions of rehydration. Gmelin and Virtanen (1961) found that thiocyanate ion and 3-indolylmethyl alcohol, as well as *iso*thiocyanate derivatives, occur in cabbage. In the presence of ascorbic acid, H_2S and organic nitriles may form as the result of the action of the myrosinase (Schwimmer, 1960, 1961; Ettlinger *et al.*, 1961).

Mitochondria and mitochondrial supernatant fractions of fresh cabbage as enzyme sources also produced an *iso*thiocyanate-like

Table 3. Summary of description of enzyme-induced flavor changes.

Precursor		Enzyme	No. of judges[a]			Flavor difference between enzyme-treated and control sample
Source	Process	Source	A	B	C	
Cabbage	Blanched & dehydrated	Mustard	20	20	20	R[b]—mustard—horseradishy—lachrymatory
Cabbage	Blanched & dehydrated	Cabbage mitochondria	6	6	6	Most like fresh cabbage
Cabbage	Blanched & dehydrated	Cabbage supernatant	10	10	10	R—mustard—horseradish
Cabbage	Blanched & dehydrated	Onion	8	8	8	Pungent—oniony
(Sinigrin)	....	Unblanched dehydrated cabbage	7	7	7	R—mustard—horseradish
Horseradish	Blanched & dehydrated	Mustard	6	6	6	Slightly pungent
Horseradish	Unblanched dehydrated	Mustard	5	5	5	R—extremely pungent[c]
Horseradish	Dehydrated $+SO_2$	Mustard	7	7	7	R—extremely pungent—lachrymatory
Broccoli	Blanched frozen	Broccoli	5	3	1	R—sweeter—more pungent
Peas	Canned puréed	Peas	8	8	8	R—grassy—stronger
Beans	Canned sliced	Beans	6	5	4	R—grassy—peppery—oily
Carrots	Blanched dehydrated	Carrots	8	8	6	R—sweeter—caramelized
Tomatoes	Heated fresh	Tomatoes	7	6	3	Less like tomato—rancid
Tomatoes	Canned juice	Tomatoes	5	5	5	R—viney—acetone-alcohol
Tomatoes	Canned whole	Tomatoes	5	5	5	R—viney
Onions	Boiled	Onions	6	6	6	R—mild onions

[a] A is the number of participants; B, the number who detected a difference; and C, the number who detected a distinct difference between enzyme-treated and control vegetable samples.
[b] R signifies "more like raw vegetable" or "more natural."
[c] Extremely pungent with or without added enzyme.

flavor, according to all members of the panel. However, the mitochondria-treated dehydrated cabbage was judged to taste more like fresh cabbage than that treated with either mitochondrial supernatant or with mustard enzyme. When both mitochondria and mitochondrial supernatant were used together, a new flavor (described as boiled chestnut, nutty, more like cooked cabbage, or cabbage heart) was detected by all members of the panel. Since much of the flavor of cooked cabbage is due to dimethyl sulfide obtained by heating methyl methionine sulfonium ion (McRorie *et al.*, 1954), it is suggested that each of these cell fractions contained components of an enzyme system capable of decomposing this flavor precursor.

Addition of onion enzyme solution to blanched dehydrated cabbage also created a distinct flavor difference. A unique, slightly choking, lachrymatory sensation, reminiscent in odor of at least some aspect of freshly sliced onion, was noted. This effect completely disappeared after 20 min. This odor most probably arose from the action of onion enzyme on the (+)−*S*-methyl-L-cysteine sulfoxide present in the cabbage (Synge and Wood, 1956).

Unblanched dehydrated cabbage rehydrated with mustard enzyme smelled and tasted the same as that which was rehydrated with water. When sinigrin, the precursor to allyl *iso*thiocyanate, was added to unblanched dehydrated cabbage, the typical *iso*thiocyanate odor was readily detectable, and was not distinguishable from the odor obtained by adding mustard enzyme to blanched, dehydrated cabbage. Apparently the naturally occurring thiohydroximyl glucoside precursors (Kjaer, 1958; Gmelin and Virtanen, 1961) were acted upon by the enzyme during processing of the cabbage so that the flavor precursors and the resulting volatile flavor constituents, but not the enzyme, were lost during processing.

Horseradish. There was unanimity of opinion concerning the following results. On rehydration, blanched, dehydrated horseradish powder lacked completely the typical *iso*thiocyanate-like, lachrymatory character of freshly prepared horseradish. Its odor was characterized by the panel as "cooked turnips" or "crushed green leaves," and the taste as "bitter and leafy." Addition of mustard enzyme regenerated the typical horseradish flavor, but at a low level of intensity as compared with fresh horseradish, even several hours after rehydration.

Unblanched, dehydrated horseradish developed the typical odor and taste of the fresh vegetable within minutes of rehydration, whether or not enzyme was added. However, the rehydrated material was quite discolored, probably from the action of the endogenous polyphenol oxidase system.

Unblanched dehydrated horseradish pretreated with SO_2 did not develop an *iso*thiocyanate flavor in the first hour after rehydration. After two hours a slight pungency could be detected. Overnight the rehydrated product became distinctly pungent but not lachrymatory. This delay in development of flavor may be somewhat more complicated than a simple inhibition of the horseradish enzyme by SO_2 since Nagashima and Uchiyama (1959) report no inhibition of the corresponding mustard enzyme (myrosinase) by 0.01*M* $NaHSO_3$.

By contrast, addition of mustard enzyme to this same rehydrated product resulted in an extremely pungent odor within a minute of addition of the enzyme. Thus, the advantage of having an undiscolored product achieved by the addition of SO_2 is nullified by the loss of strength of the product. The addition of enzyme overcomes this disadvantage to yield a rehydrated horseradish that is high in quality from the standpoint of both color and flavor.

Broccoli. A majority of the panel members could detect taste and odor differences between broccoli before and after treatment with broccoli enzyme. Only 1 detected a distinct taste difference, and two a distinct odor difference. The difference was described as "more like raw broccoli," "grassy."

Peas. All of the members of the panel agreed that the enzyme-treated puréed canned peas tasted and smelled more like raw peas than did the canned peas alone. After 10 months' storage at 10°F, the pea enzyme preparation did not, when added to canned peas, yield as desirable a flavor as when fresh enzyme preparation was added.

String beans. Nearly all of the panel members detected a distinct difference in odor and flavor between enzyme-treated and control beans. The former were described as "raw," "grassy," and "hay-like."

When incubation of string bean enzyme with string beans was carried out *in vacuo* (in a Thunberg tube), the panel agreed that the resulting odor was distinctly weaker than that of a similar sample incubated in air. After introducing air into the evacuated Thunberg tube containing the enzyme-treated string beans, an odor equivalent in strength to that in the unevacuated tube developed within 1 hr.

Carrots. When carrots were rehydrated in the presence of carrot enzyme, all members of the panel detected a difference in odor as compared with odor of carrots rehydrated without enzyme. Most considered it a distinct difference. The odor was described as "more carrot-like," "sweeter," and "caramelized." Most members of the panel detected a difference in taste, but less than half considered the difference to be distinct.

Onions. When onion enzyme was added to autoclaved fresh onion, a distinct onion-like odor developed as judged by all members of the panel. However, a lachrymatory effect, typical of freshly comminuted onion, was not detected. The enzyme-induced change in odor of canned onions was reminiscent of the odor of sauerkraut. No effect of the enzyme was observed with bottled cocktail onions as substrate, even after the highly acid product was neutralized. Onion-like odor was produced from autoclaved onion to which was added a small amount of a preparation of the L-cysteine *C*-*S*-lyase of *Albizzia lophanta* (Schwimmer and Kjaer, 1960).

Tomatoes. Addition of tomato enzyme to canned whole tomatoes or tomato juice imparted a new odor and taste as judged by all panel members. This odor was described as "more like fresh tomato," "viney," "acetone-alcohol." However, when tomato enzyme was added to laboratory-prepared tomato substrate, a distinct alteration of taste was detected by only a few members of the panel, who described it as "less tomato-like" or "more rancid."

Table 3 summarizes the judgments relating to over-all flavor alteration of the processed vegetables.

The apparent improvement in quality of processed vegetables by the addition of a source of enzyme poses something of a paradox in view of the traditional concept that all enzymes must be destroyed to maintain quality and prolong shelf life in the processed product (Joslyn, 1949; Jansen and Balls, 1951). It is suggested that the relatively short-term action of the enzymes required to regenerate flavor results in compounds that differ qualitatively from those that arise when the enzymes act on substrates in the commodity itself over extended periods of storage. Thus, storage at 0° might alter the kinetics of production of flavor substances (*cf.* Lineweaver, 1939; Schwimmer *et al.*, 1955) to yield a variety of undesirable flavor-bearing products. Further differences in flavor might arise from the relatively volative nature of the desirable flavor components which would disappear during prolonged storage periods. This would result in preferential accumulation of less volatile components contributing to off-flavor.

When a preparation of pea, bean, or tomato enzyme that had been stored for long periods (six months to one year) at 10°F was added to the corresponding processed vegetable, the flavor produced was inferior and less desirable than that produced when the same enzyme preparation was fresh. Apparently the enzyme systems involved in desirable flavorable production are less stable than those which give rise to deleterious flavors. Hultin and Proctor (1962) observed enhanced flavor deterioration in banana purées after prolonged incubation with banana enzyme and precursors although after short incubation periods, the same conditions produced a desirable fresh banana aroma. In peas, these differences could also be ascribed to a difference in enzyme preparation. Lee and Wagenknecht (1958) found that the addition to blanched peas of water-soluble, partially purified preparations of lipoxidase, peroxidase, catalase, or lipase from peas resulted in the production of off-flavors following prolonged storage at low temperature. The pea enzyme preparations used by Hewitt *et al.* (1960) and by this author consisted of the water-insoluble fraction of peas.

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Factors Influencing Quality in Pork
A. Histological Observations[a, b]

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(Manuscript received November 24, 1962)

SUMMARY

Seventy-eight carcasses of pigs, 4–42 months old, provided loins of varied intramuscular fat content. Developed experimental methods indicated that the fixation time and the staining processes of the longissimus dorsi muscle were dependent upon its structure and chemical properties. With an increase in maximum muscle fiber diameter, there was a decrease in the taste-panel tenderness scores on the cooked longissimus dorsi muscles. There were no significant relations between tenderness measurements and total amount of connective tissue; however, the coarseness of the collagenic connective tissue strands was inversely related to the tenderness of the longissimus dorsi muscle.

INTRODUCTION

Brady (1937) found a low correlation between shear values and the diameter of muscle fibers in aged muscle. This was in agreement with Beard (1924), who concluded that the inherent properties of the endomysium contributed more to tenderness than size of the fiber. The occurrence of fat in the muscle fibers of beef was reported by Bell (1909, 1910, 1911). Collagen and elastin, which are the principal proteins of connective tissue, were contributors to differences in tenderness between beef cuts as reported by Mitchell *et al.* (1927, 1928). Hiner *et al.* (1955), Nottingham (1956), and Doty and Pierce (1961) indicated the collagen content was not significantly related to tenderness.

This experiment was conducted to study the possible relations between microscopic observations and ultimate consumer acceptability of the longissimus dorsi muscle of pork. The relations and interrelations of muscle fiber and bundle size, collagenic and elastic connective tissue coarseness, fat distribution, chronological age of the animal, and organoleptic properties of the muscle are discussed. This work is an attempt to elucidate and extend histological knowledge of porcine musculature.

EXPERIMENTAL METHODS

Longissimus dorsi muscle samples from 78 carcasses varying in age and intramuscular fat content were studied histologically. Photographic standards representing five different loin marbling scores were used in categorizing the samples, with the V score representing samples with abundant marbling. Five age groups were represented: 4–4½ months, 6–7 months, 9–11 months, and packer sows of 15–18 months and 36–42 months.

Samples of the longissimus dorsi muscle from the left loin opposite the 7th rib were removed 24 hours after slaughter and transferred immediately to each of three different fixatives, 10% formalin, 1.5% picric acid, and 1% osmic acid. The picric acid was considered superior to the formalin for fixation of muscle tissue for study of muscle fibers and collagen fibers because of a lower degree of distortion and shrinkage of the muscle tissue. The picric acid was a more rapid

[a] This study was supported in part by research contract 12-25-010-577, U.S. Department of Agriculture, Marketing Research Division, and Oscar Mayer and Company, Madison, Wisconsin.

[b] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

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Contribution No. 368.

fixative. However, the reaction time was quite critical and varied with the structure of the muscle. Muscle pH was found to be a satisfactory guide for determining fixation time. All samples with a pH of over 5.80 were left in the picric acid fixative for 8 hr, while those samples with a pH range below 5.80 were removed from the fixative after 6 hr. Longer fixation times and longer reaction times in the collagenic connective tissue stain were required for the samples from the groups of 15–18 and 36–42 months.

Osmic acid was used as the fixative for the samples in the microscopic study of the quantity of intramuscular adipose tissue. After fixation, all samples were dehydrated and placed in paraffin blocks for slicing with the rotary microtome into sections six microns thick. The longitudinal and cross sections from the samples fixed in formalin and picric acid were stained by the procedure of McFarlane (1944). These samples were used for the study of the connective tissue and muscle fiber diameter.

A calibrated diaphragm was used to measure the diameter (in microns) of five of the largest muscle fibers in each of five randomly selected microscopic fields. The mean of these 25 values was recorded as the maximum muscle fiber diameter. Since fibers overlap each other, this procedure provided data that possibly represented the maximum diameter of the fibers or the diameters of the largest fibers in the sample. The muscle bundle size was subjectively scored on the basis of 1 to 5 through the use of photomicrographic standards. The thickness of collagenic and elastic connective tissue strands and the total amount of adipose tissue were also subjectively scored on a 5-point scale, with 1 representing small fibers or small amounts of adipose tissue.

Right loins from the 78 carcasses were prepared for taste panel and subsequent objective evaluations of palatability characteristics, while the center section (9th through 13th rib) of the left loin was utilized for proximate analysis. Taste-panel scores of flavor, juiciness, and tenderness were based on a 6-point hedonic scale (1 being least tender) and were compared to the histological observations of the longissimus dorsi muscle. The Warner-Bratzler shear and denture tenderometer were utilized as reported by Carpenter (1961).

RESULTS

The means and standard deviations for maximum muscle fiber diameter are presented in Table 1. The fibers ranged in diameter from 30 to 86 μ, and, although the measurement of muscle-fiber size was rather subjective in nature, the standard deviations were small. It should be re-emphasized that the largest muscle fiber cross

Table 1. Means and standard deviations of maximum fiber diameter of the porcine longissimus dorsi muscle.

Marbling score[a]	No. of carcasses	Chronological age (months)				
		4–4½	6–7	9–11	15–18	36–42
I	13	30.0[b]	44.7	62.0	69.0	72.3
		(<0.1)[c]	(1.2)	( )	(0.8)	(1.5)
II	10	32.5	44.0	61.5	68.0	76.3
		(0.5)	( )	(2.5)	(1.0)	(1.3)
III	17	33.9	47.5	63.3	72.0	81.5
		(1.1)	(1.5)	(3.4)	( )	(1.1)
IV	25	36.0	42.3	64.6	70.0	84.0
		(2.5)	(0.8)	(3.1)	( )	(0.6)
V	13	37.5	46.5	64.8	70.0	86.0
		(1.1)	(2.5)	(2.3)	(1.0)	( )

Totals:

Age (months)	No. of carcasses	Means	Std. dev.
4–4½	25	34.9	(2.7)
6–7	12	44.6	(2.4)
9–11	15	63.8	(3.0)
15–18	9	69.4	(1.4)
36–42	17	79.4	(5.0)

[a] Number V marbling score represented samples containing abundant amounts of visible intramuscular fat.
[b] Maximum fiber diameter in microns.
[c] Standard deviations of maximum fiber diameter.

sections were selected for measurement; therefore, it must be kept in mind that these data were reported as maximum fiber diameters. Analysis of variance of these data showed that the older animals produced larger maximum muscle-fiber diameters. There also tended to be an increase in fiber diameter with an increase in intramuscular fat content.

Simple correlations were computed to measure the relation of muscle fiber diameter with the other histological observations and some measurements of palatability (Table 2).

Table 3 shows the frequency of microscopic detection of elastic connective tissue fibers in the longissimus dorsi muscle samples. Elastic tissue was not detected in any of the carcasses in the 4–4½-month or 6–7-month groups. Only 20.5% of the carcasses in the study yielded microscopic sections in which elastic connective tissue fibers could be differentiated. Table 4 gives the means for the collagenic tissue coarseness and the total amount of collagenic connective tissue. The older carcasses contained significantly (<0.05) thicker or coarser collagenic connective tissue strands. Statistically, there was no difference in the total amount of collagenic connective tissue among the five age groups, which agrees with the chemical analysis of connective tissue as reported by Kauffman *et al.* (1963) in another phase of this research.

Table 2. Simple correlations of maximum muscle fiber diameter with other pork quality measurements.

Fiber diameter	r
Thickness of connective tissue	.79**
Bundle size	.36**
Shear (raw)[a]	−.20
Shear (cooked)	.23*
Denture tenderometer (raw)[a]	−.38**
Denture tenderometer (cooked)	.45**
Tenderness (taste panel)[b]	−.48**
% fat (moisture-free basis)	.24
Age (days)	.84**
Juiciness (taste panel)	−.20

[a] Higher shear and denture tenderometer values indicate less tender samples.
[b] Based on 6-point hedonic scale with 6 representing most tender samples.
* $P < 0.05$.
** $P < 0.01$.
n = 78 pork carcasses.

Table 3. Frequency of detection of elastic connective tissue in porcine loin muscle.

Age (months)	No. of carcasses		% of total
4– 4½	(25)[a]	0[b]	0
6– 7	(12)	0	0
9–11	(15)	3	3.8
15–18	(9)	4	5.1
36–42	(17)	9	11.5
Total, over-all study	(78)	16	20.5

[a] Total carcasses studied in each age group.
[b] Number of carcasses in which elastic connective tissue was found in the longissimus dorsi muscle tissue.

Table 4. Means of total amount and coarseness of collagenic connective tissue in pork muscle.

Age (months)	Total amount	Coarseness
4– 4½	2.3[a]	1.4[a]
6– 7	2.3	2.7
9–11	2.2	3.1
15–18	2.1	3.3
36–42	2.2	4.0

[a] Subjective scores, ranging 1 to 5, with 1 representing small amounts or thin connective tissue strands.

Examination of similar data consisting of mean values of coarseness of collagenic connective tissue for the various marbling scores indicated that, with an increase in intramuscular fat, there was a significant decrease in the coarseness of the connective tissue. This was possibly due to separation of collagen fibers by the formation of fat cells, thus forming a looser network.

Table 5 presents simple correlation coefficients between certain pork quality characteristics and connective tissue scores, muscle bundle size and adipose tissue scores. Coarseness of the collagenic connective tissue and the chronological age of the animal were highly correlated. Significant negative correlations were also obtained between coarseness of collagenic connective tissue and the tenderness and flavor of the loin samples. No significant relations were observed between total amount of collagenic connective tissue and the palatability factors. Muscle bundle size, because of its relation with the amount of fat, was positively correlated with juiciness. With an increase in the subjective score for the amount of fat, there was a significant increase in juiciness, flavor, and tenderness.

Muscle bundle size increased with an increase in amount of microscopic fat and marbling score. Intrafiber fat was also observed by use of the osmic acid fixative. This intrafiber fat was not considered to be true adipose tissue, since cell walls could not be detected. The microscopic evaluation of the adipose tissue showed that with an increase in fatness, fat appears to infiltrate the connective tissue, thus establishing a partial explanation for the increase in tenderness associated with an increase in the marbling score (Carpenter, 1961).

Table 5. Simple correlations of histological observations with certain pork quality characteristics.

	Collagenic connective tissue		Muscle bundle size	Amount of fat
	Coarseness	Total amount		
	(r)	(r)	(r)	(r)
Total amount of collagenic tissue	—.12			
Muscle bundle size	.25*	.02		
Amount of fat	.03	.15	.67**	
Shear (raw)[a]	—.24*	.06	.09	.12
Shear (cooked)	.22	.01	—.23*	—.44**
Denture tenderometer (raw)[a]	—.45**	.04	—.16	—.16
Denture tenderometer (cooked)	—.30**	—.15	—.17	—.37**
Flavor (panel)[b]	—.31**	.12	.14	.38**
Tenderness (panel)[b]	—.38**	.05	.19	.42**
Juiciness (panel)[b]	—.04	.17	.39**	.48**
% fat (MFB)	.15	.09	.56**	.65**
Age (days)	.66**	—.05	.26*	—.08

[a] Higher shear and denture tenderometer values indicate less tender samples.
[b] Based on 6-point hedonic scale with 6 representing most desirable tenderness, flavor and juiciness characteristics.
* $P < 0.05$.
** $P < 0.01$.

DISCUSSION

No significant relations were found between tenderness measurements and the total amount of connective tissue, especially when the variation of intramuscular fat was held constant in the regression analyses. The coarseness of the collagenic connective tissue strands was related to tenderness. However, the relation was quite low when the age and percentage of fat were held constant. These results are in partial agreement with those of Mitchell *et al.* (1928), but in disagreement with their conclusion that elastic connective tissue content was not associated with age of the animal. The observations as to the total amount of collagenic connective tissue are in agreement with those of Batterman *et al.* (1952), Herschberger *et al.* (1951), Bourne (1956), and Wilson *et al.* (1954), but in disagreement with those of Miller and Kastelic (1956), Nottingham (1956), and Bate-Smith (1942).

Adipose tissue cells were found within connective tissue strands, between muscle bundles, and within muscle bundles. Also, fat droplets were observed within muscle fibers, regardless of the loin marbling score. This was in agreement with the observations of Bell (1909, 1910, 1911), but did not substantiate the work of Robertson and Baker (1933). It seems reasonable to assume that marbling, which has infiltrated the connective tissue, may aid in the ultimate alteration of the collagen during cookery. In addition, those fats present in the muscle fibers may serve to "lubricate" the fibers and fibrils and, in so doing, enhance the juiciness and tenderness of the cooked product.

The diameter of the muscle fibers increased with age and was positively associated with the thickness of the connective tissue and bundle size. The relation between percent fat and fiber diameter was positive but not statistically significant. These data are in agreement with those of Hammond (1932), Robertson and Baker (1933), Hiner *et al.* (1953), and Tuma *et al.* (1962) from work with ovine and bovine muscles. The correlation coefficient between fiber diameter and the raw shear and denture tenderometer values (Table 2) may be explained by the fact that the ½-inch cores, taken from the muscles of those carcasses that had smaller muscle fibers,

contained more fibers in the core. Therefore, more of the sarcolemma and endomysial connective tissue was present, resulting in a less tender product. Opposite results with the cooked samples may be explained either by the selective alteration and subsequent tendering of the connective tissue in the cooked samples or by a major decrease in tenderness due to denaturation of the fibrillar proteins.

Multiple correlation analyses indicated 44% of the variation in taste-panel tenderness score was accounted for by the combination of the histological observations (maximum fiber diameter, amount of fat, and thickness of connective tissue). Therefore, it is obvious that other characteristics of pork muscle, some of which must be chemical in nature, also contribute to the degree of pork tenderness.

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Effect of Excitement, Fasting, and Sucrose Feeding on Porcine Muscle Phosphorylase and Post-Mortem Glycolysis[a, b]

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(Manuscript received November 27, 1962)

SUMMARY

Phosphorylase activities were studied in relation to pre-slaughter treatment, post-mortem glycolysis, and ultimate characteristics of porcine longissimus dorsi. Total phosphorylase activity was not affected by pre-slaughter treatment and did not appear to be associated with post-mortem muscle glycolytic rate or ultimate muscle characteristics. The Hampshire muscles which had high muscle glycogen levels immediately post-mortem also possessed especially high levels of total phosphorylase; however, when all breeds were considered, the within breed correlations for these two factors were not significant (p 0.05). Insufficient knowledge exists on the relation of the time course of phosphorylase activation and glycolytic rate in post-mortem muscle. Nevertheless, extracts of porcine muscle at 10 min post-mortem generally showed the phosphorylase to be in the *b* form.

Short-term excitement and exercise immediately prior to slaughter caused a rapid post-mortem glycolysis, indicated by a rapid pH decline and decrease of color intensity in the muscle. This rapid glycolysis resulted in muscle with inferior water-binding properties and low color and texture scores. Long- and short-term sucrose feeding elevated the glycogen level of the muscle at slaughter, which ultimately resulted in muscle that was slightly soft and pale. Fasting 70 hr prior to slaughter lowered the initial glycogen content of the muscle and also slowed pH decline and color change during post-mortem glycolysis. Correlation between pH values and the ultimate color and water-binding properties of the muscle were significant soon after death, declining thereafter.

INTRODUCTION

Unless the glycogen content is essentially depleted at slaughter the rate of glycogen breakdown post-mortem has a greater influence on the properties of muscle than the total amount of glycogen present at the time of death (Briskey and Wismer-Pedersen, 1961). Sayre *et al.* (1963a) reported data on porcine muscle glycogen structure to support the hypothesis of Lawrie *et al.* (1959) that a decreased rate of anaerobic glycolysis, may be due, in part, to an alteration in the molecular structure of the muscle glycogen. Those workers (Sayre *et al.*, 1963a) also demonstrated that sucrose feeding tended to lengthen the external chains of the muscle glycogen stores. Briskey and Lawrie (1961) also reported that glycogen samples isolated from different muscles at pre-rigor and post-rigor periods were broken down at unequal rates by phosphorylase. Conversely, in a study of six muscles, Wismer-Pedersen (1959) implied that rate of glycolysis was very highly related to total phosphorylase activity. It has been shown that muscle glycogen stores can be influenced by ante-mortem sucrose feeding

[a] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

[b] This investigation was supported in part by a research grant EF-81 (C3) from the Department of Health, Education and Welfare, Public Health Service, National Institutes of Health, and by the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation.

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(Briskey *et al.*, 1960), fasting (Sayre *et al.*, 1961), exhaustive exercise (Briskey *et al.*, 1959), and excitement (Wismer-Pedersen, 1959).

An investigation was made of: a) the relation of phosphorylase activity to rate of anaerobic glycolysis; and b) the influence of long- and short-term sucrose feeding, fasting, and excitement on muscle glycogen stores, phosphorylase activity, rate of anaerobic glycolysis, and certain associated physical properties of chilled (24 hr) porcine muscle.

EXPERIMENTAL

Three pigs from each of the Hampshire, Chester White, and Poland China breeds were randomly assigned to each of four treatments, making nine replicates per treatment. Lot I was fed a 14% protein, 50% sucrose ration (Briskey *et al.*, 1960) for two weeks and was not fasted prior to slaughter. Lots II, III, and IV were fed a 14% protein basal ration with corn as the principal source of carbohydrate. Lot II was fasted 56 hr and subsequently fed a 50% sucrose ration for 12 hr prior to slaughter. Lot III was fed the same as Lot II but was subjected to 15 min of excitement and exercise immediately prior to slaughter. The excitement and exercise consisted of placing the animal on an animal exerciser (Finner *et al.*, 1958) and subjecting it to electric shocks and canvas "slappers." These conditions were employed in an attempt to simulate conditions often encountered as animals are driven to slaughter. Lot IV was fasted 70 hr prior to slaughter. As the animals were exsanguinated, samples were taken from the longissimus dorsi and processed or frozen in dry ice within 10 min of death. Additional samples of the longissimus dorsi were removed from the carcasses at various intervals during the post-mortem chilling period.

At slaughter the activities of phosphorylase *a* and total phosphorylase were determined on an extract of the longissimus dorsi by the method of Illingworth and Cori (1953) as modified by Krebs and Fischer (1955). pH values were obtained 0, 1, 3, 6, and 24 hr post-mortem by placing a glass electrode and calomel reference electrode directly on the freshly cut cross-sectional surface of the muscle fibers. Objective color measurements were made at 0, 3, and 24 hr with a Bausch and Lomb "Spectronic 20" spectrophotometer with reflectance attachment. The color reflectance values (485 mμ) are reported as percent reflectance based on a magnesium carbonate block as a standard for 100% reflectance.

Chilled longissimus dorsi and gluteus medius were given color scores according to the subjective rating of very light to dark on a four-point scale as described by Sayre *et al.* (1961). The chilled longissimus dorsi was also scored subjectively for texture on a four-point scale (1, very soft; 2, slightly soft; 3, normal; 4, firm and dry).

Water-binding capacity of the longissimus dorsi (24 hr), measured by the filter-paper absorption technique of Grau and Hamm (1953) as modified by Briskey *et al.* (1959), is expressed as the ratio of total area to meat area. Thus, a larger ratio indicates an increase in the "watery" condition of the muscle or a decrease in its water-binding capacity. The buffer capacity (pH 5.2–4.8) of 10 g of muscle contained in a 10% homogenate was determined at 3 and 24 hr by titration with standard acid. Buffer capacity values are expressed as 10^{-5} equivalents per pH unit per g of fresh tissue.

Samples obtained at 0, 1, and 24 hr were sealed in evacuated plastic bags and frozen in a dry ice–acetone bath for later determination of glycogen by the method of Dubois *et al.* (1956). Ground and mixed muscle samples were analyzed for total moisture by measuring the weight loss after drying for 24 hr at 100°C. Ether extract was determined as the weight loss resulting from 24 hr of continuous Soxhlet extraction with diethyl ether. Fat-free, dry tissue was determined by difference.

RESULTS

The experimental treatments had no effect on total phosphorylase activities of muscle extracts (Fig. 1) and total phosphorylase activity was not significantly associated with fresh muscle glycogen level within breeds, or with rate of pH decline, or other ultimate physical properties irrespective of breed. In general, the phosphorylase activity of the muscle extracts was in the inactive form at 10 min post-mortem. Further studies,

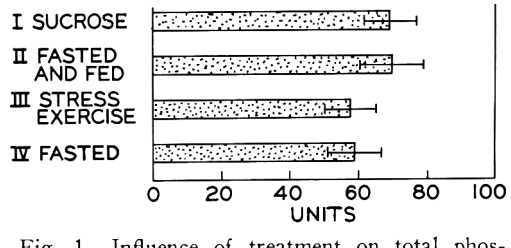

Fig. 1. Influence of treatment on total phosphorylase activity in the longissimus dorsi.

Explanation of Fig. 1. Units/ml of extract: One enzyme unit liberates 1.0 μmole of phosphate per 0.2 ml of reaction mixture in 10 min under the specified conditions of Illingworth and Cori (1953). |——| represents standard error of the mean.

however, are being conducted to determine the state of phosphorylase in porcine muscle at the time of exsanguination. The glycogen concentrations in the longissimus dorsi are illustrated in Fig. 2. Pigs fed sucrose (Lots I and II) had

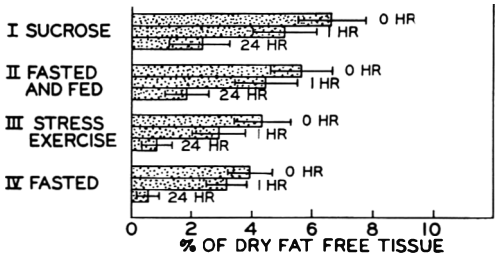

Fig. 2. Influence of treatment on glycogen level and rate of glycolysis in the longissimus dorsi.
|——| represents standard error of the mean.

higher 0 hr glycogen levels than fasted pigs (Lot IV). Sucrose feeding for 12 hr prior to slaughter resulted in muscle glycogen levels nearly equaling those found when sucrose was fed for two weeks. Lots II and III were treated identically except that Lot III was given excitement and exercise immediately prior to slaughter. Although non-significant at the 5% level of probability, the glycogen content of the longissimus dorsi tended to be lower in Lot III at the time of slaughter. The muscles from the pigs in Lot III also evidenced a faster and more extensive glycolysis.

Excitement and exercise caused the 0 hr pH to be significantly (p 0.05) lower in Lot III than in Lots I and IV, and also resulted in a pH value significantly (p 0.05) lower than in Lot IV at 1 hr (Fig. 3). Lot IV retained the highest

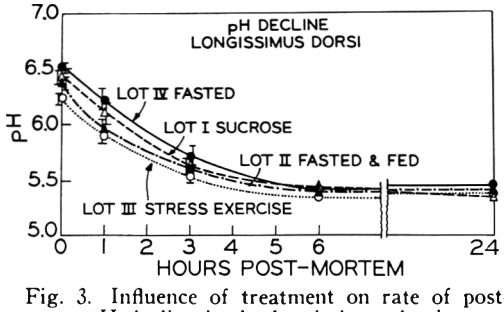

Fig. 3. Influence of treatment on rate of post-mortem pH decline in the longissimus dorsi.
|——| represents standard error of the mean

pH at 24 hr, and Lot I the lowest, reflecting the respective quantities of glycogen broken down.

Light reflectance from the muscle surface

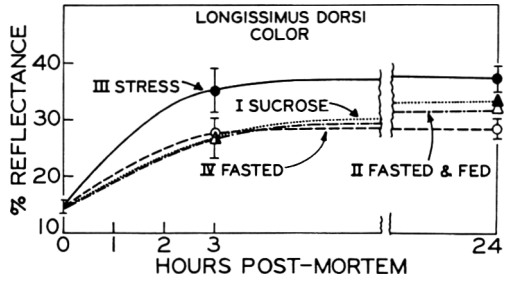

Fig. 4. Influence of treatment on the rate of post-mortem color change in the longissimus dorsi.
|——| represents standard error of the mean.

(Fig. 4) was uniform at 0 hr regardless of treatment, illustrating the general uniformity of muscle appearance at slaughter. At 3 hr, however, the longissimus dorsi muscle was significantly (p 0.05) lighter in Lot III (excited and exercised) than in the other three lots, which were still similar. After 24 hr, Lots I and II were intermediate between Lots III and IV, neither of which had changed appreciably after 3 hr.

Although some of the differences between means presented in Table 1 were not statistically significant (p 0.05), the water-binding capacity tended to be lowest in Lot III as evidenced by the higher ratio of total area to muscle film area but was similar in the other three lots. Fasting was effective in maintaining desirable texture and color in the longissimus dorsi and gluteus medius, whereas excitement and exercise caused a pale, soft muscle condition. The pigs from the two sugar-fed lots possessed muscles that were slightly pale and soft in final appearance. Buffer capacity was similar in all lots and exhibited no consistent change between 3 and 24 hr post-mortem.

Simple correlations, including all breeds and treatments, are shown in Table 2. Initial glycogen level in the muscle apparently was not associated with the initial rate of post-mortem glycogen breakdown but was significantly (p 0.01) correlated with the pH of the muscle at 3 and 24 hr. The subjective color score and the expressible-water ratio were closely associated with early pH values but exhibited a decreasing relation with later pH values, thus indicating the importance of rate of post-mortem glycolysis in establishing these characteristics.

DISCUSSION

A study of muscle phosphorylase was undertaken to determine if there was any association between this enzyme and the rate of post-mortem glycolysis. Phosphorylase, which catalyzes the first reaction in

Table 1. Effects of treatment on some post-mortem characteristics of the longissimus dorsi.

Characteristics		Treatment[a] Lot I 50% sucrose	Lot II Fasted and fed 50% sucrose	Lot III As II but excited and exercised	Lot IV Fasted
Expressible-water ratio[b]	$\bar{x}$	2.8	2.8	3.2	2.7
	$s_{\bar{x}}$	0.1	0.2	0.1	0.2
Texture score[c] (longissimus dorsi)	$\bar{x}$	2.4	2.3	1.8	2.7
	$s_{\bar{x}}$	0.2	0.2	0.3	0.2
Color score[d] (longissimus dorsi)	$\bar{x}$	2.4	2.6	2.1	2.9*
	$s_{\bar{x}}$	0.3	0.3	0.2	0.1
Color score[d] (gluteus medius)	$\bar{x}$	1.8	2.1	1.7	3.1*
	$s_{\bar{x}}$	0.3	0.3	0.2	0.3
Buffer[e] capacity 3 hr[f]	$\bar{x}$	4.50	4.75	4.50	3.25
	$s_{\bar{x}}$	0.25	0.50	0.75	0.25
Buffer[e] capacity 24 hr[f]	$\bar{x}$	4.50	5.00	4.25	4.75
	$s_{\bar{x}}$	0.25	0.25	0.25	0.25

[a] Nine pigs/treatment.
[b] Ratio of total area/muscle film.
[c] Expressed on a four-point scale (1, very soft; 4, firm and dry).
[d] Expressed on a four-point scale (1, very light; 4, dark).
[e] Expressed as 10^{-5} equivalents/pH/g of fresh tissue.
[f] Time post-mortem.
* Significantly different ($p < .05$) from the underlined observations.

Table 2. Correlations[a] between certain physical and chemical characteristics of the longissimus dorsi.

Physical or chemical characteristic	pH 0 hr[b]	pH 1 hr	pH 3 hr	pH 24 hr	Expressible-water ratio
pH 1 hr	0.69**				
pH 3 hr	0.29	0.40*			
pH 24 hr	0.14	0.29	0.43**		
Expressible-water ratio[c]	— .44**	— .57**	— .36*	— .11	
Longissimus dorsi color score[d]	0.57**	0.65**	0.38*	0.16	—.64**
Glycogen,[e] 0 hr	0.25	0.26	— .42**	— .43**	—.16

[a] Simple correlations over all treatments.
[b] Time post-mortem.
[c] Ratio of total area/muscle film area.
[d] Expressed on a four-point scale (1, very light; 4, dark).
[e] Expressed as percent of dry, fat-free tissue.
* $p < .05$, r .32.
** $p < .01$, r .41.

degradation of glycogen to lactic acid, was of interest because the conversion of inactive phosphorylase *b* to active phosphorylase *a* is stimulated by epinephrine (Sutherland, 1951). Epinephrine secretion, resulting from excitement (Hedrick *et al.*, 1957) or subcutaneous epinephrine injection (Sodal and Sarcione, 1959) has been shown to reduce muscle glycogen levels markedly.

The lack of phosphorylase *a* activity observed in muscle extracts is in agreement with the finding of Krebs and Fischer (1955) that extracts of resting muscle contain primarily phosphorylase *b*. The muscle

of animals receiving excitement and exercise treatment was presumably not resting muscle. However, no effect of treatment on occurrence of phosphorylase *a* was observed in muscle extracts 10 min post-mortem. Nevertheless, the animals receiving excitement and exercise possessed muscles with lower glycogen and pH at the time of death. These two criteria of muscle status would imply, if phosphorylase *a* were a rate limiting factor, that phosphorylase might be activated as a result of stress which would account for faster ante-mortem glycogen breakdown and lower initial post-mortem glycogen and pH. It seems pertinent, however, that these procedures, which were thoroughly substantiated by detecting expected amounts of phosphorylase *a* and *b* (Krebs and Fischer, 1955) in rabbit muscle revealed very little phosphorylase *a* in porcine muscle extracts 10 min post-mortem. It is entirely possible that pigs vary in sensitivity to the phosphorylase *b*—*a* conversion as was found in mice strains by Lyon and Porter (1963). Nevertheless, further work is being conducted to determine the phosphorylase state in living porcine muscle as well as at various post-mortem periods.

Cori (1945) pointed out that the AMP (adenosine 5′-monophosphate) concentration in the muscles of live animals is insufficient to allow appreciable conversion of glycogen to glucose-1-phosphate by catalysis of phosphorylase *b*. However, Stetten and Stetten (1958) postulated that conditions of severe muscle work or anaerobiosis may elevate AMP concentrations sufficiently to render phosphorylase *b* an active enzyme. Therefore, it is conceivable that phosphorylase *b* could function in glycogen breakdown under post-mortem anaerobic conditions. Sayre *et al.* (1963b) have shown that the muscle of a strain of Hampshire pigs contained approximately twice as much total phosphorylase and two to three times as much glycogen as the muscle of a strain of Poland China pigs. Regardless of the higher total phosphorylase activity and glycogen level of the Hampshire pigs, the Poland China muscle showed an especially faster rate of glycolysis immediately post-mortem. This is in disagreement with the report of Wismer-Pedersen (1959) who showed total phosphorylase activity to be faster in pigs which had a low pH 45 min post-mortem. It should be pointed out, however, that Wismer-Pedersen (1959) used glycogen disappearance in a muscle homogenate as a measure of phosphorylase activity. In the present study total phosphorylase activity was not implicated as a regulator of post-mortem glycolytic rate. Likewise, total activity was not altered by the nutritional variables studied or by short-term excitement and exercise. However, it is important to point out that our present knowledge of the time course of phosphorylase activation and that of glycolysis under different conditions is insufficient (Quillary and Mommaerts, 1962).

The results from long- and short-term sugar feeding were similar. The water-binding capacity, color, and texture of muscle at 24 hr were somewhat inferior for pigs receiving sugar than for pigs fasted 70 hr prior to slaughter. This agrees with results of Briskey *et al.* (1959).

Short-term excitement and exercise resulted in muscle with inferior water-binding, color and texture properties. Animals receiving this treatment were hot and respiring rapidly at slaughter. The brief strenuous exercise may have caused some glycogen breakdown and lactic acid accumulation in the muscle prior to slaughter. Ludvigsen (1957) postulated that the vasconstrictor effect of epinephrine prevents lactic acid produced in the muscle under stress conditions from entering the general circulation. The results of this experiment could be interpreted to correspond with this theory since the initial muscle pH value of the excited and exercised lot was considerably lower than observed in the other three lots. The pH of the excited and exercised lot remained lowest through the early stages of glycolysis, resulting in rapid color change and development of pale, soft, watery muscle. However, the rate of pH decline through the first 6 hr post-mortem as indicated by the slope of the curve (Fig. 3) was not greatly different from that for the other lots.

Since the initial pH values were not measured until approximately 10 min post-mortem, another interpretation of the re-

sults could be that a very rapid rate of glycolysis occurred for a short period immediately after death, resulting in the low pH at 10 min. This interpretation corresponds more closely with the conclusions of Wismer-Pedersen (1959) that fright and shock prior to slaughter are more detrimental to post-mortem muscle characteristics than mere exercise.

The similarity in buffer capacity in different lots indicates that buffer capacity was not a major factor influencing differences observed between treatments and are in agreement with the work of Bendall and Wismer-Pedersen (1962). Also, within-treatment correlations failed to show an association of buffer capacity with the ultimate characteristics of the muscle.

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Volatiles from Strawberries. I. Mass Spectral Identification of the More Volatile Components

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(Manuscript received April 5, 1963)

SUMMARY

An oil with a characteristic strawberry aroma was obtained from the condensate from the jam-making process. Programmed-temperature capillary gas-liquid chromatography (PTCGLC) showed the oil to be a very complex material of more than 150 components. A fast-scan mass spectrometer was used to analyze the material as it was eluted from the PTCGLC equipment, and some of the more volatile compounds identified are reported.

A systematic investigation of volatiles from various fruits has been in progress for some time at the Western Regional Research Laboratory, Albany, California. It became evident from early GLC work that intensive effort would have to be made to refine the methods. The preliminary efforts were toward developing methods of separation and analysis of very small quantities of the volatiles for complexity or purity (Buttery and Teranishi, 1961; Teranishi *et al.*, 1960, 1962). With the development of PTCGLC, it is now possible to analyze a small amount of an oil consisting of many constituents. With the great fractionation power of the capillary columns, fractions isolated can be analyzed for purity. With the application of infrared (IR), nuclear magnetic resonance (NMR), and mass spectrometry (MS), the assignment of molecular structure can be made with more certainty with smaller samples than with the relatively large amounts necessary when these physical methods are not available. With application of the fast-scan mass spectrometer to monitor directly the material fractionated by the capillary GLC (McFadden *et al.*, 1963), not only retention times but also molecular weights and fragmentation patterns can be obtained with submicrogram quantities. This capillary GLC-mass spectrometry (Cap-MS) method has proved to be very effective in the investigation of strawberry oil, and some of the results obtained are reported in this paper.

The volatiles from strawberries have been studied for several decades (Bidmead and Welti, 1960; Coppens and Hoejenbos, 1939; Corse and Dimick, 1958; Dimick and Makower, 1956; McGlumphy, 1951; Seidel *et al.*, 1958; Winter, 1958; Winter *et al.*, 1962). A large list of components has been compiled, and the complexity of the strawberry oil has been demonstrated (Teranishi *et al.*, 1960). Obtaining enough of this oil for separation and isolation of each constituent is a formidable task. Coupled with the difficulty of processing ton quantities of fresh strawberries is the fact that the fresh strawberry changes to a less desirable material in a matter of minutes (Winter, 1958). Elegant equipment for isolation of the volatiles from strawberries and raspberries with minimum deterioration of quality was reported by Winter *et al.* (1962).

To approach the study of the fresh strawberry aroma, GLC methods were developed so that the vapors from a single strawberry fruit could be analyzed directly (Teranishi and Buttery, 1962) without time-consuming extraction or concentration steps. This method of direct vapor analysis by GLC has been given the name "aromagram." Though this method is useful for following rapid changes, definite characterization of

the constituents requires some properties other than mere GLC retention times. To obtain such data, more than the nanogram or microgram quantities sufficient for the sensitive hydrogen flame ionization detectors must be isolated. Therefore, our composition work is on the more stable components. Work on the unstable fresh aroma constituents will be done when it is experimentally possible.

EXPERIMENTAL

Ten tons of condensate from strawberry jam pot stills was flash-heated with steam injection to remove about 10% of the solution (Brown *et al.*, 1951; Morgan and Carlson, 1960), and this evaporated material was fractionated at atmospheric pressure with a packed distillation column of about 10 theoretical plates. In this manner the volatiles were concentrated to 200 lb of an aqueous solution. This material was further concentrated by distillation through a 40-plate Oldershaw column (Dimick and Simon, 1952) to 25 lb of condensate high in alcohol content. A continuous liquid-liquid extraction with isopentane separated the neutral organic material from the aqueous-alcohol solution, and the isopentane was removed through a 10-plate Oldershaw column to minimize entrainment loss. No attempt was made to free the resulting oil completely of the isopentane to avoid further loss and heat damage. The yield from 10 tons of the original condensate was about 50 ml of the isopentane solution. This material had a strong strawberry aroma.

The chromatographic equipment necessary for the PTCGLC has been described by Teranishi *et al.* (1960). The combination of the capillary GLC with the fast-scan mass spectrometer has been reported by McFadden and Teranishi (1963) and McFadden *et al.* (1963).

In this technique, Cap-MS, one-half to one-quarter of the effluent from the capillary column enters the mass spectrometer without an intermediate detector. An auxiliary vacuum pump is used to control the amount of effluent entering and thus helps maintain a suitable vacuum level in the mass spectrometer. A component in the effluent is detected by observing the mass spectral output on an oscilloscope; and, in addition, the ionization occurring at m/e 15 from CH_3^+ (m/e is the mass to charge ratio) is used to obtain a concurrent chromatogram (Selke *et al.*, 1961). Although this is not a good quantitative method of obtaining a chromatographic record and is very insensitive for some compounds, it can be carried out without modification of the Bendix Time-of-Flight Mass Spectrometer, Model 12, used in this work. When a compound is eluted from the capillary column, the complete mass spectral pattern is recorded at a scan rate of m/e 24 to m/e 200 in 6 sec.

Because the exit pressure of the capillary GLC column is essentially zero, the inlet pressure must be reduced by about 15 lb. Prior to a complete run, this pressure is adjusted so that the "front" of the sample emerges at the same time as that obtained with the column connected to Sr-90 argon or hydrogen flame ionization detectors. In this fashion the retention times observed using the ionization at m/e 15 match those observed under normal conditions (Fig. 2).

Of the acetals studied, 1,1-diethoxymethane, 1,1-dimethoxyethane, and 1,1-diethoxyethane were commercially available materials. The larger and mixed acetals were synthesized in our laboratory. The esters used in this study were commercially available. All compounds were purified by preparative GLC and were checked for purity by capillary GLC.

RESULTS AND DISCUSSION

A PTCGLC chromatogram of the strawberry oil is shown in Fig. 1. Because of the complexity of the chromatogram, it has been divided into 4 zones—A, B, C, and D. Fig. 2 shows zone A compared with the recording of the intensity of the m/e 15 ionization obtained with the capillary column connected to the mass spectrometer. The GLC peak numbers are given above the Sr^{90} argon ionization responses, and the mass spectral chart numbers are given below the m/e 15 intensities. Although there are response differences between the two types of detectors, correlation between the GLC peaks and mass spectral charts can be obtained. Thus, the assignments of the molecular structures to materials represented by the GLC peaks can be made by interpretation of the MS fragmentation patterns and by comparisons of retention times with those of pure, known compounds.

During the Cap-MS analysis, 32 mass spectrograms were obtained in zone A. To illustrate the quality of these data, the type of information available, and the methods of interpretation, four of these are shown in Fig. 3.

Fig. 3 presents mass spectral charts 14, 15, 17, and 18. (No additional information was obtained from chart 16, since it was essentially background, so it is omitted.)

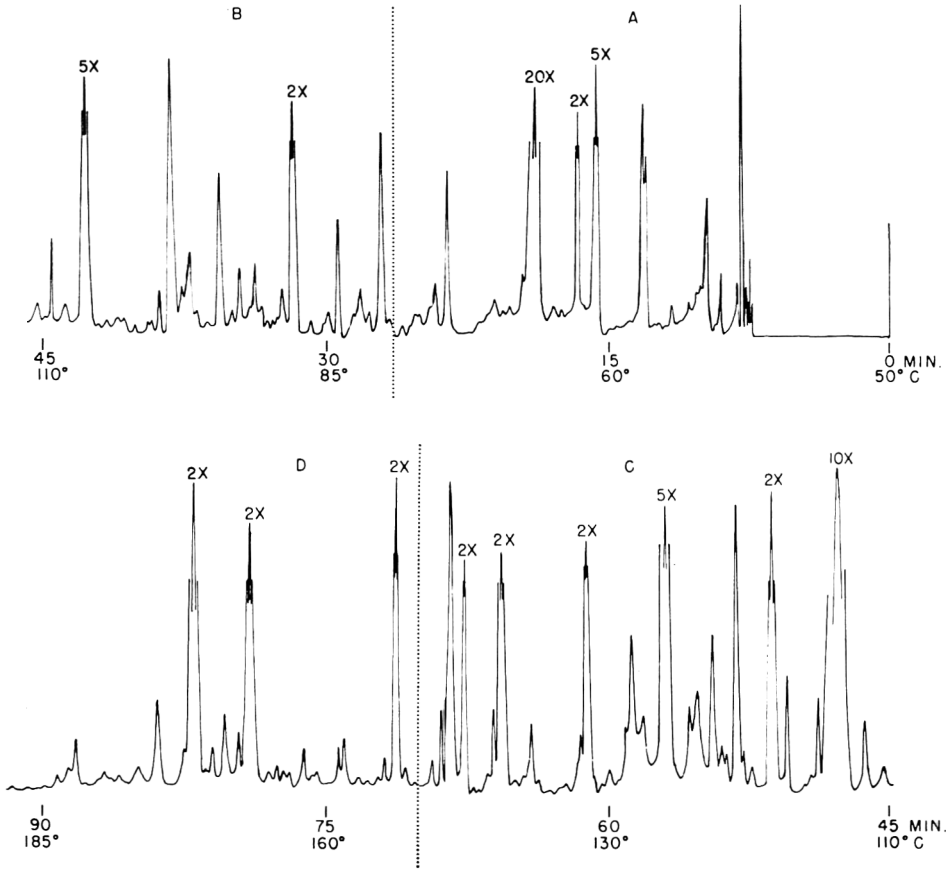

Fig. 1. Chromatogram of strawberry oil. Sr^{90} argon detector. Programmed-temperature capillary column, 200 ft, 0.01 inch I.D., coated with Tween-20.

Chart 17 was obtained for the most abundant species of the oil (MS attenuation, × 100) and serves to illustrate interpretation methods. Thus, the fragment peak at m/e 103 indicates the possible existence of two ether- or alcohol-type oxygens and five "saturated" carbons; the fragment peak at m/e 73 indicates the possibility of one such oxygen and four carbons (although a carbonyl oxygen is not ruled out *a priori*) and the m/e 45 peak indicates a possible OC_2H_5+ group. Such fragments suggest a possible acetal, and comparison of chart 17 with the mass spectrum of 1,1-diethoxyethane (Friedel and Sharkey, 1956) gave satisfactory agreement except at m/e 46 and 31. These latter suggested that ethanol also might be present. The presence of both was confirmed by GLC retention time, 1,1-diethoxyethane composing about 70% of the total material in this peak.

Chart 14 also showed (in different ratios from chart 17) ion peaks at m/e 103, 73, and 45, but in addition showed significant ion peaks at m/e 89, 61, and 59. The structures assigned to these peaks are shown in Fig. 3, and these indicate that the material is 1,1-methoxyethoxyethane, a mixed acetal. The mass spectrum of this compound had not been previously catalogued, but the data obtained from a sample synthesized in this laboratory gave complete agreement with the data of chart 14. Again, the retention time confirmed the chromatographic position.

In a similar fashion the other mass spectral patterns were interpreted. Chart 15 showed a base peak (defined as the most abundant ion peak in the spectrum) at m/e 43 and a rearrangement ion peak at m/e 61. These are characteristic of acetates, and the parent ion observed at m/e 88 indicates it

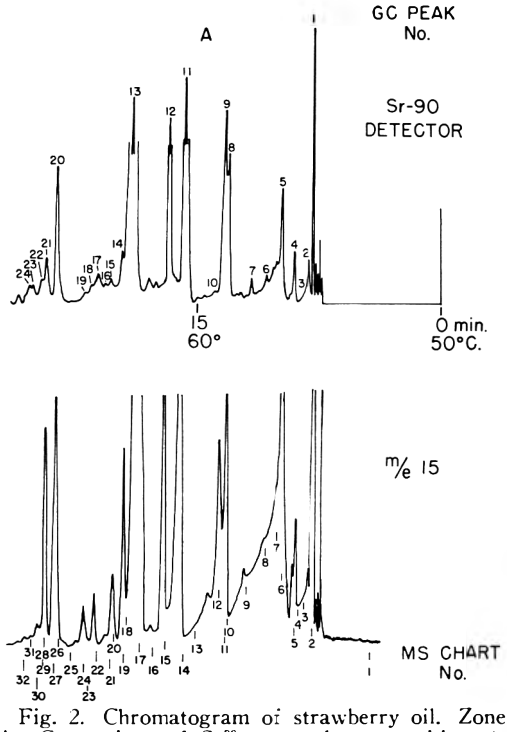

Fig. 2. Chromatogram of strawberry oil. Zone A. Comparison of Sr^{90} argon detector with m/e 15 responses.

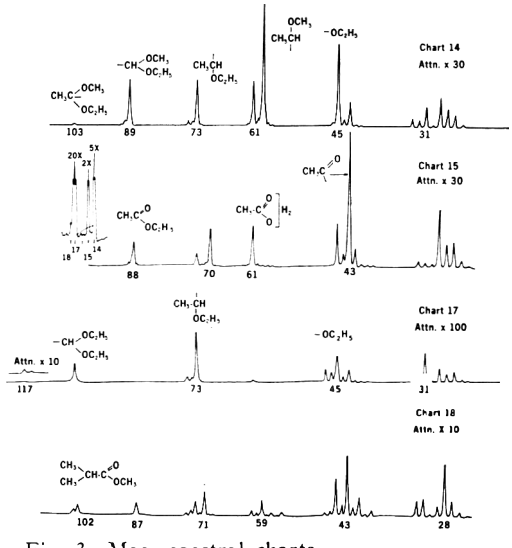

Fig. 3. Mass spectral charts.

to be ethyl acetate. This suggestion was confirmed by comparison with tabulated spectra and by GLC retention time.

The small chromatographic peak 14, observed at the base of the large 1,1-diethoxyethane peak 13 in Fig. 2, was identified from MS chart 18. It is noted that only a small residual of 1,1-diethoxyethane was recorded (attentuation of chart 18 is 1/10th that of chart 17). It is also noted that the separation of peak 14 from peak 13 is much better with the capillary column connected to the mass spectrometer. The mass peaks at m/e 102, 87, 71, 59, 43, and 41 were in the ratios corresponding to methyl isobutyrate, and the GLC retention time confirmed this identification.

The other mass spectral charts of zone A were similarly interpreted, and the results are summarized in Table 1. Both MS identification and GLC confirmation were obtained for all the compounds listed. The sources of the mass spectra used for comparisons are also given.

The compounds identified in zone A are obviously the more volatile components in strawberry oil. The hydrocarbons and diethyl ether are probably from the solvents used and are of little significance in aroma chemistry. It is of interest, however, to find methyl and ethyl acetate, ethyl propionate, methyl and ethyl isobutyrate, and methyl *n*-butyrate in this oil. It is of even greater interest that, with the Cap-MS method, 1,1-dimethoxymethane, 1,1-dimethoxyethane, 1,1-methoxyethoxyethane, and 1,1-diethoxyethane have been separated and identified. Although it is known that acetals have a wide range of odors (Moncrieff, 1951), not very many have been reported in the volatiles from natural products. It was therefore surprising to find 1,1-diethoxyethane as one of the most abundant components of our strawberry oil.

MS data of compounds in zones B, C, and D indicate the presence of higher-molecular-weight acetals, alcohols, aldehydes, and esters, and these compounds will be undoubtedly even more interesting as to their contributions to the aroma. Compounds such as benzaldehyde, ethyl benzoate, benzyl acetate, and methyl and ethyl cinnamates were readily identifiable. However, positive identification of others is understandably more difficult because of the many possible isomers in the branched aliphatic compound series. These molecular structure identifications and GLC peak assignments will be reported later.

Table 1. Compounds in zone A.

GLC peak number	Compound	Reference
1.	isopentane	Am. Petroleum Inst. MS Catalog
2.	methylpentane	Am. Petroleum Inst. MS Catalog
3.	diethyl ether	Am. Petroleum Inst. MS Catalog
4.	*n*-hexane	Am. Petroleum Inst. MS Catalog
5.	acetaldehyde	Am. Petroleum Inst. MS Catalog
6.	methylcyclopentane 2-methyl-1-pentene	Am. Petroleum Inst. MS Catalog
7.	cyclohexane	Am. Petroleum Inst. MS Catalog
8.	acetone	Am. Petroleum Inst. MS Catalog
9.	1,1-dimethoxymethane 1,1-dimethoxyethane	Friedel and Sharkey, 1956
10.	methyl acetate	Sharkey *et al.*, 1959
11.	1,1-methoxyethoxyethane	This work
12.	ethyl acetate	Sharkey *et al.*, 1959
13.	1,1-diethoxyethane	Friedel and Sharkey, 1956
14.	methyl isobutyrate	Sharkey *et al.*, 1959
15.	3-methyl-2-butanone	Sharkey *et al.*, 1956
16.	benzene	Am. Petroleum Inst. MS Catalog
17.	1,1-ethoxypropoxyethane	This work
18.	unknown (essentially background)	
19.	ethyl propionate	Sharkey *et al.*, 1956
20.	ethyl isobutyrate	This work
21.	methyl *n*-butyrate	This work
22.	unknown (acetal?)	
23.	unknown (trace of a sulfur compound)	
24.	unknown (acetal?)	

ACKNOWLEDGMENTS

The authors thank W. L. Stanley for suggestions and encouragement, and D. C. Patterson and R. A. Carlson for their help.

Reference to a company or product name does not imply approval or recommendation of the product by the U. S. Department of Agriculture to the exclusion of others that may be suitable.

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The Volatile Flavor Substances of Celery

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(Manuscript received March 13, 1963)

SUMMARY

The isolation and identification of the volatile compounds of celery are described. Non-linear temperature-programmed gas chromatography was found to give resolution superior to that of either isothermal operation or linear temperature programming. The identification of 24 compounds from celery is reported. Of the 38 compounds thus far identified from celery distillates, the following six compounds are primarily responsible for the characteristic flavor and aroma of celery: 3-isobutylidene-3a,4-dihydrophthalide; 3-isovalidene-3a,4-dihydrophthalide; 3-isobutylidene phthalide; 3-isovalidene phthalide; cis-3-hexen-l-yl pyruvate; and diacetyl. The phthalide derivatives are also implicated in the occurrence of certain celery off-flavors reported in the literature.

INTRODUCTION

The composition of the flavor of celery has not previously received intensive investigation, although celery is used in cooking and in processing primarily for its unique and characteristic flavor. Guenther (1950) listed what was known of the composition of the essential oil of celery seed. He cited sedanonic anhydride (3-butylidene-3a,4,5,6-tetrahydrophthalide) and sedanolide (3-butylidene 5,6,7,7a-tetrahydrophthalide) as the primary flavor constituents. The character of the flavor and aroma of the stem portion of the celery plant is different, however, from that of the seed. In a preliminary report, the present authors (Gold and Wilson, 1961) described collection of the volatile substances from celery. These volatile materials were divided, by differential cold-trapping, into relatively high-boiling and relatively low-boiling fractions, and the analysis of the acidic portion of the high-boiling fraction was discussed. A separate paper (Gold and Wilson, 1963) described the structural elucidation of four new phthalide derivatives from the neutral portion. The present paper describes the completion of the analysis of the high-boiling fraction and the analysis of the relatively low-boiling materials. The characteristic flavor of celery has been found to be due to six of the compounds identified.

EXPERIMENTAL

Celery juice was prepared and distilled in pilot-plant equipment, and the distillate fractions were taken to the laboratory for examination by chemical and physical means.

Preparation and distillation of juice. Details of preparation and distillation of the celery juice have been reported (Gold and Wilson, 1961). About 5,000 kg of fresh celery was used, yielding 4130 kg of juice, which was distilled in a modified continuous pilot-plant essence recovery unit (Morgan *et al.*, 1953). In this unit, the juice was flash vaporized under vacuum, the condensate given a second flash vaporization, and the liquid remaining after this second stage was fractionally distilled. Fractions were collected in the following portions of the apparatus: 1) column-bottom (material continuously withdrawn as it accumulated in the fractional distillation boiling pot); 2) chilled-water trap; 3) ice trap; 4) salt and ice trap; 5) dry-ice trap; 6) liquid nitrogen trap. All compounds found in traps 2, 3, and 4 were also found in either the column-bottom fraction or the dry-ice trap.

Gas chromatography. The analytical gas chromatographic equipment consisted of a Perkin-Elmer 154-D Vapor Fractometer combined with a Beckman Thermotrac temperature programmer.

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Mention of brand names is for identification only and does not imply recommendation by the U. S. Department of Agriculture.

The Perkin-Elmer exhaust was modified to come straight out of the bottom to prevent condensation of high-boiling materials in the line. The following columns were used: A) 0.01-inch by 150-foot capillary column, coated with polypropylene glycol; B) ⅛-inch by 16-foot column, containing 0.25% Silicone 200 on glass microbeads; C) 3/16-inch by 10-foot column containing 0.25% Silicone 200 on glass microbeads; D) ¼-inch by 16-foot column, containing 0.25% Silicone 200 on glass microbeads. Columns A, B, and C were used with a flame ionization detector, and Column D with a thermistor detector.

The preparative gas chromatographic equipment, constructed in the laboratory, consisted of eight ⅝-inch by 2-foot columns, connected in series and encased in a 4-inch-diameter galvanized sheet-metal tube. The tube was wrapped with asbestos-insulated heating wire, and placed inside a 6-inch-diameter tube. Air was circulated up through the column compartment and down through the outer concentric chamber, thereby reducing temperature gradients. Insulation was provided by 1-inch-thick pipe lagging, and temperature was controlled by a Wheelco model 292 controlling pyrometer. A four-filament diffusion-type detector was used, provided with a separate, independently heated compartment. The ⅝-inch by 16-foot column was filled with 25% silicone 200 on Gas-Chrom P.

Retention times were corrected for column hold-up before log retention-time plots were constructed. When the flame ionization detector was used, column hold-up time was estimated by the procedure of Gold (1962). The use of retention time to substantiate identifications was considered valid only when the retention time of the unknown matched with that of known material within the readability of the chart (0.03 min.).

Infrared measurements. Infrared spectra were taken with either a Perkin-Elmer 137 Infracord or a Beckman IR-7 Infrared Spectrophotometer.

Analysis of the liquid nitrogen trap fraction. The liquid nitrogen trap constituted a relatively simple system, comprising four compounds. These were separated by gas-liquid chromatography (GLC), using the preparative column at 110°C. Infrared spectra were taken on the collected compounds.

Analysis of the dry-ice trap fraction. Contents of the dry-ice trap consisted of 25 compounds, as determined by temperature-programmed GLC (column B). No acids, aldehydes, or phenolic compounds were found. Preliminary information was obtained by application of the log retention time plots of Evans *et al.* (1962). Information was obtained on alcohols and esters by the techniques of "subtractive" gas chromatography, as follows:

Alcohols. A 10-μl portion was treated with *p*-phenylazobenzoyl chloride (Woolfolk, *et al.*, 1955) to remove alcohols. Chromatograms of the alcohol-free mixture were compared with those of the original material (columns A and C).

The mixture of *p*-phenylazobenzoates was saponified (Van Etten, 1951), the reaction mixture acidified with hydrochloric acid, and the alcohols extracted with approximately 50 μl of carbon disulfide. The azo linkage of the reagent split during saponification, giving analine and *p*-amino benzoic acid. The use of hydrochloric acid as the acidifying agent caused the precipitation of these compounds as the hydrochlorides, and prevented their carry-over into the carbon disulfide. The carbon disulfide extract was examined by infrared spectrometry and by GLC.

Esters. A 10-μl portion was saponified by the microsaponification technique cited above. Chromatograms were compared with those of the original material, as described above.

The acids formed were removed with aq. sodium bicarbonate, and the bicarbonate solution acidified, extracted with 35 μl of carbon disulfide, and examined by GLC.

In order to reduce the complexity of the gas-chromatograms obtained from this fraction, thereby simplifying their interpretation and facilitating the collection of individual compounds, the remaining fraction was chromatographed on silica-gel before attempting fractionation by GLC. Maximum separation on the silica-gel column was achieved by grading the eluent polarity. The sequence of solvents used was: hexane-methylene chloride; methylene chloride; methylene chloride-acetonitrile; acetonitrile. The eluted fractions were separated by GLC on the preparative column, and infrared spectra taken on the individual compounds.

Analysis of the neutral fraction from the column-bottoms. Analysis of the acidic fractions of the column-bottoms has been reported (Gold and Wilson, 1961).

The neutral fraction, which was in ether solution, was concentrated *in vacuo* to 30 ml and treated with Girard-T reagent (Girard and Sandelusco, 1936; Hunter and Struck, 1962; Stanley *et al.*, 1961; Teitelbaum, 1958). Aldehydes were regenerated by the method of formaldehyde exchange. After separation of the aldehydes from the reaction mixture, ketones were recovered by acid regeneration. Compounds in each case were purified by GLC (column D). Fractions were collected directly in Connecticut Instrument Co. type "D" cavity cells, and infrared spectra taken.

The ketone- and aldehyde-free neutral fraction

was taken up in ether, concentrated to 30 ml, and chromatographed on silica-gel, as described for the dry-ice trap. The eluted fractions were submitted to GLC (column D), and infrared spectra were taken on the collected compounds.

Flavor evaluations. The contributions of individual fractions and compounds to the overall flavor of celery were evaluated by a group of five to seven members of the laboratory staff.

RESULTS AND DISCUSSION

When it was necessary to examine complicated mixtures directly by GLC, the technique of programming the temperature non-linearly was found to give resolution superior to that of isothermal operation or of linear temperature programming. This is demonstrated by a comparison of the isothermal, linear temperature-programmed and non-linear temperature-programmed chromatography of a mixture of normal alcohols (Fig. 1).

Compounds identified from celery are listed in Table 1. In most cases, identification is based upon comparison of both GLC retention times and infrared spectra with those of commercially available compounds.

Exceptions are as follows: Cis-3-hexen-1-yl pyruvate was identified by comparing its infrared spectrum with that of the synthetically prepared ester (Weisberger and Kibler, 1955), comparison of the infrared spectrum of the saponified mixture with that of a mixture of cis-3-hexen-1-ol and pyruvic acid, and by retention times of the parent compound and the saponification products. Ozonolysis, followed by reduction of the ozonide with Raney nickel, gave propionaldehyde, thus confirming that the double bond is in the 3 position.

The identifications of ethyl isovalerate, carvyl acetate, and neryl acetate were based upon the retention times of the parent compounds and of the saponification products.

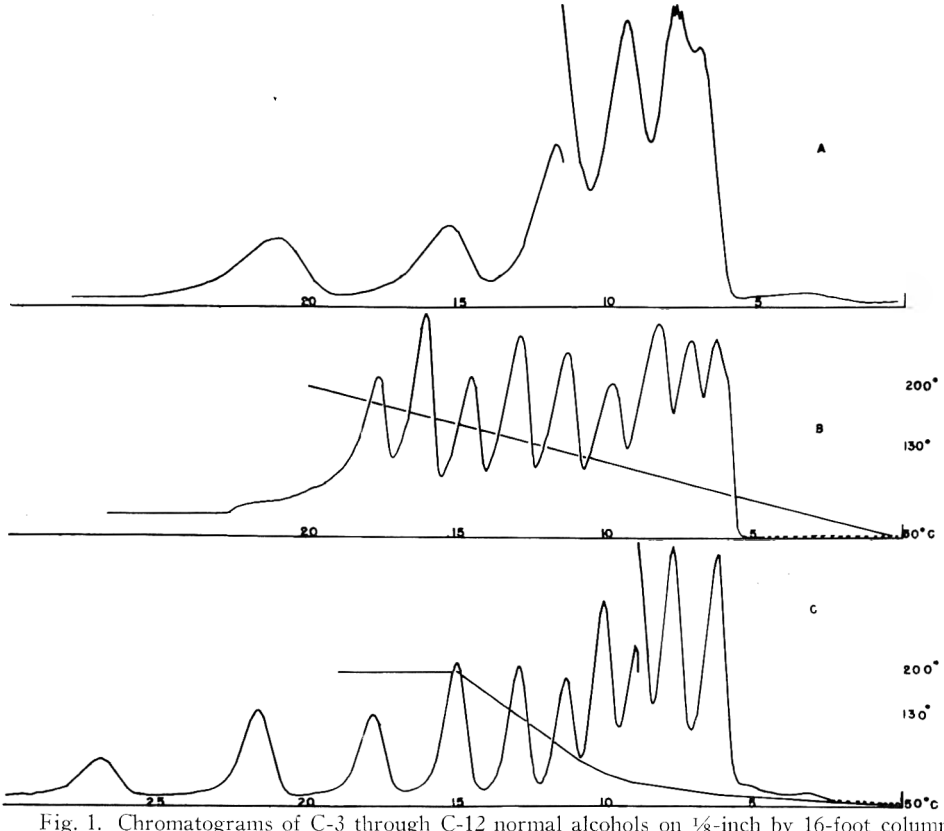

Fig. 1. Chromatograms of C-3 through C-12 normal alcohols on ⅛-inch by 16-foot column containing 0.25% silicone 200 on glass microbeads. A) isothermal operation at 150°C; B) temperature linearly programmed, according to the superimposed line; C) temperature non-linearly programmed, according to the superimposed curve.

Table 1. Volatile substances from celery.

Aldehydes	*Esters*	*Alcohols*
formaldehyde (6)	ethyl isovalerate (5)	iso-amyl alcohol (5)
acetaldehyde (6)	cis-3-hexen-1-yl pyruvate (5)	hexanol (2,3,5)
propionaldehyde (6)	decyl acetate (5)	heptanol (1,2,3,5)[a]
hexanal (1)	linalyl acetate (5)	
heptanal (1,4)	terpinyl acetate (5)	*Acids*
octanal (1,4)[a]	geranyl acetate (5)	n-valeric (1)[a]
undecanal (1)	citronellyl acetate (5)	iso-butyric (1)[a]
dodecanal (1,4)	neryl acetate (5)	pyruvic (1)[a]
neral (1)[a]	carvyl acetate (5)	
citronellal (1)	terpinyl propionate (4,5)	*Phenol*
	geranyl butyrate (4,5)	guiacol (1)[a]
	benzoyl benzoate (1)	
Hydrocarbons	*Phthalides*	
d-limonene (5)[a]	3-isobutylidene-3a,4-dihydrophthalide (1,2,3)[b]	
myrcene (5)[a]	3-isovalidene-3a,4-dihydrophthalide (1,2,3)[b]	
Ketones	3-isobutylidene phthalide (1,2,3)[b]	
carvone (5)	3-isovalidene phthalide (1,2,3)[b]	
diacetyl (6)	sedanonic anhydride (1)[a]	

(1) column-bottoms; (2) chilled-water trap; (3) ice trap; (4) salt-ice trap; (5) dry-ice trap; (6) liquid nitrogen trap: a) Gold and Wilson, 1961; b) Gold and Wilson, 1963.

In addition to the compounds listed in Table 1, valeraldehyde, nonanal, geranial, and the C-3, C-4, C-8, C-9, C-10, C-11, and C-12 normal alcohols were indicated by functional group determination and by GLC retention time. Sufficient quantities of these materials were not available for detailed study. An additional compound gave, upon saponification, acetic acid and an unidentified branched-chain alcohol. The alcohol retention time coincided with that predicted for 2-methylhexanol by a log retention-time plot for 2-methyl alkanols, and the ester retention time approximately coincided with that predicted for 2-methylhexanyl acetate by a log retention-time plot for acetates of the 2-methyl alkanols (predicted, 12.0 min; observed, 12.4 min).

Contribution of compounds to flavor and aroma. Many of the compounds listed in Table 1 are normally considered rather malodorous. Their odors, however, become less objectionable, and even pleasant, when dilution is sufficiently high (Gold and Wilson, 1961). While most of the compounds listed probably make some contribution to the over-all flavor and aroma of celery, six are of primary importance: the 3-isobutylidene-3a,4- and 3-isovalidene-3a,4-dihydrophthalides, the 3-isobutylidene and 3-isovalidene phthalides, cis-3-hexen-1-yl pyruvate, and diacetyl.

Early in this work, it was noted that the column-bottoms exhibited a strong characteristic celery odor. The principal odor constituents of this fraction were the four phthalide derivatives listed above. They were isolated in the ratio of 6:3:1:1. It has been found (Berlingozzi, 1927; Kariyone and Shimizu, 1953) that a celery-like odor is generally characteristic of synthetically prepared 3-alkylidene phthalides and hydrophthalides. The presently reported compounds exhibit a readily detectable celery aroma in concentrations as low as 0.1 ppm in water. Such solutions are characterized by a mild bitterness, and an occasionally noted burning sensation. When tasted in neat form, the dihydrophthalides exhibited the intense burning-numbing sensation described by Pan (1960), the isobutylidene compound being more intense than the isovalidene. Examination of the infrared spectrum presented by Pan for a burning-numbing fraction from celery, showed it to be similar to the spectrum presented by the present authors (Gold and Wilson 1963) for the isoalkylidene dihydrophthalides.

Notwithstanding the characteristic celery odor of the column-bottoms fraction, addi-

tion of this material to tomato juice did not reproduce the flavor of tomato-celery juice blends unless material from the dry-ice and liquid-nitrogen traps was included. As indicated above, the principal odor constituent of the dry-ice trap was cis-3-hexen-1-yl pyruvate, and that of the liquid-nitrogen trap was diacetyl. When 2 μl of the following mixture was added to 100 ml of tomato juice, a strong celery flavor and aroma were imparted: 3-isobutylidene-3a,4-dihydrophthalide (26 parts); 3-isovalidene-3a,4-dihydrophthalide (13 parts); cis-3-hexen-1-yl pyruvate (4 parts); diacetyl (1 part). The contribution of the aromatic phthalides could not be assessed, because of insufficient material.

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