A TEST FOR DETECTING WHEAT PASTURE FLAVOR IN COW'S MILK

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TABLE OF CONTENTS

LIST OF TABLES

Table | Page

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Consumption of certain feed stuffs by dairy cows often results in the appearance of flavor defects in milk. Grazing on wheat pasture during fall and spring in wheat growing areas can cause an undesirable flavor defect in milk. This undesirable milk flavor resulting from grazing cows on wheat pasture has been characterized as a "fishy" flavor.

The "off flavor" subsequently appears in processed products which is unacceptable to consumers. Thus such a flavor defect ("fishy") results in the milk being rejected by processing plants.

The only means available to most people for detecting wheat pasture flavor in raw milk has been by subjective evaluation. Such sensory evaluation has not gained wide acceptance in the dairy field. An easy and rapid objective test to check the milk at the farm would be desirable.

The objective of this study was to find an easy objective method to detect the compound associated with wheat pasture flavor in milk and confirm this method by comparing it with sensory evaluation.

CHAPTER II

REVIEW OF THE LITERATURE

Use of wheat pasture for grazing dairy cattle is routine in wheat growing areas. However, consumption of this forage by cows has been shown to result in the presence of an undesirable flavor, characterized as "fishy" in their milk.

Studies on the "fishy flavor in milk" have been undertaken for many years. Strobel et al (1953) reported that pasturing of dairy cattle on common rye caused fishiness in milk. An undesirable milk flavor resulting from grazing cows on brome grass pasture was also reported by Loney et al (1963). Johnson et al (1973) found that the intensity of wheat flavor in milk was related to the amount of time the cows grazed on wheat pasture.

The compound that causes the fishy flavor in milk was identified as trimethylamine (TMA) by Johnson et al (1973, 1974). The relationship between TMA and fishy flavor in milk from cows pastured on wheat was confirmed by Mehta et al (1974). Even though the compound that causes the fishy flavor was identified as TMA, detection of low concentration of TMA in milk under field conditions remains a problem in the dairy industry (Von Gunten et al, 1976).

Bassette (1965) advocated a test procedure involving addition of an alkali to aid detecting amines in sensory evaluation. However, this method has not gained wide acceptance in the industry. Small

amounts of TMA in milk could be detected by gas chromatography (Mehta et al, 1974, 1977), but these instruments are not available to the milk tank drivers for use in the field.

Many methods for determining trimethylamine (TMA) in fish have been reported. Dyer (1945, 1950, 1959) used a colorimetric method with picrate salt. Hansen et al (1947) tried steam distillation to separate the TMA from the fish. Beatty et al (1937) used a modification of the Conway microdiffusion method and Cromwell et al (1950) attempted a microestimation of TMA using cis-aconitic anhydride as a reactant. Sass et al (1958) used colorimetric estimations involving a picric acid system, cis-aconitic anhydride system and chloranil system. Gas chromatographic methods have been employed by many investigators (Mehta, 1974; Nonaka et al, 1967; Groninger, 1958; Wong et al, 1967; Miller III, 1972). None of these methods except gas chromatographic methods have been applied to cow's milk.

TMA in milk can be released by adding a releasing agent like potassium hydroxide (Bassette, 1965). Various releasing agents including saturated K_2CO_3, 10N NaOH, saturated Na_3PO_4 and saturated $Na_3PO_4 \cdot KOH$ were compared in the modified Conway microdiffusion method for fish (Cobb III et al, 1973). The use of saturated sodium phosphate solution as the releasing agent reduced the interference of dimethylamine (DMA) and ammonia in the analysis for TMA. Addition of potassium hydroxide to the saturated sodium phosphate solution (designated $Na_3PO_4 \cdot KOH$) was necessary for complete distillation of TMA in the modified Conway microdiffusion method. Sodium hydroxide could also be used as a complete releasing agent for TMA.

TMA analysis by the microdiffusion procedure (Beatty, 1937) depended upon separation by distillation and trapping of TMA. The ammonia, primary amines and secondary amines reacted with formaldehyde (Sprung, 1940) and were not released for distillation. Dyer (1945) treated fish tissue with formaldehyde to prevent extraction of ammonia, primary amines and secondary amines when the tissue was further treated with saturated potassium carbonate and extracted with toluene. Tozawa et al (1971) have suggested that saturated potassium carbonate be replaced with 25% potassium hydroxide to prevent DMA interference, but interference of DMA is not a problem in milk. This was shown by Mehta et al (1974) who used gas liquid chromatography to quantitate only TMA and primary amines in steam distillates of milk from two cows which had been on winter wheat pasture for 8 days.

Various methods for estimating TMA in fish were compared by Shewan et al (1971). The automated method of Murray and Burt (1964) using the "Technicon" autoanalyzer system, in which a trichloroacetic acid extract of the fish muscle was treated with formaldehyde followed by potassium hydroxide to release the volatile amines. A fixed proportion of volatile phase at 75°C (167°F) was passed into a stream of bromothymol blue solution at pH 6.0. The increase in pH caused by the volatile amines was monitored colorimetrically at 618 mμ and the color was automatically measured and recorded.

Trimethyl amine is a tertiary amine. It has a low boiling point (3.5°C) thus it is considered a volatile compound. Amines are alkaline in nature due to an unshared electron pair on the nitrogen atom (Hart and Schuetz, 1972).

CHAPTER III

EXPERIMENTAL PROCEDURES

Reagent Solution and Indicator

Source and Preparation of Reagents

Five percent sodium hydroxide solution was prepared by dissolving 5 g of NaOH (Mallinckrodt Inc., St. Louis, Missouri) in distilled water and diluting to 100 ml. Formaldehyde (37%) containing 10% methanol as a preservative was obtained from Mallinckrodt Inc., St. Louis, Missouri. Both solutions were stored at room temperature.

A stock solution containing 1 mg TMA per ml was prepared by dissolving 1.62 g of trimethylamine hydrochloride (Eastman Organic Chemical Co., New York) and 1 ml of hydrochloric acid (Baker Chemical Co., Phillipsburg, N. J.) in a small portion of distilled water and adjusting the final volume to 1000 ml with additional distilled water. A standard solution containing 0.1 mg of TMA per ml (100 ppm) was prepared by diluting 10 ml of the stock solution with 90 ml of distilled water. The solutions were stored in the refrigerator.

Indicator Test Strips

Indicator test strips were prepared by dipping fiber cords in 0.1% alcoholic solution of bromcresol green (BCG) for two minutes. The saturated cords were dried for one hour at room temperature in

front of a fan. The dried test strips were cut and inserted into glass tubing (0.2 cm internal diameter) of the desired length. The fiber cords used were: 100% virgin orlon acrylic fiber; shiny cotton (DMC-3); cotton (Bucilla six strand, color; 2003); acrylic; wool; nylon (Nevada, acrylamide 65%). All are white and were purchased from local department stores.

One other pH indicator, methyl red, was evaluated for preparing test strips. The indicator solutions were tested in different concentrations (0.1, 0.2% of each solution). Various combinations of the two indicators were also tested by preparing mixed indicator solutions at the ratio of 0:1, 1:1, 1:2, 2:1, 1:0 (Methyl red:BCG).

Measurement of Trimethylamine

Assay System

A schematic drawing of the test system is shown in Figure 1. A test tube (2.5 x 15 cm) was plugged with a rubber stopper through which two glass tubes were inserted. One of the tubes (0.4 cm ID) extended to the bottom of the test tube and was connected to an air pump. The other tube contained the indicator test strip and was inserted into the stopper so that its lower end was flush with the bottom of the stopper and the entire test strip was visable above the stopper. The test system was placed in the waterbath during the experiment so that the level of water was above that of the sample in the test tube.

Assay Procedure

Twenty ml of milk to be assayed was placed in the tube of the test system. One ml of 37% formaldehyde and 1 ml of 5% sodium hydroxide

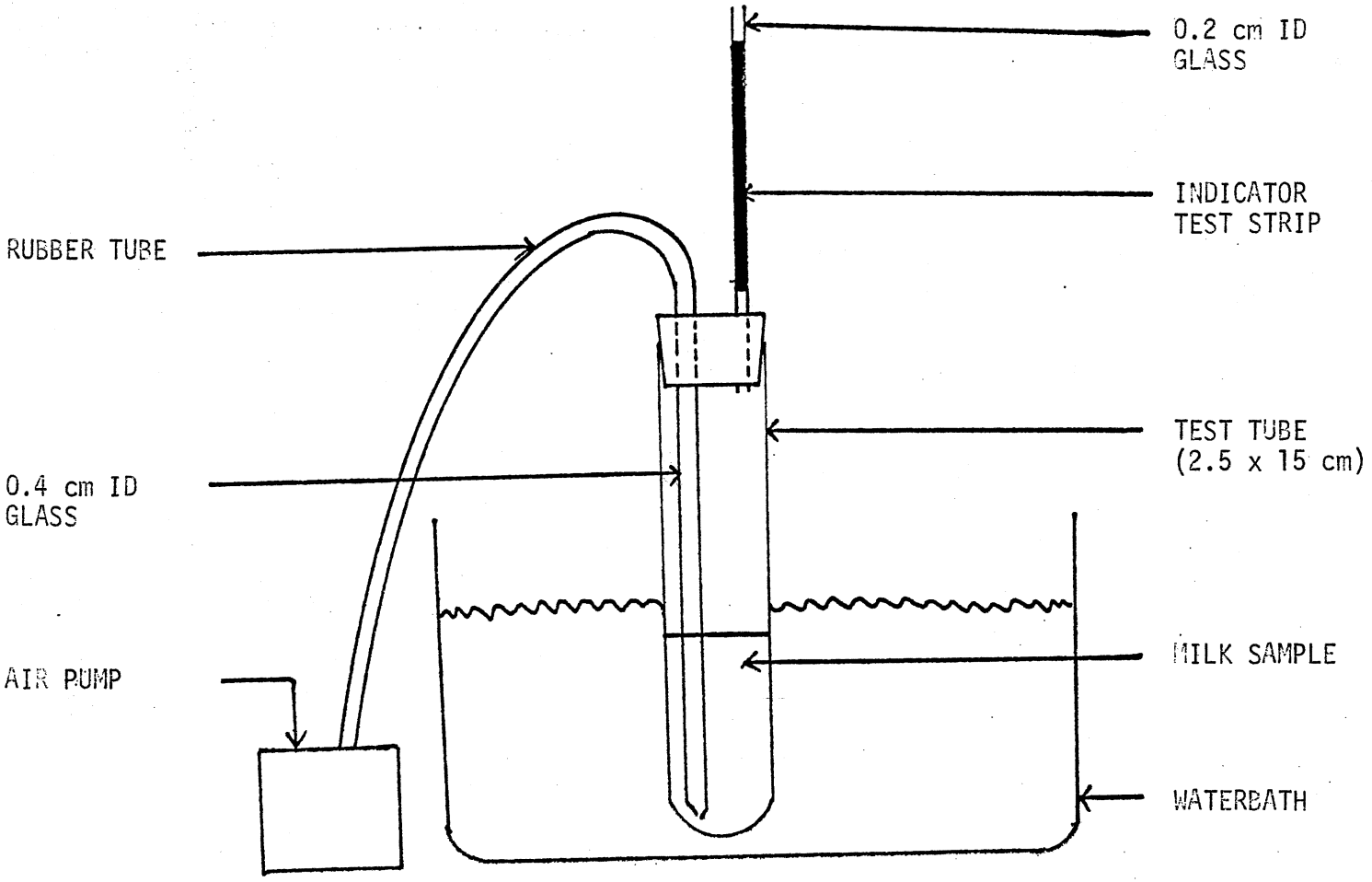

Figure 1. Test System for Detecting Volatile Alkaline Materials in Milk

were added to the sample. After the stopper was inserted, the test tube was placed in the water bath (28°C) and the air pump connected. The system was aerated (5.8 ml/min) for the desired time. The height of color change (from orange to green for BCG) in the indicator test strip was measured and recorded at one minute intervals.

Various types of fiber cords and pH indicators were compared to select the best indicator test strip. The length of the indicator test strips used in these experiments was 10 cm. The heights of color change in these experiments were measured at 1 minute intervals for 10 minutes using milk samples containing 5 ppm of TMA.

In order to confirm the necessity of each reagent in this assay, milk samples from cows which had grazed on wheat pasture were assayed with and without each of the following: 1 ml of formaldehyde, 1 ml of 5% sodium hydroxide or 1 ml of both reagents. The height of color change on the indicator strip was measured and recorded at 1 minute intervals for 6 minutes during the assay.

Determination of Optimum Aeration Time

Twenty ml of milk containing no TMA (from cows not on wheat pasture) was taken from a healthy cow in the evening milking and kept in the refrigerator (5°C) until the next day. This milk was used to prepare samples containing known amounts of TMA for use in preparing a response curve.

Concentrations of 1, 2, 3, 4 and 5 ppm of TMA in milk were prepared. The amounts of milk, TMA and distilled water used for each standard are shown in Table 1.

TABLE I

THE AMOUNTS OF MILK, STOCK SOLUTION OF TMA AND DISTILLED WATER FOR EACH STANDARD

Tube No.	ml milk	ml of stock[a] Solution of TMA	ml distilled H_2O	ppm TMA
1	19	0	1.0	0
2	19	0.2	0.8	1
3	19	0.4	0.6	2
4	19	0.6	0.4	3
5	19	0.8	0.2	4
6	19	1.0	0	5

[a]Stock solution of TMA contained 100 ppm TMA.

Twenty four test samples (4 samples of 6 different concentrations) were randomized and then tested. The heights (cm) of the color change on the indicator test strip were measured and recorded at 1 minute intervals for 6 minutes. To determine the optimum aeration time, the height of color change for each concentration at each of the 6 different aeration times were plotted as height of color change versus concentration of TMA.

Analysis of Samples From Cows on Wheat Pasture

Raw milk was obtained from a group of 10 cows that had been grazing on wheat pasture and from 2 cows not on wheat pasture (for control samples). The wheat pasture was seeded in September 1978 with Triumph 64 and the cows were first pastured when the wheat was 5-8 inches tall.

Lactating Holstein cows from the dairy herd of Oklahoma State University were used in this study.

The group of 10 cows was allowed to graze for 2 hours on wheat pasture, the other group (2 cows) were never allowed to graze on wheat pasture. The cows were removed from the pasture two hours before milking and milk samples were taken from each cow two times per week during the evening milking time. Duplicate samples from the 12 cows were randomized and tested the next day.

Comparison of the Assay with Sensory Evaluation to Detect TMA in Milk

Sensory evaluation of the same milk samples from the 12 cows described in the previous section was performed by 5 trained panelists. The samples were served in coded containers to the panelists. They evaluated and recorded the intensity of the fishy flavor using the following numerical scale: 1 (none detected); 2 (slightly detectable); 3 (detectable); 4 (strong); and 5 (very strong) (Guilford, 1954).

In order to compare the chemical test with sensory evaluation for detecting the intensity of TMA, milk samples containing known amounts of TMA were used. Two different systems were used to record the intensity of the TMA in the sensory evaluation and chemical test. One system was a ranking system in which a set of milk samples was ranked from 1 through 5 based on increasing intensities of TMA. The other system was the scoring system previously described. Scores for the chemical test were based on the height of color change in the indicator test strip (range of 0 - 0.3 cm = 1; 0.4 - 0.9 cm = 2; 1.0 - 1.5 cm = 3; 1.6 - 2.0 cm = 4; and > 2.1 cm = 5).

Fifteen samples were used for the ranking system during 3 days (5 samples per day) and twelve samples were used for the scoring system during 2 days (6 samples per day). The concentrations of all the samples used in these experiments were within 0 to 4 ppm of TMA.

Statistical Evaluation

The effects of aeration time and concentration of TMA on the height of color change were studied by using split-plot design in which the main plot had 6 concentration levels set up in a completely randomized design having four milk samples for each concentration of TMA. The subplot consisted of 6 aeration times. The results obtained from this experiment were used to decide the optimum aeration time.

The data obtained from chemical test and sensory test by using 10 same cows on wheat pasture and 2 cows not on wheat pasture during 2 weeks period (4 trials) were analyzed as a completely randomized design. Correlations between the chemical test and the panel scores in which the cows on wheat pasture were considered. The methods for these analyses are outlined in Principles and Procedures of Statistics (Steel and Torrie, 1960).

CHAPTER IV

RESULTS

Indicator Test Strips

To find the best fiber cord for preparing the indicator test strips for this chemical assay, various kinds were used to prepare the strips. The heights of color change were measured and recorded at one minute intervals for ten minutes during the analysis of milk samples containing 5 ppm of TMA. Bromcresol green was the pH indicator used for these comparisons. The results are shown in Table II. After ten minutes, the heights of color change for test strips prepared from wool and acrylic strips were much shorter than those of the other strips. The color changes in these two test strips were not distinct. A sharp color change was also not noted in the shiny cotton and the nylon strips. While the color change for the cotton strip was definite (yellowish green → strong green), the change for the virgin orlon acrylic fiber (orange → green) was even more distinct. The height of the color change was also greatest for the latter. Thus the orlon acrylic fiber yarn was used for the remainder of the study.

A one-tenth percent solution of Bromcresol green was the best pH indicator for preparing the test strips for this chemical assay. The use of methyl red or various combinations of methyl red and bromcresol green did not appear to improve the indicator test strips. The color change observed in the strips prepared using bromcresol green pro-

TABLE II

SELECTION OF FIBER CORD FOR PREPARING THE INDICATOR TEST STRIP FOR DETECTING VOLATILE ALKALINE COMPOUNDS IN MILK

Kinds of String	Height[a] of Color Change[b] for 10 Minutes										Appearance of Indicator Test Strip	
	1	2	3	4	5	6	7	8	9	10	Color Change	Clarity of End Point
Shiny Cotton	0	0.5	1.0	2.2	3.3	5.0	6.0	7.0	8.0	9.0	Slight green → green	Poor
Acrylic	0	0.4	0.5	0.6	0.8	1.1	1.3	1.5	1.8	1.9	Green → Strong green	Poor
Cotton	0.2	1.3	2.0	2.4	2.6	3.2	3.5	4.2	4.6	5.4	Yellowish green → Strong green	Good
Wool	0	0.4	0.5	0.7	0.8	0.9	1.0	1.2	1.3	1.5	Slight green → Strong green	Poor
Nylon	1.5	2.5	3.8	5.8	7.5	8.7	9.3	>10	>10	>10	Yellowish green → Green	Poor
Virgin Orlon Acrylic Fiber	1.2	2.5	3.8	5.0	6.0	8.2	9.2	>10	>10	>10	Orange → Strong green	Good

[a]Height in cm.

[b]Bromcresol green was the pH indicator.

vided the sharpest color change of any of the combinations when exposed to volatile alkaline material (TMA).

Confirmation of the Necessity of Reagents

The selection of reagents for this assay was based on those used in assaying fish products for TMA. To determine the necessity of each of the reagents, the assay was conducted with and without each. Data in Table III shows the effect of reagents on detection of volatile alkaline compounds in milk from a cow which had grazed on wheat pasture and one which had not (control). Both milk samples to which no reagents were added or just 1 ml of formaldehyde was added showed no color changes during the test procedure. The addition of 1 ml of sodium hydroxide to the milk samples resulted in the appearance of color changes in the indicator test strips for both samples. The color changes was greater in the sample from the cow which had been on wheat pasture when formaldehyde and sodium hydroxide were added. No color change was observed in the test strip for the control sample containing both formaldehyde and sodium hydroxide.

Influence of TMA Concentration and Aeration Time on the Height of Color Change of This Test

Twenty four samples (4 samples each of 6 different concentrations) containing 0, 1, 2, 3, 4 and 5 ppm of TMA were analyzed randomly by chemical test at 28°C. The heights of color change on the 5 cm indicator test strips were measured and recorded at 1 minute intervals for 6 minutes (Table IV). These data (average values for each concentration in Table IV) was plotted as concentration of TMA versus height of

TABLE III

EFFECTS OF REAGENTS ON THE HEIGHT OF COLOR CHANGE FOR MILK SAMPLE FROM COW ON WHEAT PASTURE AND FOR CONTROL MILK

Treatments	Replicate No.	Height[a] of color change at 6 minutes	
		Wheat Pasture[b]	Control[c]
20 ml milk	A	0	0
	B	0	0
20 ml milk + 1 ml HCHO	A	0	0
	B	0	0
20 ml milk + 1 ml NaOH	A	>5.0	3.0
	B	>5.0	2.7
20 ml milk + 1 ml HCHO + 1 ml NaOH	A	3.2	0
	B	3.5	0

[a]Height in cm.

[b]Sample from cow on wheat pasture

[c]Sample from cow not on wheat pasture

TABLE IV

THE HEIGHTS OF COLOR CHANGE OF KNOWN CONCENTRATIONS OF TMA BY THE CHEMICAL TEST AT 28°C

[a]Conc	Sample No. / Height of Color Change[b]	Aeration Time[c] 1	2	3	4	5	6
0	1	0	0	0	0	0	0.1
	2	0	0	0	0.1	0.2	0.2
	3	0	0	0	0	0.1	0.1
	4	0	0	0	0.1	0.2	0.2
	Average	0	0	0	0.05	0.125	0.15
1	1	0	0.1	0.2	0.3	0.5	0.6
	2	0	0.1	0.2	0.4	0.5	0.6
	3	0.1	0.2	0.4	0.6	0.7	0.8
	4	0	0.1	0.2	0.3	0.4	0.5
	Average	0.025	0.125	0.25	0.4	0.525	0.625
2	1	0	0.2	0.4	0.6	0.8	1.0
	2	0.1	0.2	0.4	0.7	0.9	1.2
	3	0.1	0.3	0.5	0.8	1.0	1.2
	4	0	0.1	0.3	0.5	0.7	0.9
	Average	0.05	0.2	0.4	0.65	0.85	1.075
3	1	0.1	0.3	0.6	0.9	1.3	1.6
	2	0.2	0.5	0.8	1.1	1.5	1.8
	3	0.1	0.4	0.6	0.9	1.3	1.7
	4	0.2	0.5	0.7	1.0	1.4	1.8
	Average	0.15	0.425	0.675	0.975	1.375	1.725
4	1	0.3	0.7	1.0	1.5	1.9	2.3
	2	0.3	0.7	1.2	1.7	2.1	2.5
	3	0.2	0.6	1.1	1.6	1.9	2.3
	4	0.3	0.7	1.0	1.5	1.8	2.2
	Average	0.275	0.675	1.075	1.575	1.925	2.325
5	1	0.4	0.9	1.4	1.9	2.4	2.8
	2	0.3	0.7	1.3	1.7	2.1	2.5
	3	0.5	1.0	1.5	2.0	2.4	2.8
	4	0.5	1.1	1.5	2.0	2.4	2.9
	Average	0.425	0.925	1.425	1.9	2.325	2.75

[a]ppm

[b]cm

[c]minute

color change in the indicator test strip to identify general shape of the resulting curves in an effect to establish a useable response curve (Figure 2). The heights of 6 minutes aeration time showed the most linear line among the lines of 6 different intervals aeration time.

The analysis of variance of these results (Table V) showed a highly significant interaction between concentration and aeration time ($P < .005$). It can be seen from Figure 2 that the response curve for 2 minutes of aeration was always higher than the response curve for 1 minute of aeration. The response curves were higher at each succeeding aeration time with the highest curve being for 6 minutes of aeration. The differences in the heights were greater as the concentration of TMA increased for each aeration time.

The combined effects of aeration time and concentration may be seen in Figure 3. In this figure the heights of color change for each concentration at each of the 6 different aeration times were plotted in a three dimensional diagram in which the height of color change was the vertical axis. The horizontal axes were aeration time in minutes and TMA concentration in ppm. It is obvious that greater response occurred at the higher concentration of TMA as long as the aeration time increased. As both aeration time and concentration increased so response of the height of color change was increased.

Comparison of Chemical Test and Sensory Evaluation for Detecting Known Amounts of TMA in Milk

The accuracy of the sensory evaluation and of the chemical test were compared by using ranking and scoring system. Five samples of

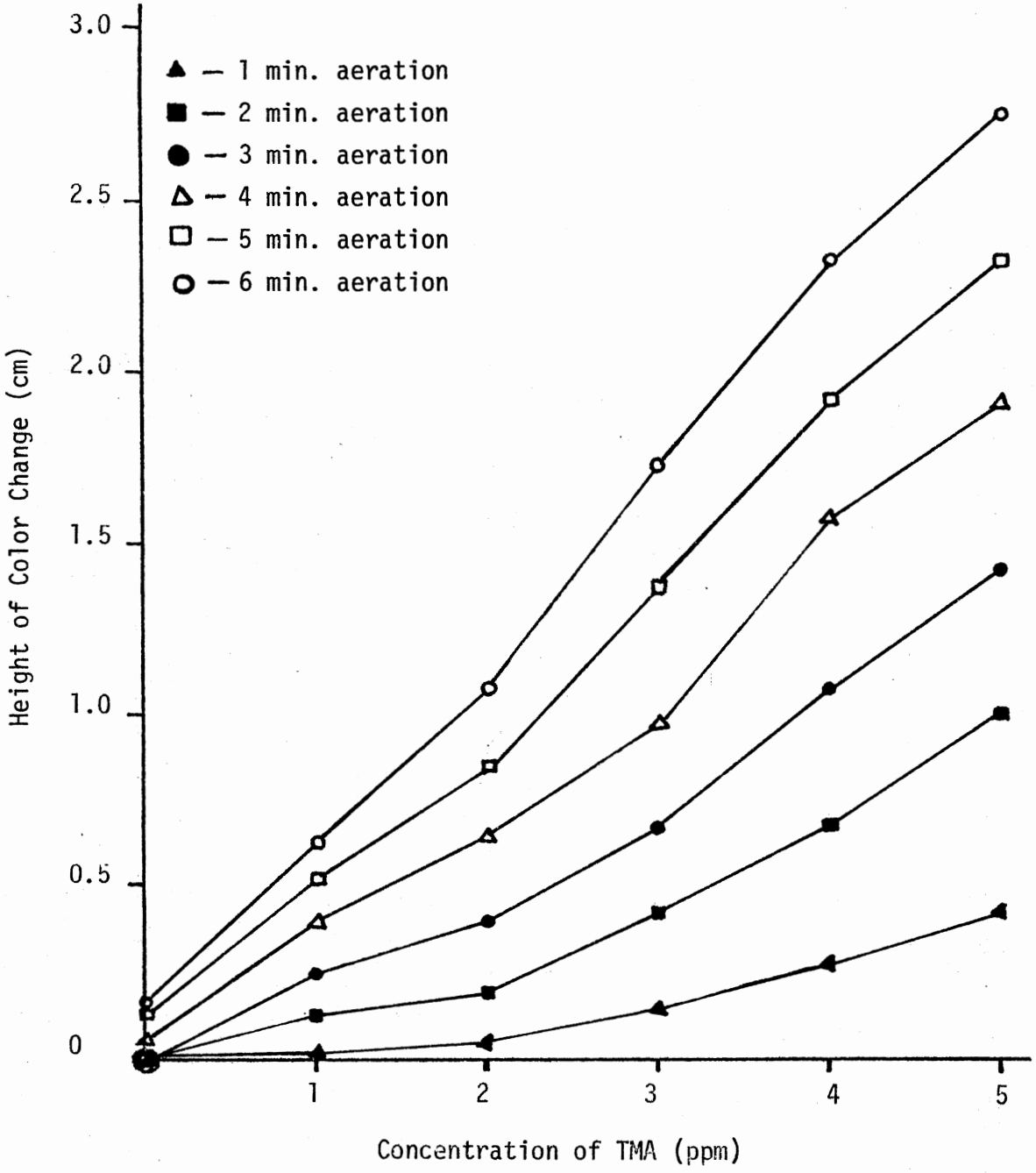

Figure 2. The Height of Color Change by Different Aeration Time of the Chemical Test for Identification of General Response Curve

TABLE V

ANALYSIS OF VARIANCE OF TMA CONCENTRATION AND AERATION TIME ON THE HEIGHT OF COLOR CHANGE IN THE CHEMICAL TEST AT 28°C

SOURCE	df	SS	MS	F*
TOTAL (CORR)	143	82.754		
Concentration	5	43.124	8.625	
Sample (Conc)	18	0.929	0.052	
Time	5	28.491	5.698	
Conc * Time	25	10.002	0.400	192.49
Sample * Time (Conc)	90	0.209	0.002	

*F value required for significance of concentration * time interaction = 2.17 at 0.5% level.

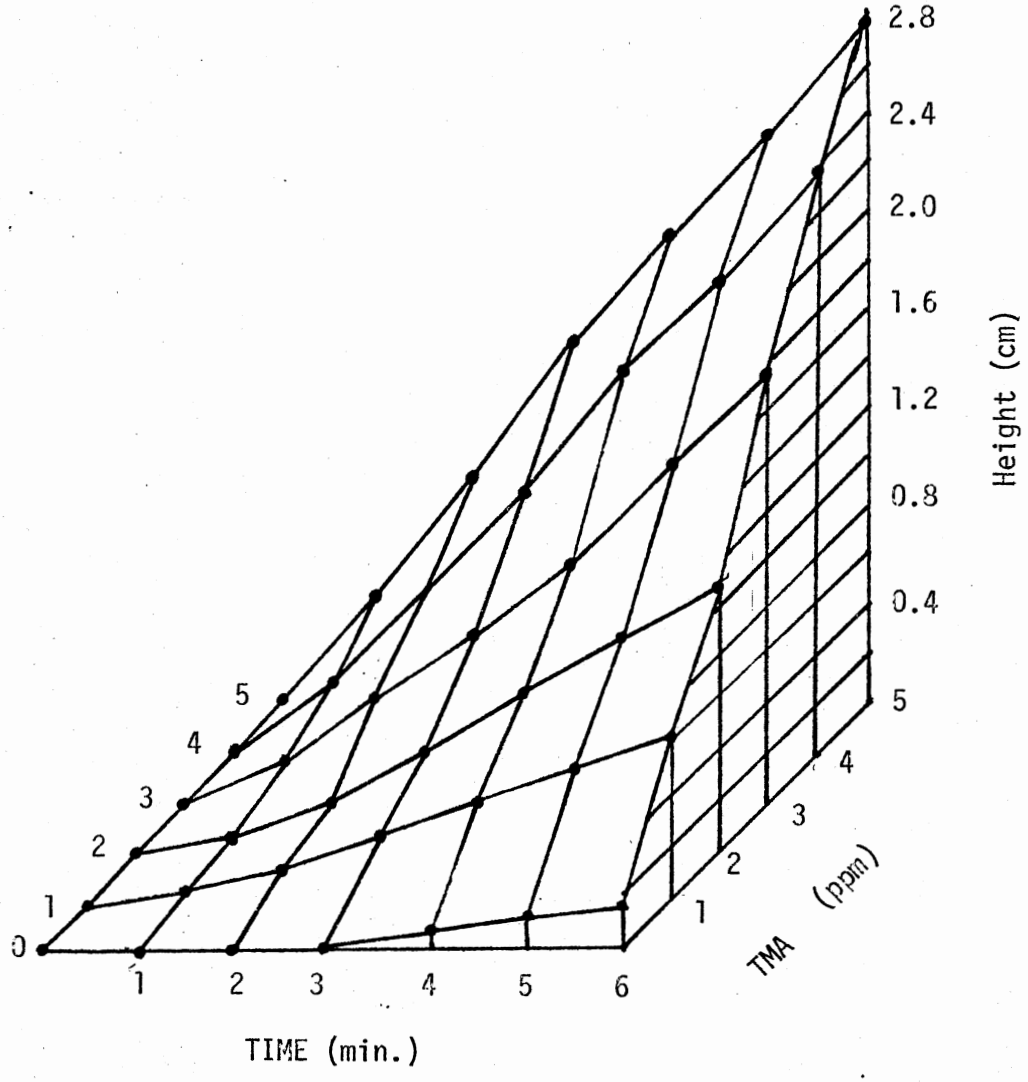

Figure 3. Surface Representation: The Influence of Aeration Time and Concentration on the Height of Color Change

milk containing known amounts of TMA were prepared and coded. The coded samples were subjected to the chemical test and to sensory evaluation. This was repeated on 3 different days using fresh samples. The results are shown in Tables VI and VII.

By the ranking system, the chemical test had an 86.7% accuracy and the sensory evaluation had a 51.7% accuracy. Comparison by the scoring system showed similar results (91.7% accuracy for the chemical test and 50% for the sensory evaluation).

Evaluation of Milk Samples From Cows Which had Been on Wheat Pasture

The chemical test and sensory evaluation of milk samples from cows which had grazed on wheat pasture were done 4 times during the period from December 8 to December 21, 1978. The comparisons between the chemical tests and sensory evaluations are shown in Tables VIII, IX, X and XI.

At the beginning of the wheat pasturing period, the amounts of volatile alkaline materials were low as measured by the chemical test. No fishy flavor was detected in these samples (Table VIII). After one week on wheat pasture, the amounts of volatile alkaline materials had increased (Table IX). Also the intensities of the fishy flavor detected by the sensory panelists had increased. However, none of the five panelists could detect fishy flavor in samples which exhibited less than a 1.0 cm height of color change in the indicator test strip.

On December 14, three of the five panelists detected fishy flavor (average score of 1.9 on a scale of 1 to 5) in the samples which exhibited 1.2 cm or greater of color change in the indicator test

TABLE VI

COMPARISON OF CHEMICAL TEST AND SENSORY EVALUATION FOR DETECTING KNOWN AMOUNTS OF TMA IN MILK BY RANKING SYSTEM

Day	Sample		Rank				
	No	ppm TMA	Chemical Test[a]	Sensory panelists			
				I	II	III	IV
	1	1	2	3	2	4	2
	2	2	3	4	4	5	3
1-26-79	3	3	4	2	3	1	4
	4	0	1	1	1	2	1
	5	4	5	5	5	3	5
	6	4	5	5	5	5	3
	7	2	4	4	2	1	5
1-31-79	8	0	1	3	1	4	1
	9	3	3	1	4	3	4
	10	1	2	2	3	2	2
	11	4	5	4	5	5	4
	12	0	1	1	1	1	1
2-2-79	13	2	3	5	4	3	2
	14	1	2	2	2	2	3
	15	3	4	3	3	4	5
				40%	60%	46.7%	60%
	Accuracy		86.7%	51.7%			

[a]Based on height of color change in the test strip

TABLE VII

COMPARISON OF CHEMICAL TEST AND SENSORY EVALUATION FOR DETECTING KNOWN AMOUNTS OF TMA IN MILK BY SCORING SYSTEM

Day	Sample		Score				
	No	ppm TMA	Chemical Test[a]	Sensory Panelists I	II	III	IV
	1	1	2	2	1	1	2
	2	4	5	3	2	4	4
2-7-79	3	3	4	4	3	3	4
	4	3	4	3	3	2	4
	5	0	1	3	1	2	1
	6	0	1	1	1	1	2
	7	1	3	3	1	2	1
	8	2	3	5	2	2	2
2-9-79	9	0	1	1	1	1	1
	10	0	1	2	1	1	1
	11	0	1	1	1	1	1
	12	1	2	4	2	2	2
				41.7%	50%	50%	58.3%
	Accuracy		91.7%	50%			

[a]Based on height of color change in the test strip

TABLE VIII

THE COMPARISON BETWEEN THE CHEMICAL TEST AND SENSORY EVALUATION AT 28°C ON DECEMBER 8, 1978

Cow's No	Wheat Pasture	Replicate No	Height[a] of color change at 6 min. aeration	Score[b] of individual panelist				
				I	II	III	IV	V
865	yes	1	0.3	1	1	1	1	1
		2	0.2					
870	yes	1	0.3	1	1	1	1	1
		2	0.4					
876	yes	1	0.3	1	1	1	1	1
		2	0.2					
877	yes	1	0.8	1	1	1	1	1
		2	0.7					
911	yes	1	0.3	1	1	1	1	1
		2	0.3					
913	yes	1	0.2	1	1	1	1	1
		2	0.1					
923	yes	1	0.4	1	1	1	1	1
		2	0.4					
926	yes	1	0.3	1	1	1	1	1
		2	0.2					
933	yes	1	0.3	1	1	1	1	1
		2	0.3					
936	yes	1	0.5	1	1	1	1	1
		2	0.4					
019	no	1	0.6	1	1	1	1	1
		2	0.8					
499	no	1	0.5	1	1	1	1	1
		2	0.7					

[a]Height of color change expressed in cm

[b]Scoring scale: 1(none); 2(slightly detectable); 3(detectable); 4(strong); 5(very strong).

TABLE IX

THE COMPARISON BETWEEN THE CHEMICAL TEST AND SENSORY EVALUATION AT 28°C ON DECEMBER 14, 1978

Cow's No	Wheat Pasture	Replicate No	Height[a] of color change at 6 min. aeration	Score[b] of individual panelist				
				I	II	III	IV	V
865	yes	1	2.1	1	3	2	2	2
		2	2.0					
870	yes	1	1.4	2	1	2	3	2
		2	1.3					
876	yes	1	1.3	2	1	3	3	1
		2	1.2					
877	yes	1	1.5	1	2	2	2	1
		2	1.6					
911	yes	1	0.8	1	1	1	1	1
		2	0.9					
913	yes	1	0.2	1	1	1	1	1
		2	0.2					
923	yes	1	3.6	2	3	2	2	2
		2	3.8					
926	yes	1	1.3	2	1	3	3	1
		2	1.3					
933	yes	1	0.9	1	1	1	1	1
		2	0.9					
936	yes	1	1.7	1	2	2	2	2
		2	1.5					
519	no	1	0.5	1	1	1	1	1
		2	0.5					
770	no	1	0.4	1	1	1	1	1
		2	0.5					

[a]Height of color change expressed in cm.

[b]Scoring scale: 1(none); 2(slightly detectable); 3(detectable); 4(strong); 5(very strong).

TABLE X

THE COMPARISON BETWEEN THE CHEMICAL TEST AND SENSORY EVALUATION AT 28°C ON DECEMBER 15, 1978

Cow's No	Wheat Pasture	Replicate No	Height[a] of color change at 6 min. aeration	Score[b] of individual panelist				
				I	II	III	IV	V
865	yes	1	>5	3	3	3	3	2
		2	>5					
870	yes	1	3.5	2	3	2	3	2
		2	3.7					
876	yes	1	2.0	1	2	3	2	2
		2	2.7					
877	yes	1	3.7	2	2	2	2	1
		2	3.5					
911	yes	1	2.3	1	1	2	2	1
		2	2.5					
913	yes	1	1.7	1	3	3	3	2
		2	1.4					
923	yes	1	>5	1	2	2	2	2
		2	>5					
926	yes	1	2.9	1	2	1	2	2
		2	2.6					
933	yes	1	1.7	1	3	1	2	2
		2	1.7					
936	yes	1	>5	1	3	2	1	2
		2	>5					
019	no	1	0.2	1	1	1	1	1
		2	0.2					
129	no	1	0.3	1	1	1	1	1
		2	0.4					

[a]Height of color change expressed in cm.

[b]Scoring scale: 1(none); 2(slightly detectable); 3(detectable); 4(strong); 5(very strong).

TABLE XI

THE COMPARISON BETWEEN THE CHEMICAL TEST AND SENSORY EVALUATION AT 28°C ON DECEMBER 21, 1978

Cow's No	Wheat Pasture	Replicate No	Height[a] of color change at 6 min. aeration	Score[b] of individual panelist				
				I	II	III	IV	V
865	yes	1 2	2.1 2.2	4	2	3	3	
870	yes	1 2	2.0 2.0	3	3	4	3	
876	yes	1 2	1.1 1.2	1	1	1	2	
877	yes	1 2	2.2 1.9	2	2	2	2	
911	yes	1 2	2.0 2.2	2	1	3	2	
913	yes	1 2	2.4 2.2	3	2	2	3	
923	yes	1 2	4.0 3.6	3	2	3	3	
926	yes	1 2	2.0 2.4	3	2	3	2	
933	yes	1 2	1.8 1.5	2	3	2	2	
936	yes	1 2	1.4 1.5	2	3	2	3	
423	no	1 2	0 0.1	1	1	1	1	
484	no	1 2	0.2 0.3	1	1	1	1	

[a]Height of color change expressed in cm.

[b]Scoring scale: 1(none); 2(slightly detectable); 3(detectable); 4(strong); 5(very strong).

strips. On December 15, three of the five panelists detected fishy flavor (average score of 2.1) in the samples which exhibited 1.7 cm or greater of color change by the chemical test. On the final day, two of the four panelists detected fishy flavor (average score of 2.5) in the samples which exhibited 1.4 cm or greater of color change in the indicator test strips. It appears that as more vegetation was available from the growing wheat, the sensory panelists were able to detect greater intensities of fishy flavor. This occurred in conjunction with increases in the relative amounts of volatile alkaline materials.

The analysis of variances of the data in Tables VIII, IX, X and XI appear respectively in Tables XII, XIII, XIV, XV and XVI in the Appendix. The analysis of the December 8 trial indicate that no significant differences ($P < 0.17$) occurred between the responses due to the 10 cows on wheat pasture and the 2 dry lot cows.

The results of chemical test and the sensory evaluation showed that the differences between the effects due to wheat pasture and dry lot rations increased from the first week through the final period.

The chemical test detected lower levels of TMA than the sensory panel. There was a correlation of 0.745 ($P < .01$) between sensory evaluation and chemical test with regard to the scores of the panelists and the height of color changes in the test strips.

CHAPTER V

DISCUSSION

TMA in milk can be detected by gas chromatography, although this does not appear to be a useful method for field work. It requires highly trained technicians and expensive equipment A rapid and easy test in which some reagents are mixed with a sample of milk for detection of TMA would be very useful for its detection in the field. The chemical test described in the present study for rapid detection of TMA could fulfill this need.

Johnson et al (1973) using mass spectrophotometry identified the compound that causes the fishy flavor in milk from cows on wheat pasture as TMA. Mehta et al (1974) confirmed that TMA was responsible for the fishy flavor in milk using gas chromatography. Bassette et al (1965) advocated a sensory test procedure which involved the addition of an alkali to the milk to enhance detection of fishy flavor in milk. These results indicated that alkali acted to release the TMA in milk or at least to increase its volatility.

The selection of reagents for the assay described in the present study was based on those used in methods for detecting TMA in fish. The following have been used as agents to release TMA: KOH, NaOH, K_2CO_3, $Na_3PO_4 \cdot KOH$ (Cobb III et al, 1973; Tozawa et al, 1971). Formaldehyde has been used to complex or bind other volatile compounds that might be present in the fish (Dyer, 1945; Tozawa et al,

1971; Cobb III et al, 1973). The apparent mechanism which permitted separation of TMA from other volatile alkaline materials was that formaldehyde complexed with ammonia, primary and secondary aimes but not with tertiary amines (Sprung, 1940) and the alkali released or enhanced the volatility of tertiary amines. He indicated that when ammonia and formaldehyde are mixed in aqueous solution, hexamethylenetetramine breaks down through a succession of stages involving mono, di and trimethylolamines. It was postulated that primary and secondary aliphatic amines react in the following way:

$$RNH_2 + HCHO \xrightarrow{OH^-} RNHCH_2OH$$

$$R_2NH + HCHO \xrightarrow{OH^-} R_2NCH_2OH$$

They can be condensed with a second molecule of amine to form bis(akkylamino) - or bis (dialkylamino) - methanes. The bis methanes can also be formed directly from amines and formaldehyde (Sprung, 1940). These types of compounds are apparently not volatile or do not behave in a manner like that of tertiary amines.

The tertiary amines including TMA which do not complex with formaldehyde are "released" after adding sodium hydroxide. Results of the present study suggest that a similar mechanism functions when formaldehyde and sodium hydroxide are added to milk containing TMA. Sparging the milk with air sweeps the tertiary amines from milk. When these volatiles are passed over a test strip containing a pH indicator, as in the assay system, a color change is observed.

A possible problem may exist with the formaldehyde. If the formaldehyde contained more than 10% of methanol a false positive

test was observed. This happened when a lot of formaldehyde containing 11.2% methanol was used.

It is assumed that TMA was the only volatile alkaline material released by adding formaldehyde and sodium hydroxide to milk containing wheat pasture flavor. No positive test resulted from control milk and the amounts of alkaline material as indicated by increased color change increased as intensity of fishy flavor increased. Cole et al (1961) reported that all fresh raw milk contained ammonia, propyl and hexylamines (primary amines). Mehta et al (1974) using gas chromatography quantitated TMA as the only tertiary amine in milk from cows on wheat pasture.

The indicator test strip was the sensitive part of the test system. Variations among types of fiber cord tested may have been due to non-uniformity of each and the relative ability to retain sufficient pH indicator. A uniform support for the pH indicator should provide more control over the height of color change for a given concentration of TMA in milk. Precaution must be exercised regarding exposure of the test strips to ammonia or other alkaline material prior to use since this will result in change in the color of the strip.

The aeration rate and temperature might affect the height of color change in this test. While a higher flow rate of air might reduce the aeration time required to remove all of the TMA from the sample, it might also cause the gaseous material to diffuse more in the test strip. Diffusion of the gas in the test strip material could certainly reduce the accuracy of the test. Unless this can be controlled the test would be limited with regard to a high degree of accuracy. The removal of all the volatile alkaline material from the sample by

sparging could be hastened by the increasing of temperature of the sample. However, excess moisture might occur in the volatile material causing problems in relation to appearance of the test strip by dissolving the indicator and allowing it to migrate up the test strip. The chemical test appeared to be more sensitive than sensory evaluation in detecting TMA in milk, especially at lower concentrations. Both methods can predict qualitatively the intensities of TMA at higher concentrations. Comparison of the sensory evaluation and chemical test in the assay samples containing known amounts of TMA showed the chemical test to be more accurate than the sensory test.

None of the five panelists detected fishy flavor in samples which exhibited less than 1.0 cm height of color change and three of the five experienced panelists detected fishy flavor in the sample which exhibited 1.7 cm height of color change. It thus could be assumed that color change in the range of 1.0 - 1.7 cm of height on the strip would correspond to a slight level of wheat pasture flavor.

To be of practical use a dairy processing plant would need to establish their own limits with regard to the range of color change needed to accept or reject raw milk. In establishing such a range, careful attention should be given to the material used for making the indicator test strip, the aeration time, the flow rate of the air and the temperature. Sensory evaluation should be compared to the test results as done in this study to establish the desired range of color change to be used in accepting or rejecting milk.

CHAPTER VI

SUMMARY AND CONCLUSIONS

The objective of this study was to find an easy method to detect TMA (the compound associated with "wheat pasture" flavor) in milk and confirm this method by comparing it with sensory evaluation.

A specially designed assay system consisting of a pH indicator test strip (BCG), test tube (2.5 x 15 cm) and air pump was devised. The system permitted separation of volatiles from the milk such that they were passed over a test strip saturated with a pH indicator. Twenty ml of milk sample to be assayed was placed in test tube of the test system at 28°C. One ml of 37% formaldehyde and 1 ml of 5% sodium hydroxide were added to the sample. After inserting the stopper into the test tube, this system was aerated (5.8 ml/min) for the desired time. The air carrying the volatiles was forced over the test strip. The height of color change from orange to green in the indicator test strip was measured.

Samples from cows on wheat pasture were compared. There was no color change produced when control milk was assayed. The height of the color change increased as the intensity of fishiness (wheat pasture flavor) in the milk increased.

Virgin orlon acrylic fiber cord was the best material of those tested for the pH indicator test strip and 0.1% BCG alcohol solution was selected for pH indicator.

This chemical test was more sensitive than sensory evaluation by an experienced panel, especially at lower concentrations of TMA. It was also more precise than sensory evaluation.

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A P P E N D I X

TABLE XII

ANALYSIS OF VARIANCE OF SCORES AND HEIGHT OF COLOR CHANGE
FOR SAMPLES TAKEN DECEMBER 8, 1978

Source	df	SCORE*			Height of color change			Mean Product	Correlation
		MS	F	PROB>F	MS	F	PROB>F		
TOTAL (CORR)	47	0	-	-	0.035	-	-	0	0
TRT	1	0	-	-	0.620	6.146	0.031	0	0
COW (TRT)	10	0	-	-	0.100	-	-	0	0
JUDGE	3	0	-	-	0.000	-	-	0	0
TRT * JUDGE	3	0	-	-	0.000	-	-	0	0
COW * JUDGE (TRT)	30	0	-	-	0.000	-	-	0	0

* MS of score showed zero because all judge had same score

TABLE XIII

ANALYSIS OF VARIANCE OF SCORES AND HEIGHT OF COLOR CHANGE
FOR SAMPLES TAKEN DECEMBER 14, 1978

Source	df	SCORE			Height of color change			Mean Product	Correlation
		MS	F	PROB>F	MS	F	PROB>F		
TOTAL (CORR)	47	0.542	-	-	-	-	-	0.348	0.528
TRT	1	3,504	3.427	0.091	6.667	2.151	0.171	4.833	1.000
COW (TRT)	10	1.023	-	-	3.099	-	-	1.153	0.047
JUDGE	3	0.632	2.001	0.134	0	-	-	0	0
TRT * JUDGE	3	0.126	0.401	0.757	0	-	-	0	0
COW * JUDGE (TRT)	30	0.316	-	-	0	-	-	0	0

TABLE XIV

ANALYSIS OF VARIANCE OF SCORES AND HEIGHT OF COLOR CHANGE
FOR SAMPLES TAKEN DECEMBER 15, 1978

Source	DF	SCORE*			Height of color change			Mean Product	Correlation
		MS	F	PROB>F	MS	F	PROB>F		
TOTAL (CORR)	47	-	-	-	-	-	-	0.90	0.641
TRT	1	7.35	7.424	0.021	65.313	7.826	0.018	21.910	1.000
COW (TRT)	10	0.99	-	-	8.345	-	-	2.039	0.709
JUDGE	3	1.25	5.000	0.006	0	-	-	0	0
TRT * JUDGE	3	0.25	1.000	0.59	0	-	-	0	0
COW * JUDGE (TRT)	30	0.25	-	-	0	-	-	0	0

TABLE XV

ANALYSIS OF VARIANCE OF SCORES AND HEIGHT OF COLOR CHANGE
FOR SAMPLES TAKEN DECEMBER 21, 1978

Source	df	SCORE*			Height of color change			Mean Product	Correlation
		MS	F	PROB>F	MS	F	PROB>F		
TOTAL (CORR)	47	-	-	-	-	-	-	0.528	0.643
TRT	1	13.067	11.264	0.007	24.962	13.867	0.004	18.060	1.000
COW (TRT)	10	1.160	-	-	1.800	-	-	0.674	0.466
JUDGE	3	0.333	1.136	0.351	0.000	-	-	0.000	0.000
TRT * JUDGE	3	0.067	0.227	0.877	0.000	-	-	0.000	0.000
COW * JUDGE (TRT)	30	0.293	-	-	-0.000	-	-	-0.000	0.000

TABLE XVI

ANALYSIS OF VARIANCE OF SCORES AND HEIGHT OF COLOR CHANGE FOR SAMPLES TAKEN FROM WHOLE PERIOD

Source	df	SCORE*			Height of color change			Mean Products	Correlation
		MS	F	PROB>F	MS	F	PROB>F		
TOTAL (CORR)	159	-	-	-	-	-	-	0.686	0.578
COW	9	1.514	-	-	8.201	-	-	2.627	0.745
JUDGE	3	0.806	2.694	0.024	0	-	-	0	0
COW * JUDGE	27	0.218	-	-	0	-	-	0	0
DATE	3	14.239	40.497	0.0001	65.025	118.028	0.0001	23.484	0.772
JUDGE * DATE	9	0.617	1.756	0.085	0	-	-	0	0
COW * DATE	27	0.670	-	-	2.204	-	-	0.556	0.457
COW * JUDGE * DATE	81	0.245	-	-	0	-	-	0	0
C * D + C * J * D	108	0.352	-	-	0.551	-	-	0.139	0.316

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