were taken at 1.5 hours were found to contain about 0.2 to 0.25 p.p.m. of cycloheximide. This indicates that the fruit cannot hold more than about 1% of its weight in spray solution. From Figure 1 it may be noted that the amount of

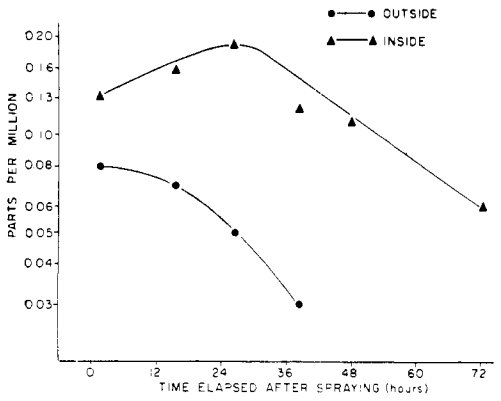

Figure 1. Persistence of cycloheximide spray residue on cherries sprayed with 30 p.p.m. solution

Average temperature 68° F.

antibiotic on the outside of the fruit which was removed by soaking in water decreased steadily, while the amount on the inside increased during the first 24 hours and then decreased. The relatively high proportion of the antibiotic found inside the fruit 1.5 hours after spraying may indicate a rapid absorption of the antibiotic during this period. Another possible explanation is that the antibiotic was absorbed by the fruit during the overnight soaking in water.

At the temperature of the experiment (61° to 77° F.) the half-life period of the cycloheximide residue was about 24 hours after the first day. This was estimated from the slope of the lines in Figure 1. As solutions of cycloheximide in dilute acetic acid (pH 3.3) have been found (*4*) to retain about two thirds of their activity after 3.5 months at 25° C., the rate of inactivation in the ripe cherries is much more rapid and may be due to the presence of an enzyme system in the fruit. The enzyme hypothesis receives further support from the fact that the antibiotic in canned cherries stored at room temperature has been found to lose about one fourth of its activity in 6 months (*12*).

Interfering Substances

Several products that are frequently applied to cherry trees for the control of other diseases were diluted with water to give the concentrations recommended by the manufacturer. The resulting suspensions were extracted with chloroform, the solvent was removed from the extracts, and the residues were bioassayed by the procedure used for the cycloheximide residues. Dieldrin, methoxychlor, lead arsenate, basic copper, and glyodin all failed to give inhibition zones on the *S. pastorianus* assay plates. The ferbam and captan both gave zones of inhibition and these substances would interfere with the assays for cycloheximide if they were present.

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TASTE PERCEPTION

Reliability of Phenylthiocarbamide–Sodium Benzoate Method of Determining Taste Classifications

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The phenylthiocarbamide–sodium benzoate method of determining taste classifications of individuals is unreliable, because most persons do not give reproducible taste reactions with at least one of the two chemical compounds involved. One of the principal factors affecting the reproducibility is the inability of some individuals to define standard tastes correctly. Therefore the problem of classifying individuals into groups according to their taste perceptions remains unsolved and is much more complex than might be inferred from methods proposed earlier.

In 1954 Fox (*3*) proposed a method for determining taste classifications of individuals based on interpretation of the flavor produced by two chemical compounds, phenylthiocarbamide (PTC) and sodium benzoate. The former is described by some people as intensely bitter, while others find it absolutely tasteless. This condition is known as "taste blindness" (*4*) and has been adequately shown to be caused by hereditary differences in individuals (*5, 7, 8*). Sodium benzoate, on the other hand, has been found either to be tasteless or to produce any of the four primary taste sensations—sweet, bitter, sour, or salty—depending upon the individual taster. This compound is utilized to divide each of the two classes obtained with phenylthiocarbamide (bitter or tasteless) into five separate subgroups. Thus, the

human race may be divided into ten classifications according to taste perception.

From the taste classifications of over 1000 individuals as determined by this method, Fox (*3*) calculated the percentage distribution of each of the groups throughout the human race.

If Fox's data were assumed to be valid, it would be possible to assemble a taste panel which, although composed of relatively small numbers, would be completely representative of the human race. Thus, consumer reactions to a given food product could be accurately predicted without the necessity of conducting long and complicated market surveys. As the food industry is a highly competitive field, consumer preferences are very important to the manufacturer. The phenylthiocarbamide–sodium benzoate method of taste classification seemed to present a simpler and more accurate means of studying consumer reactions and preferences.

Initially, the only work undertaken in this laboratory was to determine the taste classification of members of the sensory panel employed by this company. However, when the classification test was duplicated, many of the panel members reacted differently toward sodium benzoate from in the first test. The study described in this paper was therefore undertaken to determine the actual reproducibility of the test and the various factors affecting it.

Experimental

The experimental technique used in this study was essentially the same as that used by Fox in his original work (*2, 3*). Phenylthiocarbamide was prepared as a saturated solution in hot ethyl alcohol. Sodium benzoate was prepared as a hot saturated aqueous solution. Strips of Whatman No. 1 filter paper were saturated with the appropriate reagents and allowed to dry in air. The paper strips were then cut into small sections about $^3/_4$ inch long and $^1/_4$ inch wide. The papers were stored in covered glass containers prior to use.

In addition to phenylthiocarbamide and sodium benzoate, similar papers impregnated with sucrose, sodium chloride, citric acid, and quinine sulfate were prepared for the purpose of determining individuals' ability to define standard tastes. The sucrose, sodium chloride, and citric acid reagents were prepared as cold saturated aqueous solutions. The quinine sulfate solution used contained 10.0 mg. of alkaloid per milliliter of hot ethyl alcohol. (The quantity of this reagent per individual filter paper square was calculated to be not more than 1.5 mg.)

In making the taste reaction tests, each individual was asked to place one of the treated papers on his tongue. After allowing it to become thoroughly saturated with saliva, he was asked to indicate his conception of the flavor produced as being sweet, bitter, sour, salty, or tasteless. If more than one flavor was detected, the taster was asked to note the most predominant and record that as the flavor produced. All individuals being tested were asked to refrain from eating, drinking, or smoking immediately before the test.

Replicate tests with phenylthiocarbamide and sodium benzoate were made at irregular intervals over a period of about 8 weeks until a total of three phenylthiocarbamide tests and six sodium benzoate tests had been recorded for each member of the taste panel. In all cases only one type of treated paper was given to each person per day and efforts were made to stagger the tests, so that a particular chemical would not be repeated on successive days. In the case of the known taste standards, only single observations were made for each panel member.

The taste panel used in this study is regularly employed by the Wise Potato Chip Co. for evaluating its manufactured products. It is composed of ten male and ten female members who were chosen at random from regular company employees. The members meet in two groups of ten each twice a day and normally evaluate five to eight samples of potato chips and allied products. Coffee or milk is used as a mouth rinse by the panel. In the present paper when references are made to particular members of the panel, numbers 1 to 10 represent male panel members and numbers 11 to 20 represent female panel members.

Table I. Taste Multiples

Taste Multiple	Number of Persons: PTC (3 replications)	Number of Persons: Sodium benzoate (6 replications)
I	15	3
II	4	6
III	1	7
IV	..	4
V	..	0

Results and Discussion

When the results of the replicate tests with phenylthiocarbamide and sodium benzoate were combined and tabulated, it was found that only a very small number of the individuals tested reported sodium benzoate to give the same flavor sensation each time it was tested. As many as four different primary tastes were observed in a total of six replications of this compound.

Phenylthiocarbamide, while it also exhibited a tendency to produce varied results, was much more consistent than sodium benzoate.

In Table I the 20 individual taste panel members are arranged in groups according to the number of different taste sensations produced by phenylthiocarbamide and sodium benzoate. For convenience, the term "taste multiple" used in this table is defined as the number of different taste sensations observed.

Obviously, the most desirable condition would be for all persons tested to fall into taste multiple I, which would indicate that all gave reproducible taste ratings with both compounds. With phenylthiocarbamide about 75% of the individuals fell into this desirable class while the remaining 25% did not. With sodium benzoate, on the other hand, only 15% of the persons tested gave consistent taste ratings. The remaining 85% observed from two to four different sensations in successive tests with the compound.

Table II shows the variations from the normal reactions to phenylthiocarbamide observed by the individuals tested in the present work.

As noted in Table I, five persons from the 20-member sensory panel did not give consistent interpretations of the flavor produced by this compound. There is no definite explanation for these variations, as many factors may affect an individual's sense of taste. Certain of these factors are discussed later in this paper.

Table II. Replicate Taste Ratings with Phenylthiocarbamide

Individual Code No.	PTC, 3 Replications: T	SW	SR	B	SL
Normal reaction	...	...	...	3	...
Normal reaction	3	...	...	...	...
2	1	...	1	1	...
10	2	...	...	1	...
11	...	...	...	2	1
14	...	...	1	2	...
18	...	...	1	2	...

SW. Sweet. SR. Sour. B. Bitter.
SL. Salty. T. Tasteless.

Table III. Replicate Taste Ratings with Sodium Benzoate

Individual	Sodium Benzoate, 6 Replications				
	T	SW	SR	B	SL
	Male Panel Members				
1	1	1	...	1	3
2	...	...	6	...	...
3	...	1	1	1	3
4	...	2	1	...	3
5	1	4	...	...	1
6	...	...	2	1	3
7	...	4	1	...	1
8	...	5	...	1	...
9	1	2	...	2	1
10	...	...	5	...	1
	Female Panel Members				
11	...	1	...	2	3
12	...	5	...	1	...
13	...	2	...	...	4
14	...	6	...	...	...
15	1	1	...	1	3
16	...	6	...	...	...
17	...	2	...	1	3
18	...	3	...	2	1
19	...	3	...	...	3
20	...	5	...	1	...

SW. Sweet. SR. Sour. B. Bitter.
SL. Salty. T. Tasteless.

In an early publication Blakeslee and Fox (*1*) indicated that, while about 65% of the people tested found phenylthiocarbamide bitter and about 28% found it tasteless, a small group of persons (about 6% of the total) described phenylthiocarbamide as being something other than bitter or tasteless. This is also shown in these data. However, here the differences seem to occur as variations within individuals and not as entirely separate groups. The fact that no replications were made in the earlier work may have precluded the observation of the variables at that time.

Sodium benzoate was suggested by Fox (*3*) as a means of subdividing each of the two principal groups from the phenylthiocarbamide classification into five separate subgroups. This technique then resolved taste perception into ten statistically significant classifications into which the human race could be separated. However, in repeated determinations of certain individuals' reactions to sodium benzoate in this laboratory, consistent taste ratings were not always obtained from day to day.

In Table III the data obtained from six replicate determinations of each panel member's reaction to sodium benzoate are given.

Table III illustrates the extreme variability of individuals' reactions to this compound. Only one of the ten male panel members and only two of the female members gave consistent results. With the female panel members there were no sour ratings and only one tasteless rating for sodium benzoate. Furthermore, the compound was classified as predominantly sweet. This was in divergence to the data given for male panel members, where the variations were more or less evenly distributed throughout all five classifications.

Over the entire 20-member sensory panel, only three individuals gave consistent taste ratings for sodium benzoate through six replications (Nos. 2, 14, and 16). The remaining members varied from one to four exceptions in six replications. However, two of the three persons who gave consistent reactions to sodium benzoate were inconsistent in their reactions to phenylthiocarbamide; thus only one person in a total of 20 individuals had absolutely consistent results with both phenylthiocarbamide and sodium benzoate. Only this single individual could be accurately classified by Fox's method. Obviously, then, attempts to classify these individuals according to their taste perception by the method originally proposed were unsuccessful.

Attempts by the author and other workers in this laboratory and also by Jacob (*6*) to analyze the sodium benzoate data statistically were unsuccessful. It was shown to be impractical to attempt to assign mathematical values to an intangible system such as taste. The significance of the variations in individuals' taste reactions to sodium benzoate is, however, obvious.

If the data given in Tables II and III, are considered on a cumulative basis and a total of nine replications is assumed, the following becomes apparent:

One person (No. 16) gave consistent replications with both phenylthiocarbamide and sodium benzoate with no variations in flavor perception.

Four persons of the original 20 (Nos. 8, 12, 14, and 20) gave consistent replications with the two compounds with but one variation.

Six persons (Nos. 2, 3, 5, 7, 10, and 13) had only two variations in the nine replications.

The remaining nine persons ranged from three to five variations.

In Table IV the data of Tables II and III are considered on a single observation basis and are arranged as percentages of the total observations made with each compound.

Table IV. Distribution of Total Observations

I. PTC (Phenylthiocarbamide)		
Total observations	60	%
Total bitter	53	88.3
Total tasteless	3	5.0
Total sour	3	5.0
Total salty	1	1.7
Total sweet	0	0.0
II. Sodium Benzoate		
Total observations	120	
Total sweet	53	44.2
Total salty	33	27.5
Total sour	16	13.3
Total bitter	14	11.7
Total tasteless	4	3.3

The sodium benzoate data from this table were compared with similar data from Fox's original work (*3*) and are given in Table V.

Fox's data are based on single observations of 1062 individuals, while the data of this laboratory are based on six replicate tests of 20 individuals. When the subjects were treated in this manner, it was noted that the percentages of salty and sweet observations were nearly exactly reversed in the two sets of figures.

Table V. Sodium Benzoate Taste Ratings Comparison with Results of Fox

	%, Present Study		%, Results of Fox	
Total observations	120		1062	
Salty	27.5	71.7	40.2	66.6
Sweet	44.2		26.4	
Sour	13.3		7.8	
Bitter	11.7	28.3	17.5	33.4
Tasteless	3.3		8.1	
Total	100.0		100.0	

The three remaining classifications were in fairly close agreement. On a cumulative basis, groups composed of the two most predominant and the three lesser classifications were in very close agreement in the two summaries.

The number of subjects used in this study was very small in comparison with

Table VI. Taste Panel Reactions on Known Standards

Individual Code No.	Known Standards				
	Sweet	Salty	Sour	Bitter	Tasteless
Normal reaction	SW	SL	SR	B	T
2	...	SR	...	...	...
3	...	...	B	...	...
5	...	B	...	...	...
7	...	...	B	SR	...
11	...	...	B	...	...
14	...	...	...	T	...
16	...	SW	...	...	...
19	...	...	SL	...	...

that of Fox. However, the results seem to be strikingly significant.

Fox (*3*) compared the likes and dislikes of individuals for various types of foods with the taste classifications of those individuals. He found that, in nearly all cases, the largest group of persons liking a certain type of food were members of one particular taste group, while the greatest number of persons disliking the same type of food were members of another taste group. This constituted a very striking finding and presented a logical explanation of the varying food preferences of individuals. However, the present work seemed to indicate that the taste classification technique is unreliable and that wide variations in a person's reactions to sodium benzoate or phenylthiocarbamide may be observed. If the method is as unreliable as these data seem to indicate, it would be assumed that the apparent relationship between food preferences and taste classification as shown by Fox could not exist. Yet the relationships have been adequately shown to be statistically significant. Fox made only single determinations of individuals' taste reactions to phenylthiocarbamide and sodium benzoate. The possibility exists (*2*) that in most cases the persons reacted to the two chemicals on the first test in the same manner as they would have reacted on a majority of the replications. This may explain why Fox's results were so significant statistically.

It had been suggested that one of the principal reasons for variations in the taste classification test might be that some individuals could not distinguish between certain taste sensations. It seemed logical to assume that some difficulty might be experienced in differentiating among salty, sour, and bitter, since these sensations may be termed somewhat similar. In order to evaluate this theory experimentally, it was decided to determine the sensory panel's ability to define known standards of taste. Common sucrose was utilized to represent sweet, sodium chloride for salty, citric acid for sour, and quinine sulfate for bitter. Untreated Whatman No. 1 filter paper was used for an absolutely tasteless medium. The tests with each compound (single determinations) were made in the same manner as the previous tests with phenylthiocarbamide and sodium benzoate. Table VI shows only the variations from the normal taste sensations produced by each of the five standards.

If, for sake of convenience, these variations from normal taste may be termed "errors," the following may be observed:

1. From a total of 100 observations (20 individuals × five standards) there were nine errors. This may or may not be significant, but it was interesting to note that eight individuals or about 40% of the persons tested were unable, in some manner, to differentiate between certain of the taste standards.
2. All errors fell within the salty, sour, or bitter standards. There were no errors in classifying sucrose as sweet or untreated filter paper as tasteless. This, in effect, bears out the previously stated hypothesis that errors are more likely to occur in classifying salty, sour, or bitter standards because of their somewhat vague likeness to each other.
3. Citric acid, which, by normal taste, is extremely sour, was classified as bitter by three individuals. Either these persons could find no difference between the bitter standard (quinine) and the citric acid or else they actually interpreted the sour taste as bitter. Two of these three errors seem to agree with the first theory, as both the sour and bitter standards were classified as bitter. However, the misinterpretation theory seems to have confirmation in the third error, where citric acid was classified as bitter and quinine was classified as sour. With this one exception, all errors appearing in this test seemed to be due to the fact that no difference could be found between certain of the standards.
4. Of particular interest was the fact that one individual (No. 16) classified sodium chloride as sweet. This particular person found phenylthiocarbamide bitter and sodium benzoate sweet on all replications and was the only person whose taste ability could be accurately classified by Fox's proposed method. Another individual (No. 2) classified sodium chloride as sour. This person also found sodium benzoate sour on each of the six replicate tests made with that compound.

From this work it seemed that an individual's flavor perception varies widely, as was amply demonstrated by Fox's work with food preferences. However, the work also emphasizes the fact that any attempts to classify taste by chemical means will be complicated by this very erratic but important variable. Undoubtedly, the observation that a person's reaction to sodium benzoate is, in most cases, not reproducible from day to day is partially a result of inability to define taste standards correctly. Threshold values for taste perception were not considered in the present study because of the high reagent concentration used.

The group of persons used for the present study was very small. Similar studies made with larger, more diversified groups may yield results which, proportionally, are quite unlike those shown here. It is believed, however, that the extreme variability shown by these individuals would also be noted in larger groups and is of sufficient magnitude to warrant the conclusion that the test is unreliable.

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