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ON THE COVER: The product quality of OARDC-originated tomato selections and cultivars is evaluated to determine their merit and response to cultural treatments by Brad Hair and Mike Huber, graduate research associates, and Bill Stone, lab supervisor in the Food Processing Pilot Plant.

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Evaluation of Tomato Cultivars for Processing

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INTRODUCTION

In Ohio tomatoes continue to be the most important processed crop with planted acreage of 22,000 acres and one-half million ton production. Ohio ranks second only to California in processed tomato production.

The transition to new field production methods and new processing practices continues to create needs for a choice of better suited cultivars. This research continues to be especially directed toward improvement of the whole-pack product. Also of importance is the development of improved types for use in juice, sauce, and paste.

The objective of this study was to determine the suitability of 20 Ohio-grown tomato cultivars for processing and the quality of the canned products. The cultivars included were classified as established and/or new cultivars to Ohio tomato growers.

MATERIALS AND METHODS

The 1977 processing tomato project included 20 cultivars grown in replicated plots under acceptable commercial practices at the Ohio Agricultural Research and Development Center's Northwestern Branch near Hoytville. Each cultivar was machine harvested (with FMC Western Model) with little or no sort on the harvester and bulk handled. Following harvest the tomatoes were transported by truck (approximately 100 miles) to the Food Processing Pilot Plant at The Ohio State University, Columbus, for processing. All lots were processed after 24 hours' hold following harvest as peeled whole tomatoes.

A. About 10 to 12 field-run tomatoes were randomly selected and used for objective and subjective raw quality evaluation.
   1. Size was determined by weighing the sample and then calculating the number for a 1 lb sample. In addition, the tomatoes were subjectively classed for shape, fruit surface, core, firmness, defects, and number and size of locules.
   2. Objectively, stem scar length, stylar scar length, and stem length were determined by measuring the average length in inches and wall thickness in centimeters.
   3. Percent red color was determined by counting the number of tomatoes with full red color in the sample.
   4. E-5 cut surface color was determined on an Agtron E-5 instrument after making a crosswise cut into the tomato and reading the values after standardizing the instrument at 48.
   5. The sample was then quartered, extracted in a Food Processing Equipment Co. laboratory pulper, and deaerated.
      a) The deaerated pulp was presented to the Agtron E-5 instrument in a sample cup with the instrument calibrated at 48. The color reading was taken directly and recorded as such.
      b) The deaerated pulp was also presented to the Hunter Color Difference Meter D25D3A in a standard plastic sample cup and the Hunter L, a, b values and TCM value were taken directly and recorded as such.
      c) *Percent soluble solids:* An Abbe refractometer was used for direct determinations of percent soluble solids. The instrument was standardized with distilled water and all readings were converted to 70° C.
      d) *pH:* The pH was determined by the glass electrode method (Beckman Zeromatic pH meter) using 10 ml of tomato juice diluted with 90 ml of distilled water.
      e) *Percent total acid as citric:* The sample used for pH determination was directly titrated using 0.1 normal sodium hydroxide solution to a pH of 8.1. Calculations using the following equation were made:

$$\% \text{ acid} = \frac{(\text{No. of ml of 0.1N NaOH})\ (.0064)}{10 \text{ ml sample}} \times 100$$

      f) *Ascorbic acid:* Ten ml aliquots of tomato juice were diluted with 90 ml of 1% metaphosphoric acid and filtered. A 10 ml aliquot of the filtrate was titrated with 0.2% 2,6-dichlorophenolindophenol indicator solution. Milligrams of Vitamin C were determined by the following formula:

$$\text{Dye factor} \times \text{ml of dye} \times 100 = \frac{\text{mg Vitamin C}}{100 \text{ g}}$$

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Score Chart for Canned Tomatoes

Factors	Maximum Points	Grade A (Fancy)	Grade A (Whole)	Grade B (Ex. Std.)	Grade C (Std.)	Sub-standard
Drained Wt.	20	18-20	16-20***	16-17	14-15*	0-13*
Wholeness	20	18-19	20	16-17	14-15*	—
Color	30	27-30	27-30	24-26*	21-23*	0-20*
Defects	30	27-30	27-30	24-26	21-23*	0-20*
Minimum Score	—	90	90	80	70	—

*Limiting rule. Canned tomatoes falling into these classifications may not be graded higher regardless of total score.
**Special limiting rule. Sample units of canned tomatoes falling into this classification may not be graded higher than U. S. Grade B, regardless of the total score.
***Applicable to individual sample units only. To apply drained weight schedule for "Grade A Whole" provided, lot must also meet provision for Grade A for the factors of color and defects. Lot compliance requires minimum drained weight index of entire sample to score no less than 18 points for GRADE A WHOLE.

B. *Preparation and processing of the tomato:* All tomatoes were prepared for canning by washing, lye peeling (18% caustic soda and Faspeel at 88° C for 20 seconds) and processed as whole tomatoes. Each lot of whole tomatoes was filled to 10.0-10.5 oz. in No. 303 x 406 size fruit enamel tin cans with a 50-grain salt tablet containing 44.5% NaCl, 15% $CaSo_4.H_2O$, 37% citric acid, and 3.5% sodium bicarbonate.

C. *Grades of Canned Tomatoes:* Grades were determined in accordance with the U. S. Standards for Grades of Canned Tomatoes (1).

RESULTS AND DISCUSSION

The results are presented in Table 1. Of the 20 cultivars in the 1977 trials, 11 were rated Grade A for canned tomatoes. Of these 11 cultivars, five had pH above 4.6, indicating a definite need for acidification. Outstanding cultivars for processing were Chico III, 07664, 07667, 07678, 076120, 076121, 076122, 076170, and 076156.

With these new cultivars, a processor now has a choice based on maturity for tomatoes of variable size with no or little core for packing Fancy or Grade A tomatoes.

REFERENCE

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TABLE 1.—Tomato Cultivar Evaluation, Raw Product and Canned Whole Pack, 1977.

	Cultivar				
	C-37	**Chico III**	**Wakefield**	**Pu-74-74**	**07630**
RAW					
Fruit shape	Oblong	Pear	Oblong	Oblong	Oblong
No./lb	5	6-7	6	7	4
Stem scar	- 1/4	- 1/4	- 1/4	- 1/4	- 1/4
Stylar scar	1/8	None	None	None	1/8
Firmness	Hard	Medium	Medium	Medium	Hard
E5 pulp color	35.5	31.0	53.5	47.0	30.0
TCM color	68.1	76.6	65.4	77.6	75.3
pH	4.6	4.48	4.60	4.66	4.5
T.A.	0.32	0.36	0.28	0.31	0.33
S.S.	4.8	4.6	3.8	4.6	4.1
Vitamin C	18.3	22.6	15.4	20.1	16.6
CANNED					
Ct./can	6	7	7	6	4
Drained wt.	17	18	16	17	17
Wholeness	19	20	18	20	19
Color	27	28	27	27	26*
Defects	25*	30	28	29	25*
Total score	88*	96	89	93	87*
Grade	B	A	B	A	B
Comments	Large core, stem scar excessive		Soft	Blotchy	Stem scar excessive

	Cultivar				
	07635	**07663**	**07664**	**07667**	**07668**
RAW					
Fruit shape	Globe	Variable	Variable	Globe	Oblong
No./lb	4	6	5	7	12
Stem scar	1/4 - 1/2	1/4 - 1/8	1/4 - 1/2	- 1/4	- 1/4
Stylar scar	1/4	1/8	None	None	1/8
Firmness	Hard	Hard	Medium	Hard	Hard
E5 pulp color	32.0	49.0	37.5	34.0	42.0
TCM color	74.6	61.2	75.0	75.9	80.3
pH	4.5	4.55	4.55	4.3	4.62
T.A.	0.36	0.35	0.34	0.31	0.44
S.S.	4.3	4.1	3.8	4.6	4.3
Vitamin C		11.6	20.1	18.9	17.4
CANNED					
Ct./can	5	6	7	5	7
Drained wt.	16	15**	17	16	17
Wholeness	19	20	20	19	20
Color	26*	28	29	28	29
Defects	25*	30	29	29	29
Total score	86*	93**	95	92	95
Grade	B	B	A	A	A
Comments	Stem scar excessive				Dark core

*Limiting rule.

**Partial limiting rule.

TABLE 1. (Continued)—Tomato Cultivar Evaluation, Raw Products and Canned Whole Pack, 1977.

	Cultivar				
	07678	**07681**	**076120**	**076121**	**076122**
RAW					
Fruit shape	Oblong	Glo-Obl	Oblong	Oblong	Obl-Glo
No./lb	9	3	7	6	5
Stem scar	-¼	¼ - ½	¼ - ½	¼ - ½	¼ - ½
Stylar scar	None	⅛	⅛	⅛	None
Firmness	Hard	Hard	Hard	Hard	Medium
E5 pulp color	31.5	47.0	26.0	32.0	28.0
TCM color	77.3	69.4	78.2		77.1
pH	4.5	4.6	4.61	4.57	4.5
T.A.	0.34	0.27	0.40	0.42	0.40
S.S.	4.0	4.2	4.6	4.3	4.2
Vitamin C	23.3	13.0	17.4	13.5	16.2
CANNED					
Ct./can	7	3	6	7	6
Drained wt.	17	17	16	16	18
Wholeness	20	20	20	20	20
Color	28	27	28	28	28
Defects	29	26*	29	29	29
Total score	94	90*	93	93	95
Grade	A	B	A	A	A
Comments	Firm core	Stem scar excessive, needs coring	Soft		Dark stem scar

	Cultivar				
	076123	**076156**	**076170**	**076G1069**	**076G1034**
RAW					
Fruit shape	Oblong	Oblong	Globe	Oblong	Glo-Obl
No./lb	4-7	8	8	6	6
Stem scar	¼ - ½	¼ - ½	-¼	-¼	-¼
Stylar scar	⅛	⅛	None	None	None
Firmness	Medium	Hard	Soft	Hard	Hard
E-5 pulp color	43.0	30.0	25.5	31.0	38.0
TCM color	71.3	81.1	81.3	84.6	70.8
pH	4.6	4.58	4.6	4.36	4.6
T.A.	0.31	0.37	0.31	0.28	0.30
S.S.	3.5	4.2	4.4	4.3	4.4
Vitamin C	17.5	18.7	22.6	17.7	14.2
CANNED					
Ct./can	4	6	6	4	6
Drained wt.	16	16	18	16	16
Wholeness	20	20	20	19	19
Color	27	29	29	28	26*
Defects	28	29	30	25*	27
Total Score	91	94	97	90*	88*
Grade	A	A	A	B	B
Comments	Stem scar excessive			Stem scar excessive, veiny	Soft

*Limiting rule.

**Partial limiting rule.

Geotrichum candidum in Midwestern Tomato Processing Plants and Various Methods of Inhibiting its Growth

JAMES G. FOX and WILBUR A. GOULD[1]

INTRODUCTION

For many years sanitation was only a major concern in the home, but with the increasing number of people depending upon others to process their foods, government agencies have enacted regulations establishing sanitation guidelines for the food processing industry. The Good Manufacturing Practices, Part 128-Sanitation (Recodified 110, 3/15/77), which were promulgated in 1969 are an example.

Through the years, sanitation has often been defined in terms of what was not sanitary rather in terms of what was sanitary. Also, according to R. K. Guthrie, "The Act (The Food, Drug, and Cosmetic Act of 1938) considers food sanitation in terms which appear to make 'unsanitary' and adulterated almost synonymous." This usage is a result of the recognition that sanitation is concerned with the prevention of the spread of infectious disease, and is also concerned with chemical and physical characteristics of the food which may affect health. Further, the Food and Drug Act defines (in part) food as being adulterated if it ". . . consists in whole or in part of any filthy, putrid, or decomposed substance, or if it is otherwise unfit for food; or if it has been prepared, contaminated with filth, or whereby it may have been rendered injurious to health. . ." The phrases "may have become" or "may have been" are key phrases because they infer that only the possibility of contamination need be shown by regulatory agencies.

Geotrichum candidum or machinery mold is an example of an organism being utilized by the U. S. Food and Drug Administration and other regulatory agencies as an indicator of whether a food may have been prepared, packed, or held under unsanitary conditions whereby it may have been contaminated with filth. Therefore, the objectives of this study were: 1) to determine whether machinery mold was present in midwestern tomato processing plants, 2) to monitor the growth of machinery mold on various plant areas over a 5-week period to determine possible critical points in the processing operation, and 3) to determine the effect of temperature and chlorine inhibitory effects on the growth of *Geotrichum candidum*.

MATERIALS AND METHODS

The study involved monitoring the growth of machinery mold in two tomato processing plants over a 5-week period for various unit operations. The two processing facilities were chosen partially on the basis of their size and product line and also on the basis of the management's concern for improving potential deficiencies which might exist in their sanitation program.

One processing plant (Plant A) was a whole pack tomato processor who operated for one shift a day at a rate of approximately 15 tons per hour. The other processor (Plant B) was a concentrated tomato product processor operating three shifts a day at a rate in excess of 100 tons per hour.

The area monitored at the smaller processing facility (Plant A) included:

Site 1—The elevator coming out of the receiving tank
Site 2—The inside of a flume at water level
Site 3—The angle iron framework of the inspection table
Site 4—The conveyor belt on the inspection table
Site 5—The chopper used for comminuting the tomatoes for juice.

The areas monitored at the larger processing facility (Plant B) were identical except for three changes:

Site 2 (a-d)—Four flumes were monitored instead of one
Site 4—The inspection table used rollers instead of a rubber conveyor belt
Site 6—A sixth sample site was added—the wooden retaining boards present on one inspection table.

Samples were drawn hourly at each sample site by using a swab technique with a 1-square-inch template, and enumerating with the FDA's official staining procedure as described in the J. AOAC 57:957. At Plant A, duplicate samples were drawn hourly at each sample site for an 8-hour period while at Plant B, duplicate samples were drawn at each sample site for a 12-hour period.

RESULTS AND DISCUSSION

As the data in Figure 1 indicate, growth of machinery mold was found at all monitored areas in Plant A. The 5-week average after 8 hours' operation at the elevator coming out of the receiving tank (Site 1) was approximately 60 mycelial fragments

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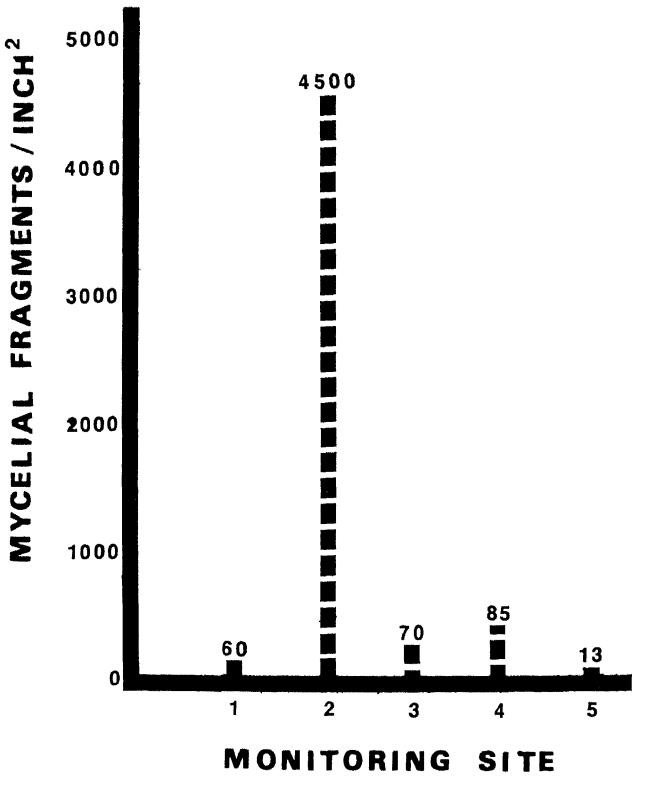

FIG. 1.—Growth levels at Plant A.

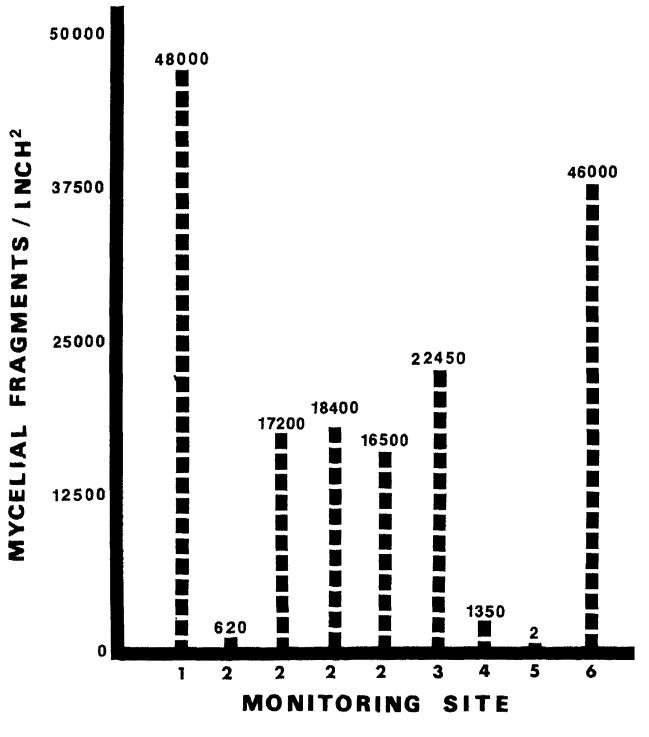

FIG. 3.—Growth levels at Plant B.

per square inch. On the inside of the flume (Site 2), approximately 4,500 mycelial fragments per square inch were enumerated; on the angle iron framework (Site 3), about 70 mycelial fragments per square inch were found. On the conveyor belt (Site 4), about 85 mycelial fragments per square inch, and on the chopper (Site 5) approximately 13 mycelial fragments per square inch were enumerated. However, it must be remembered that these figures represent the average of results obtained over a 5-week period. As the data in Figure 2 indicate, the total count after 8 hours steadily increased over the period of the season, indicating that the processor's control measures were less effective as the processing season continued. The exact reason for this effect could not be determined, although the lack of a separate and trained sanitation crew probably had some effect.

The data in Figure 3 indiate that machinery mold was found on all monitored areas at Plant B. The 5-week average growth after 12 hours operation on Site 1 was approximately 48,000 mycelial frag-

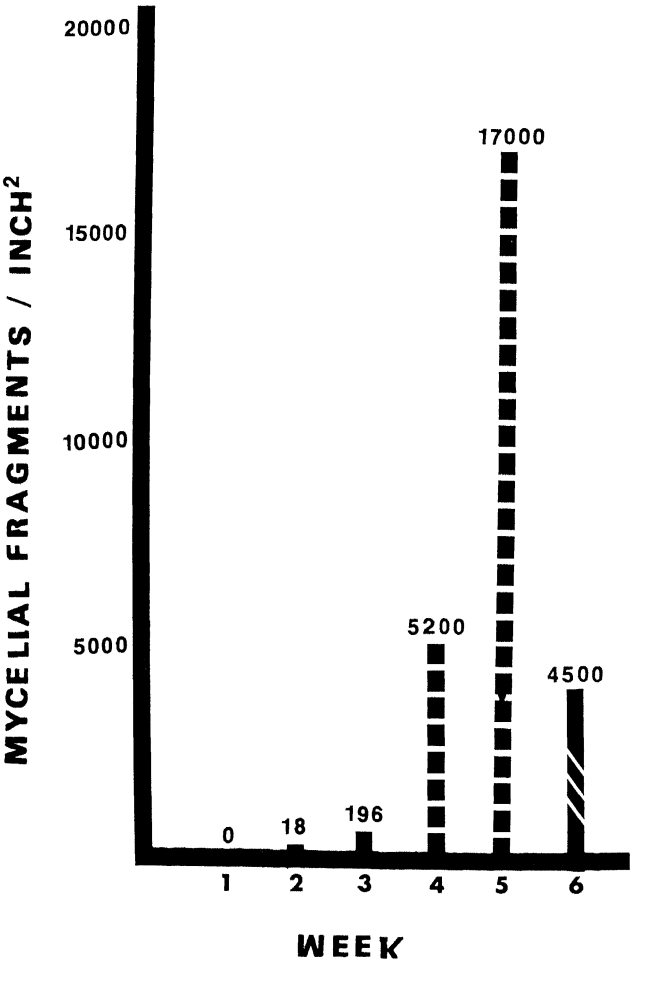

FIG. 2.—Weekly increase of growth on flume of Plant A.

ments per square inch. The level of growth on the four monitored flumes fluctuated as indicated by the following results of 620 at Site 2a, 17,200 at Site 2b, 18,400 at Site 2c, and 16,500 mycelial fragments per square inch at Site 2d. The samples collected at Site 3 averaged 22,450 mycelial fragments per square inch and Site 4 had an average of 1,350 mycelial fragments per square inch. The average for Site 5 was only 2 mycelial fragments per square inch, and the wooden retaining walls of the conveyor (Site 6) had approximately 46,000 mycelial fragments per square inch. These wooden boards were removed after 2 weeks by the management and replaced with stainless steel material. While the weekly averages fluctuated for all areas monitored, there was no steady increase of mold growth as observed at Plant A. However, except for the chopper, all *total* levels of growth were higher at Plant B than at Plant A.

It was also determined that machinery mold (isolated from a Plant B sample) grew well at temperatures of 20 to 40° C (*in vitro*); growth was retarded at 15 and 42° C and attenuated by 5 minutes' exposure to 5 ppm free residual chlorine (from calcium hypochlorite).

Geotrichum candidum is an ubiquitous organism that will grow on the surface of food processing equipment in midwestern tomato processing plants, as indicated in the results previously presented. The level of growth varied between sampling areas within the two processing plants, with Plant B having higher total growth levels at all but one sampling area. However, Plant A had an increase of growth as the processing season continued, while Plant B maintained relatively stable levels of growth throughout the season. The large difference in mold growth at Sites 2b-d at Plant B was believed to have been due to the inefficient counterflow recycled water system vs. a more efficient filtering system to remove settleable solids in the Site 2a flume. All other observed factors such as flow rate, water temperature, cleaning schedule, and tomato tonnage were the same.

The *in vitro* study of temperature growth range indicates that the organism will grow quite well at ambient temperatures normally associated with processing facilities and therefore cannot be viewed as a mechanism for general control, although some specific applications might exist for temperature control of the organism. Use of in-plant chlorination of water supplies during 1976 essentially eliminated the machinery mold problem at these two plants. Plant B found 2.5 ppm and 1.0 ppm residual chlorine effective in controlling machinery mold in water spray areas and the flume system respectively.

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Effects of Various Qualities of Raw Tomatoes (Subjective Evaluation) on Color of Extractable Juice (Objective Evaluation)

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INTRODUCTION

Color of tomato juice is one of the most important factors in quality determination according to U. S. Standards for grades and inspection manuals (1) for both raw and final processed products. The segregation of raw tomatoes by the evaluation of color before processing is necessary to establish final product quality. For this reason, contracts between growers and processors involve quality criteria based on color and other factors for the acceptance of raw materials going to the processing plant.

An accurate and reliable method of evaluating raw tomato color is needed. There are several instruments and methods of juice extraction in use for raw tomato color determination. The extraction method and maturity of the fruit are considered the greatest factors influencing the color of the extracted juice.

The purpose of this study was to evaluate the effects of method of extraction of raw tomatoes on level of quality and to objectively measure color with the aid of the Hunter Color Difference Meter and Agtron E-5.

The specific objectives of this study were:

a) To study the effects of method of extraction of raw tomatoes on color of juice
b) To study the effects of method of color evaluation (instruments) on raw product color
c) To study the effects of various blends of *red*, *pink,* and *green* mature fruit on the color of extractable juice with and without application of heat to the raw tomatoes prior to extraction
d) To determine the effects of deaeration on the color of extractable juice.

MATERIALS AND METHODS

Raw tomatoes used for this study were mechanically harvested, delivered to the Food Processing Pilot Plant at Ohio State University, and held for up to 24 hours before evaluation. Several cultivars were mixed and then sorted into full red, mature pink, and green (grass green to partially pink) fruits. After sorting and washing by hand, blends of red, pink, and green tomatoes (20 lb total each blend) of known percentages were made (Table 1). Tomatoes of each blend were cut into quarters, stem end to blossom end, each quarter being used for the following extraction methods:

1) Berkle extractor (B) with a 0.033 inch screen, half of each sample was evaluated directly and half deaerated under vacuum before evaluation (B/D)
2) OSU (Ohio State University) Century pulper and deaeration (OSU/D) for 2 minutes under vacuum
3) California Blendor method (CB)—tomatoes were blended for 1 minute under 27 inch vacuum in a Waring Blendor and screened with a 14 mesh screen
4) Microwave heating (M) of the tomatoes to 190° F followed by extracting with M/OSU and M/B methods.

Color evaluation was performed with a Hunter Color and Color Difference Meter D25D3A standardized on Hunter tile D33C-1585 (L 25.6, a 27.7, b 12.10, and TC 71.58), and an Agtron E-5 standardized at 48 on a scale of 0-100. Linear regression analyses were used to determine the relationship of the various extraction methods to color.

RESULTS AND DISCUSSION

The results indicate that the B/D extraction method resulted in the best scores as indicated by lower E-5, higher a/b ratios, and higher TC values. However, the CB method appeared to result in the least amount of incorporated air, although objective evaluation indicated no advantage. The OSU method was found to definitely require the deaeration step due to incorporation of air by the Century extractor used in this method. Microwave heating of tomatoes appeared to have adverse effects on tomato color as indicated by the data for the M/OSU and M/B methods and resulted in lower a/b ratios and E-5 values (Tables 1 and 2).

Regression analyses of extraction methods for color values showed that the method correlated best when using the E-5 or a/b values, but correlated poorly when TC values were used (Table 3, Figures 1 and 2).

The percentage of mature pink and green tomatoes had a definite effect upon the color of extracted juice (Figure 3). As either mature pink or green

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tomato percentages were increased, the a/b ratio decreased and E-5 values increased.

Thus, in summary, the Berkle (B) or Berkle/Deaeration (B/D), CB, and OSU/D were found to be satisfactory extraction methods when used in conjunction with a/b and E-5 values. However, microwave heating used with M/OSU and M/B methods was found to be undesirable. Higher correlation of (B) and (B/D) methods indicated that deaeration may not be necessary with these methods to obtain a reliable color index. It also was found that full red, mature pink, and green tomatoes could be used in various combinations to obtain a target color index.

REFERENCE

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TABLE 1.—Effects of Tomato Maturity Blends and Extraction Method on Color of Extracted Juice.

Blend	Percent Fruits (by Maturity)			Extraction Method*	Color Values		
	Red	Pink	Green		a/b	E-5	T.C.
1	100			B	2.60	27.0	79.71
				B/D	2.60	22.5	83.24
				CB	2.67	24.5	79.12
				OSU/D	2.72	27.0	70.76
				M/OSU	2.36	27.0	72.76
				M/B			
2	90	10		B	2.71	25.0	76.88
				B/D	2.75	23.5	79.90
				CB	2.72	27.0	77.32
				OSU/D	2.73	30.5	70.03
				M/OSU	2.29	30.0	72.76
				M/B	2.38	25.5	78.88
3	90	5	5	B	2.63	28.3	75.39
				B/D	2.69	27.8	78.38
				CB	2.61	30.8	78.88
				OSU/D	2.63	35.3	66.42
				M/OSU	2.18	48.5	65.97
				M/B	2.17	31.0	76.02
4	80	20		B	2.61	29.3	75.01
				B/D	2.70	27.3	77.97
				CB	2.65	30.3	77.45
				OSU/D	2.59	36.5	65.93
				M/OSU	2.20	33.5	68.45
				M/B	2.21	31.5	75.30
5	80	10	10	B	2.58	31.3	75.87
				B/D	2.60	28.5	80.65
				CB	2.50	32.3	78.52
				OSU/D	2.54	34.3	69.35
				M/OSU	2.08	37.0	67.82
				M/B	2.16	33.0	76.65
6	70	30		B	2.70	27.5	74.73
				B/D	2.74	24.5	79.36
				CB	2.66	29.5	75.70
				OSU/D	2.80	27.5	73.47
				M/OSU	2.39	32.5	70.52
				M/B	2.37	28.5	73.81
7	70	20	10	B	2.53	31.8	75.47
				B/D	2.57	29.5	79.06
				CB	2.21	34.0	75.57
				OSU/D	2.54	35.8	70.86
				M/OSU	1.87	47.5	64.62
				M/B	2.02	38.5	73.28

*See text for definitions.

TABLE 2.—Effects of Various Blends of Mature Fruits on Color Values With and Without Heating for Extraction.

Blend Composition				
% Red	% Pink	% Green	Hunter a/b	Agtron E-5
		Without Heat		
90	10	10	2.73	26.5
80	20	0	2.64	30 8
90	5	5	2.64	30.5
80	10	10	2.56	31.6
70	20	10	2.46	32.7
		With Heat by Microwave Oven		
90	10	0	2.36	28.9
80	20	0	2.27	33.3
90	5	5	2.26	32.5
80	10	10	2.21	36.3
70	20	10	2.11	39.9

TABLE 3.—Correlation of Extraction Methods.

		Hunter a/b		Agtron E-5		Hunter T.C.	
X	Y	r	y =	r	y =	r	y =
B	B/D	0 987	1.08X — 0 18	0.845	0 85X + 2.13	0.676	0.55X + 37.55
B	CB	0.811	1.41X — 1.15	0.872	1 21X — 4.66	0.663	0.81X + 15.98
B	OSU/D	0.941	1.43X — 1.10	0.746	1.29X — 3.97	0.234	0.43X + 101.65
CB	B/D	0.781	0.49X + 1.40	0.896	0 65X + 7.01	0.271	0.18X + 65.49
CB	OSU/D	0 756	0.87X + 0.27	0.843	0 68X + 7.75	0.654	—0.44X + 107.70
B/D	OSU/D	0.919	0.67X + 0.91	0.824	0.48X + 10.73	0.225	0.10X + 72.60

B = Berkle Method
B/D = Berkle and Deaeration
CB = California Blendor Method
OSU/D = OSU Pulp Method and Deaeration

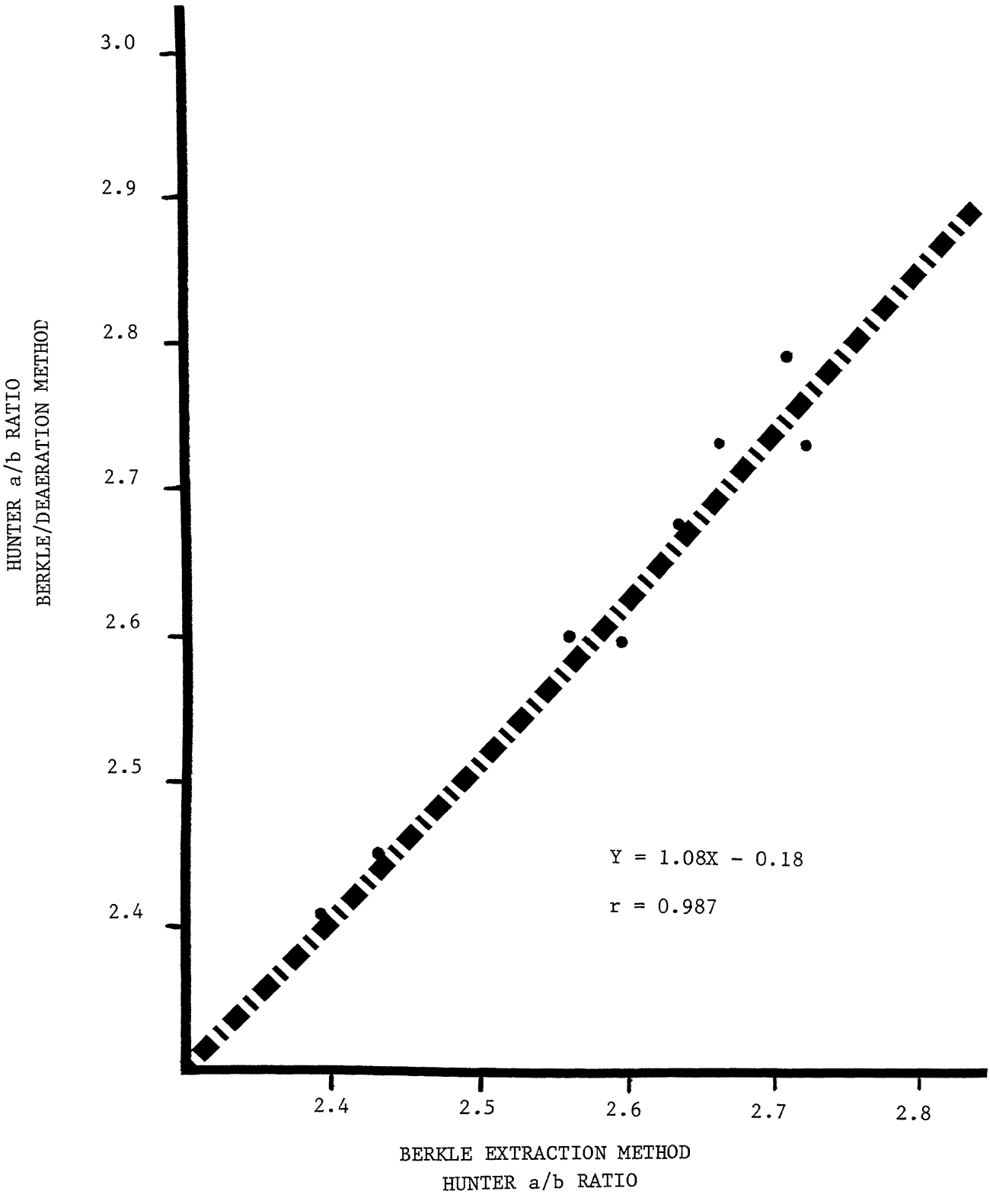

FIG. 1.—Relationship of Hunter a/b ratios of tomato juice extracted by Berkle (B) and Berkle-Deaeration (B/D) methods.

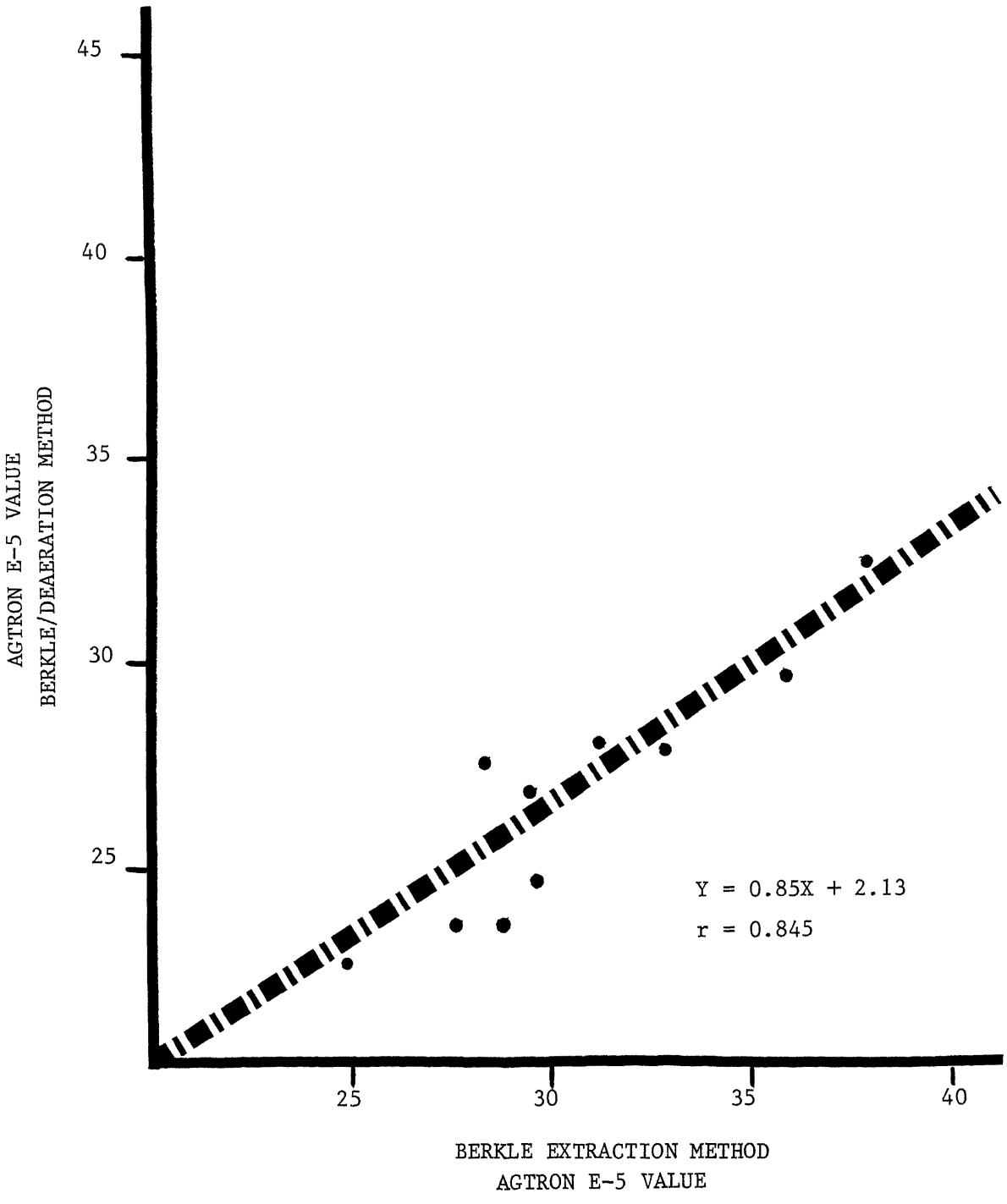

FIG. 2.—Relationship of Agtron E-5 values of tomato juice extracted by Berkle (B) and Berkle-Deaeration (B/D) methods.

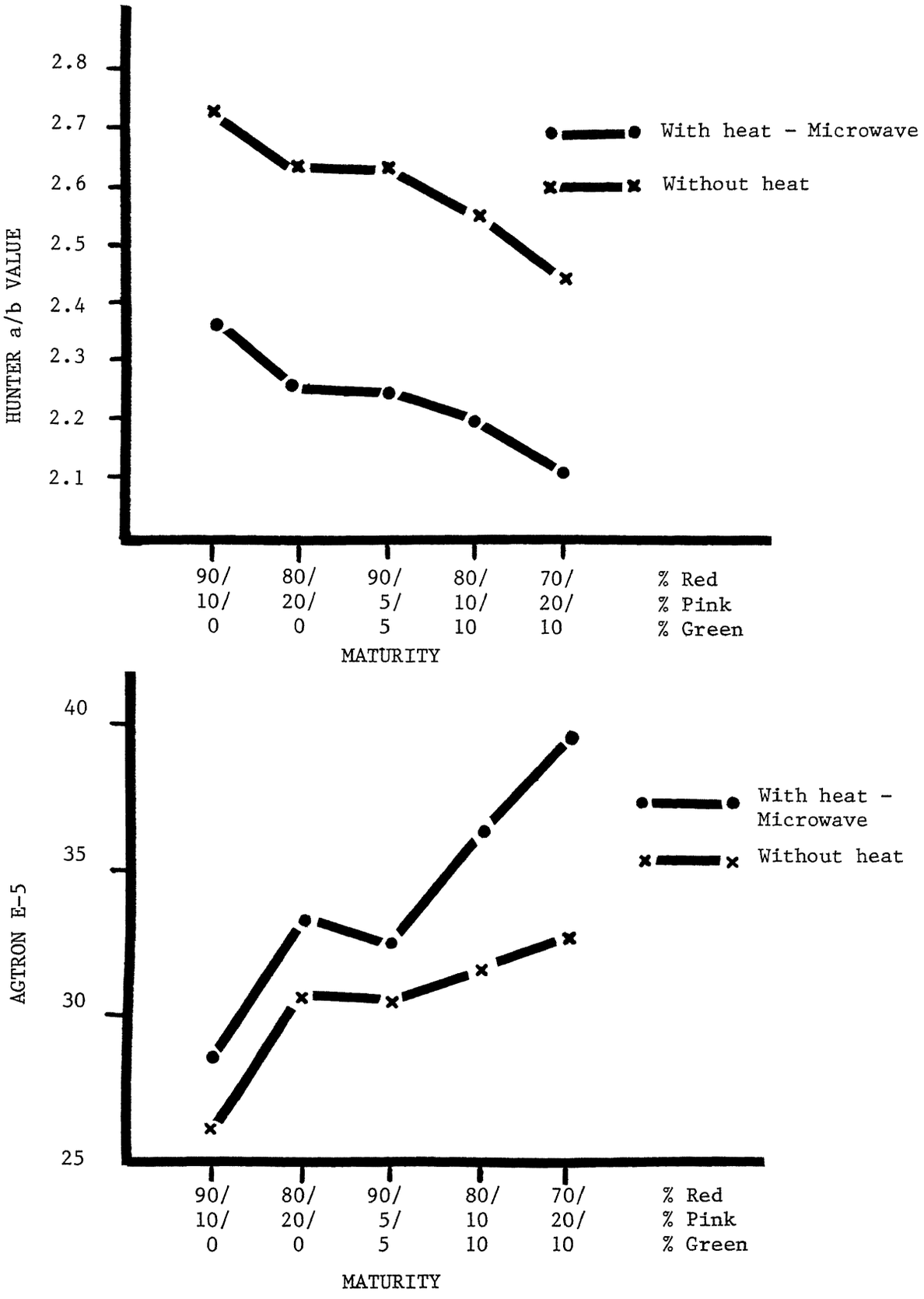
HUNTER a/b VALUE
2.8
2.7
2.6
2.5
2.4
2.3
2.2
2.1
With heat - Microwave
Without heat
90/ 10/ 0
80/ 20/ 0
90/ 5/ 5
80/ 10/ 10
70/ 20/ 10
% Red
% Pink
% Green
MATURITY
AGTRON E-5
40
35
30
25
With heat - Microwave
Without heat
90/ 10/ 0
80/ 20/ 0
90/ 5/ 5
80/ 10 10
70/ 20/ 10
% Red
% Pink
% Green
MATURITY

FIGURE 3

Protein Content of Seeds Taken from Tomatoes at Various Stages of Ripeness

DANIEL BRODOWSKI and J. R. GEISMAN[1]

INTRODUCTION

A majority of the processing tomatoes are made into liquid products such as juice, catsup, paste, and puree. The solid waste remaining after the extraction process consists of culls, peels, cores, seeds, and trimmings. The utilization of these solid waste products as a food source has only recently begun to be explored.

Solid waste is one of the few readily available and untapped resources in this country. Tomato pomace is now landfilled, incinerated, or fed to animals. Since tomato seeds comprise a majority of the solid waste generated, the utilization of tomato seeds as a protein source is an intriguing prospect for both limiting environmental pollution and feeding the world's expanding population. With this in mind, a project was undertaken to provide preliminary information which will be necessary in the eventual use of tomato seed protein. The main purpose was to determine the protein content and amino acid profile of tomato seeds at various stages of ripening in the tomato and also to determine the lipid content of tomato seeds at these stages.

MATERIALS AND METHODS

Two cultivars of tomatoes, Campbell 28 and Heinz 1350, were hand picked from The Ohio State University horticultural plot in Columbus. These cultivars were selected because of their popularity with the industry and were grown under accepted commercial practices for this area.

The tomatoes were sorted for maturity based on color observations. The Michigan State University classification for grading fresh market tomatoes was used (2). This includes six categories as follows: green—complete green in color; breakers—a definite change in color from green to tannish yellow, pink, or red on not more than 10% of the surfaces; turning—more than 10% but not more than 30% of the total surface shows a definite color change from green to tannish yellow, pink, red or combination thereof; pink—more than 30% but not more than 60% of the total surface shows pink or red; light red—more than 60% of the total surface shows pinkish red or red provided that not more than 90% of the surface is red; red—90% or more of the total surface shows red color.

After sorting the tomatoes according to color, the seeds were removed by cutting each fruit in half, squeezing the seeds and the surrounding gel into a large beaker. Approximately 25 ml of water were added to 200 ml of seeds and gel to decrease the consistency. This mixture was then blended in a Waring Blendor at low speed for 5 seconds to separate the gel from the seeds (1). The mixture was filtered and washed with water through a 12 mesh screen to remove the gel.

The seeds were then placed in containers and allowed to dry for 72 hours at 30° C. After drying, the seeds were ground in a Wiley mill fitted with a 20 mesh screen. Half of the ground seed meal was stored in sealed containers at 22° C until analyzed for amino acids and protein. The remainder was defatted with a 2:1 (v/v) ratio of chloroform and methanol. This defatted seed meal was dried in a Forester oven for 48 hours at 30° C.

Micro-Kjeldahl was used to determine the amount of crude protein in the dried whole and defatted seed samples. The amino acid profile was determined with the aid of a Durrum D-500 amino acid analyzer. The solvent used to defat the seed meal was filtered and filtrate placed in preweighed 250 ml beakers. The solvent was evaporated in a water bath at 60° C. Beakers were dried and weighed to determine the amount of lipid present in each sample.

RESULTS AND DISCUSSION

The results of the protein determinations are shown in Table 1 for both whole and defatted tomato seed meal. These results indicated that defatted tomato seed meal was slightly higher in protein content than the whole tomato seed meal. There was no dif-

TABLE 1.—Average Percent Protein Content of Whole and Defatted Tomato Seed Meal by Stage of Ripeness for Campbell 28 and Heinz 1350 Cultivars.

Ripeness	Campbell 28		Heinz 1350	
	Whole	Defatted	Whole	Defatted
	Percent Protein*			
Green	23.4	27.5	23.2	27.4
Breaking	23.7	27.4	23.4	26.9
Turning	23.3	26.7	22.9	26.7
Pink	23.5	27.1	23.5	27.0
Light Red	23.1	27.0	22.9	27.5
Red	23.1	27.2	23.2	27.0

*Dry weight basis, gm/100 gm.

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TABLE 2.—Average Amino Acid Content of Defatted Campbell 28 Tomato Seed Meal at Various Stages of Ripeness.

Amino Acid	Stage of Ripeness					
	Green	Breaking	Turning	Pink	Light Red	Red
			Percent*			
Aspartic Acid	5.06	4.98	4.06	4.76	4.02	4.06
Threonine	1.39	1.46	1.30	1.30	1.21	1.20
Serine	2.08	2.02	1.88	1.90	1.82	1.81
Glutamic Acid	6.76	6.68	6.41	6.50	6.47	6.45
Proline	2.02	1.99	1.85	1.85	1.86	1.87
Glycine	1.82	1.74	1.61	1.69	1.62	1.65
Alanine	1.61	1.57	1.45	1.48	1.42	1.43
Cystine	0.56	0.54	0.55	0.55	0.56	0.56
Valine	1.63	1.62	1.49	1.53	1.42	1.41
Methionine	0.51	0.49	0.52	0.49	0.56	0.55
Isoleucine	1.39	1.36	1.28	1.28	1.26	1.27
Leucine	2.33	2.30	2.17	2.14	2.07	2.09
Tyrosine	1.75	1.66	1.63	1.57	1.66	1.65
Phenylalanine	1.77	1.72	1.62	1.63	1.58	1.60
Histidine	0.86	0.80	0.77	0.77	0.82	0.81
Lysine	2.48	2.31	2.14	2.16	2.19	2.19
Arginine	3.72	3.64	3.39	3.49	3.31	3.37
Ammonia	0.83	0.81	0.72	0.80	0.74	0.72
Total	38.57	37.71	34.82	35.89	34.54	34.68

*Dry weight basis, gm/100 gm.

ference in protein content between cultivars. However, the most important conclusion from these data was that the change in stage of ripeness does not affect the protein content of the seeds. This means that any stage of maturity could be used for seed collection and would allow green tomatoes from mechanically harvested fields to be utilized as a source of seed protein.

Defatted tomato seed meal was found to be high in both glutamic acid and aspartic acid (Table 2). In general there was little change in amino acid composition of the seed as the tomato ripened. These results also indicated the same trend as pointed out for the data in Table 1. The higher total protein content (Table 2) is due to the utilization of a more purified protein sample. It should also be noted that tomato seeds were found to be an excellent source of lysine.

TABLE 3.—Average Percent Lipid from Campbell 28 Tomato Seeds at Various Stages of Ripeness.

Ripeness	Percent Lipid*
Green	28.1
Breaking	28.4
Turning	28.3
Pink	30.1
Light Red	31.3
Red	31.7

*Dry weight basis, gm/100 gm.

The results of the lipid composition indicate that the lipid content of tomato seed increases as the tomato ripens (Table 3). Since high quantities of lipid may interfere with protein extraction, it would be desirable to extract lipid material prior to grinding or milling for protein recovery. The lipid content that exists in red tomatoes would indicate that protein from green tomatoes could be extracted more readily than protein from the ripe tomatoes.

SUMMARY AND CONCLUSIONS

Disposal of solid waste is a major problem for tomato processors. One solution is to reduce the amount of tomato pomace by converting tomato seeds into usable by-products. Relatively high protein content of the seeds suggests their possible value as a food source.

The protein content and amino acid profile of tomato seeds indicated that there was little change as the fruit ripened. This is most beneficial in that culls from mechanical harvest as well as immature fruit can be used as a source of tomato seeds for protein extraction.

The lipid content of seeds increased slightly as the tomato ripened. Lipid accumulates to provide energy for the rapidly growing seedling during germination. Tomato seed oil is similar chemically and physically to a number of commercial vegetable oils (3). It is pale yellow in color and has a fatty nut-like taste with no bitter aftertaste. These character-

istics would allow tomato seed oil to also be used as a food product.

Solid waste has been described as a resource out of place. With current waste disposal and food problems, it is important to investigate the use of new resources. The study has described some basic information about tomato seed protein, amino acids, and lipids. These products could be utilized for human food, which would alleviate the disposal problem for a tomato processor as well as provide additional resources to feed the expanding world population.

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Effects of Cultivar, Break Temperature, and Extraction Methods on the Viscosity of Tomato Juice

HASHMY BEL-HAJ and WILBUR A. GOULD[1]

INTRODUCTION

Viscosity or consistency is one of the most important factors to be considered in determining the overall quality and acceptability of many tomato products. Viscosity is paramount as a quality attribute in determining the acceptability of tomato products by the consumer and is an integral part of the quality grade standard requirements (2).

Several factors have a great influence on the viscosity of tomato juice. Both the serum and the suspended solid particles contribute to the viscosity; the viscosity is further influenced by the character, size, and proportion of the suspended particles. In addition, many variations are possible in the chemical composition of the suspended solids, which will be partly governed by maturity and other characteristics of the tomato used and/or by the method of manufacture. Thus, the possible variations in the consistency and viscous particles of tomato juice are practically unlimited (2).

It has been shown that pectic substances are of considerable importance in determining consistency in tomato juice. In tomato juice manufacture, the freshly crushed tomato fruit (macerate) must be quickly heated to 180° F in order to inactivate pectic enzymes (2). If a lower temperature than this is employed, pectic enzymes remain active and rapidly alter the pectic substances and the resulting tomato juice is characteristically thin and watery.

McColloch *et al.* (7) found that there is a considerable loss in pectic substances when preheating temperatures are too low and the pectic enzymes are not inactivated, which leads to lower consistency. Luh *et al.* (6) reported that tomato pulp consistency depends on tomato variety and the quantity of pectic materials present in the puree. Luh *et al.* (5) reported that juices made from soft-ripe tomatoes were thinner in consistency than those made from firm-ripe fruits. This might be partially explained by the higher protopectic content in firm-ripe tomatoes.

Juice with proper consistency should have a smooth mouth feel and its solids should not separate readily from the serum. Consequently, the objectives of this study were to determine the effect of the following on consistency:

a) New cultivars
b) Break temperature
c) Extraction and pulping methods using different screen size openings at different extractor speeds.

MATERIALS AND METHODS

The experimental work described in this study was carried out in the Food Processing Pilot Plant and laboratories of the Department of Horticulture, The Ohio State University.

Tomato cultivars (C-37, 07667, 076170, 076-G1069, and 076G1034) were produced under the direction of the Vegetable Crops staff of the Department of Horticulture and were mechanically harvested at the OARDC Northwestern Branch near Hoytville. They were transported to the Pilot Plant in Columbus for processing.

Upon arrival, the tomatoes were sorted for quality (removal of defected and grass green fruits only)

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and were placed in an agitated soaker wash at 105° F. After approximately 3 minutes in the soak tank, fruits were moved on a roller conveyor under high pressure sprays (130 psi, square spray pattern) with each fruit turning about twice under the spray. After washing, the fruits were sorted to remove any other defective fruits. After sorting, the tomatoes were chopped or crushed followed by hot break at three different temperatures, 200° F, 175° F, and 150° F for 15, 36, and 45 seconds, respectively.

Following the heat treatment (hot break), the juice was extruded in a Langsenkamp extractor (screw type) with 0.023 screen size and a Langsenkamp pulper at different screen sizes and paddle speeds, ranging from 0.023 to 0.033 with varying speeds between 1150 to 1650 rpm. Following extraction, the juice was pumped to a holding tank where a sample of the juice was immediately filled in No. 303 plain tin cans and frozen at —17° F for extraction and detection of enzyme activity.

The remainder of the juice was then pumped to a Walker-Wallace paraflow heat exchanger (Model HT—four pairs of stainless steel plates HTST sterilization in which the juice was held at 250° F for 42 seconds). The juice was then filled in No. 303 fruit enamel cans with a 50 gram NaCl tablet added. After closing and holding for 2 minutes, they were spin cooled to a temperature of 100-105° F and then cased and stored for analysis of the following:

1) *Gross Viscosity:* Gross viscosity was measured with a GOSUC efflux-tube viscometer which has been described by Kluter (3). Viscosity values were expressed as time in seconds.

2) *Serum Viscosity:* Serum was obtained from juice by centrifugation at 10,000 rpm for 30 minutes. The serum viscocity was measured using an Ostwald-Cannon-Fenske viscometer. Viscosity was expressed as the ratio of the flow rate of serum to flow rate.

3) *pH:* The pH was measured by the glass electrode method (Beckman Zeromatic pH Meter), using 10 ml of tomato juice diluted with 90 ml of distilled water.

4) *Percent Total Acid as Citric*: The sample which was used for pH determination was titrated directly using 0.1N NaOH to a pH of 8.1 and calculating the total acid using the formula:

$$\% \text{ Acid} = \frac{(\text{No. ml of 0.1N NaOH}) \times 0.0064}{10 \text{ ml sample}} \times 100$$

Correlations were calculated and standard statistical methods (4) for viscosity by the Ostwald and GOSUC.

TABLE 1.—Effects of Break Temperature on Total Acidity and pH of Tomato Juice from Different Cultivars.

	150° F		175° F		200° F	
Cultivar	pH	T.A.	pH	T.A.	pH	T.A.
22	4.44	0.347	4.45	0.341	4.5	0.301
21	4.41	0.385	4.43	0.373	4.55	0.291
20	4.48	0.362	4.50	0.351	4.51	0.315
10	4.30	0.371	4.31	0.360	4.5	0.310

RESULTS AND DISCUSSION

Reported in Table 1 are pH and total acid values for the three break temperatures from four different cultivars. In every instance, the total acidity for the 200° F break temperature was lower than that for the 175° F or 150° F break temperatures. The difference in total acidity for the three different break juices may be explained by the enzymatic deesterification of pectin in the tomato pulp to form pectic and galacturonic acid by pectin esterase (PE) and polygalacturonase (PG). Preheating at 200-220° F causes rapid heat inactivation of PE and PG and this resulted in lower titratable acidity values in the product.

The effects of cultivar and break temperature are presented in Figure 1. The observed variation between cultivars is due to the nature of the chemical composition. The decrease of total pectin at lower break temperature was caused by the action of the enzyme PG on the pectic materials during maceration (5). The sample extracted at the highest break temperature (200° F) was higher in pectin retention due to the efficient heat inactivation of PG.

The effects of cultivar on gross viscosity and serum viscosity are presented in Figure 2. Juice from cultivars with high gross viscosity showed high serum viscosity; however, variation was observed between the cultivars. The relationship between gross viscosity and serum viscosity is presented in Figure 3. There was a good correlation between gross and serum viscosity.

Finisher screen size and paddle speed also had an apparent effect on gross and serum viscosity. The data in Figures 4 and 5 indicate that, in general, the higher the paddle speed and the smaller the screen size, the higher the gross and serum viscosity. This may be explained on the basis of relative numbers of particles present in the juice. When the finisher was operated at low speed (1150 rpm), fewer particles were incorporated into the juice (most of which were spherical) and the juice obtained was of low viscosity. Conversely, when the finisher speed was high (1650 rpm), greater numbers and concentrations of

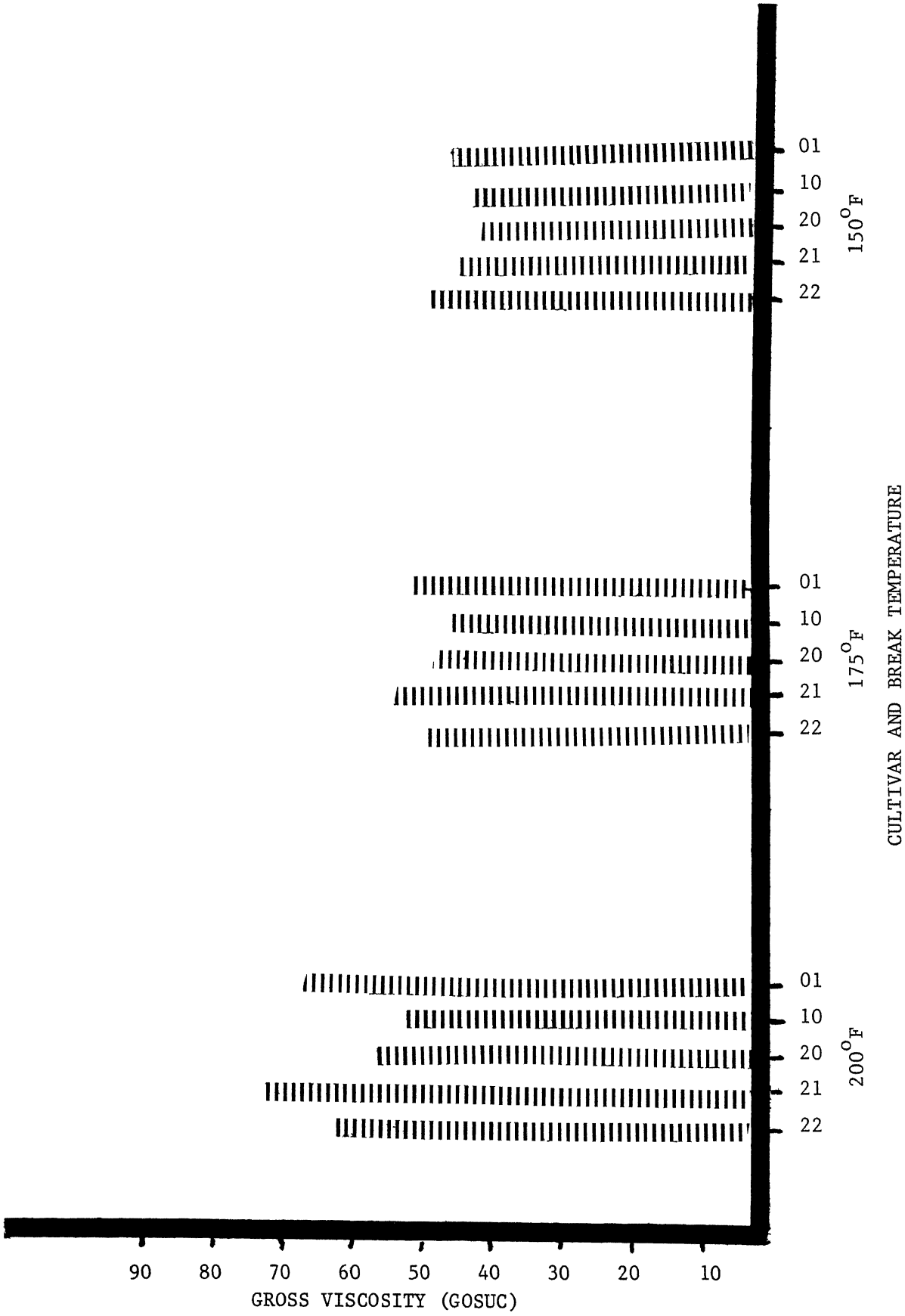

FIG. 1.—Effects of cultivar and break temperature on the gross viscosity of tomato juice.

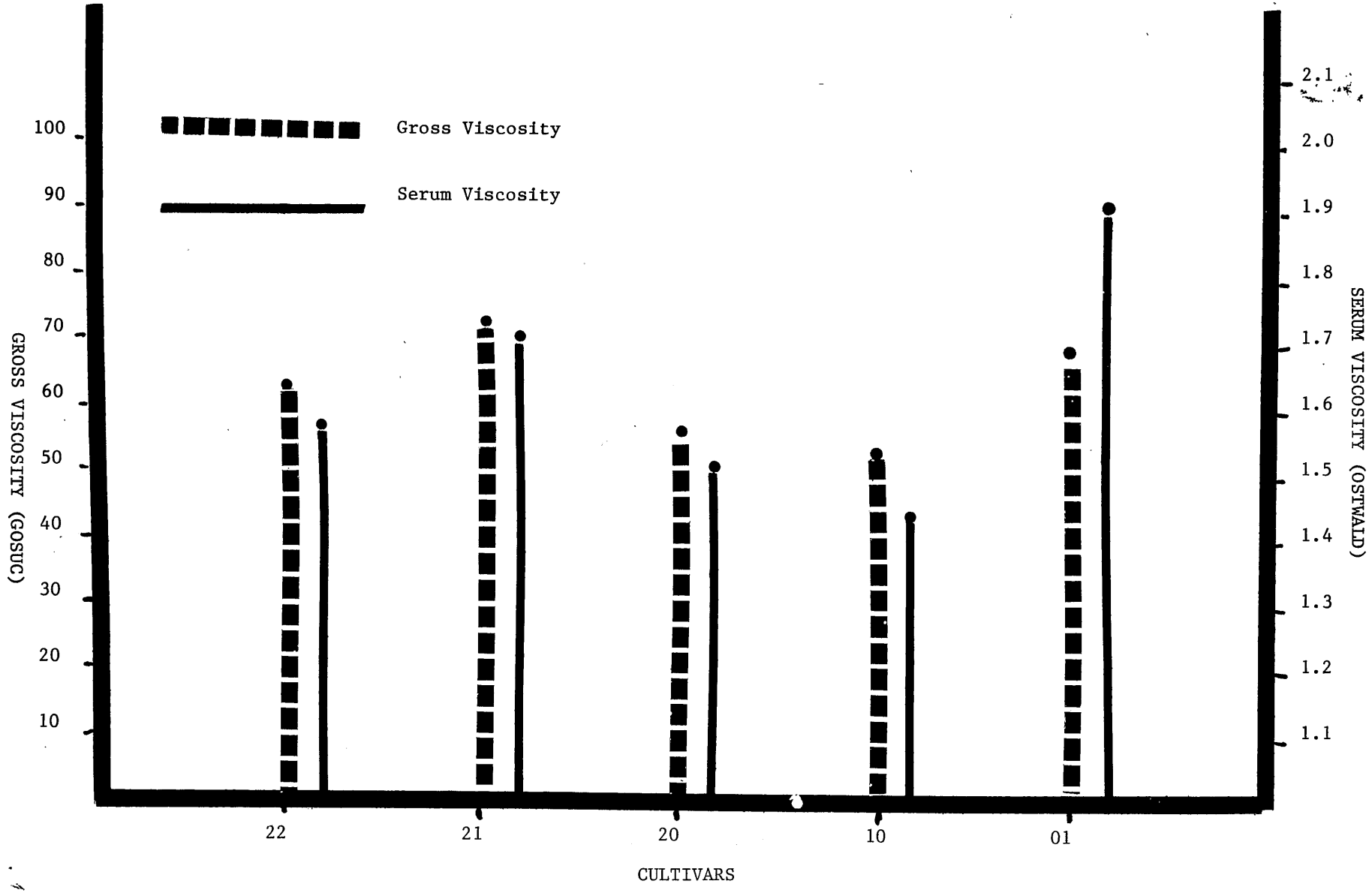

FIG. 2.—Effects of cultivar on the gross viscosity and serum viscosity of tomato juice.

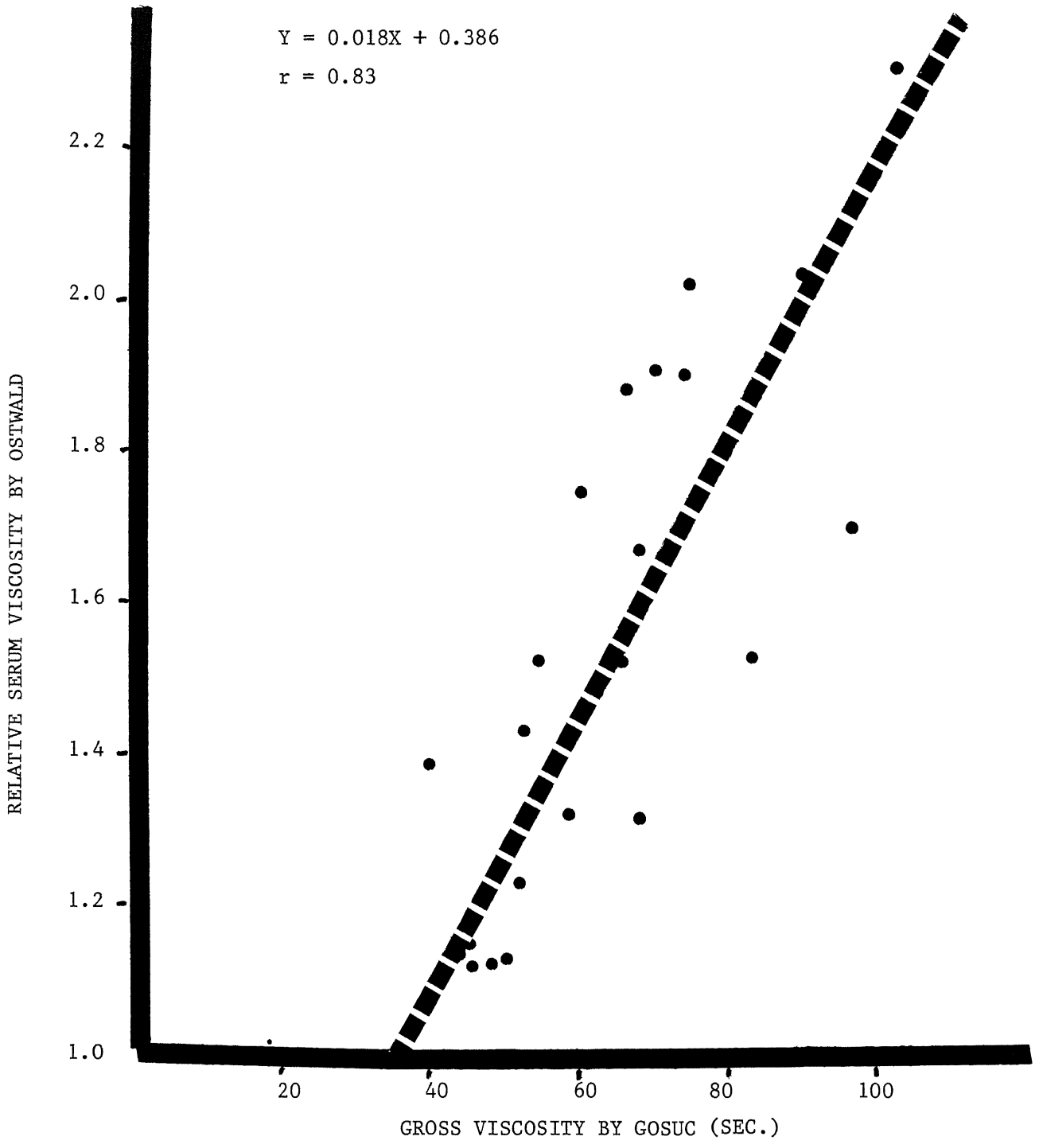

FIG. 3.—Relation between serum viscosity and gross viscosity.

particles were incorporated into the juice (most of which were elongated) resulting in higher viscosity. In addition, the finisher operated at 1650 rpm gave a higher gross and serum viscosity than the extractor operated at the same speed and screen size (0.023 inch).

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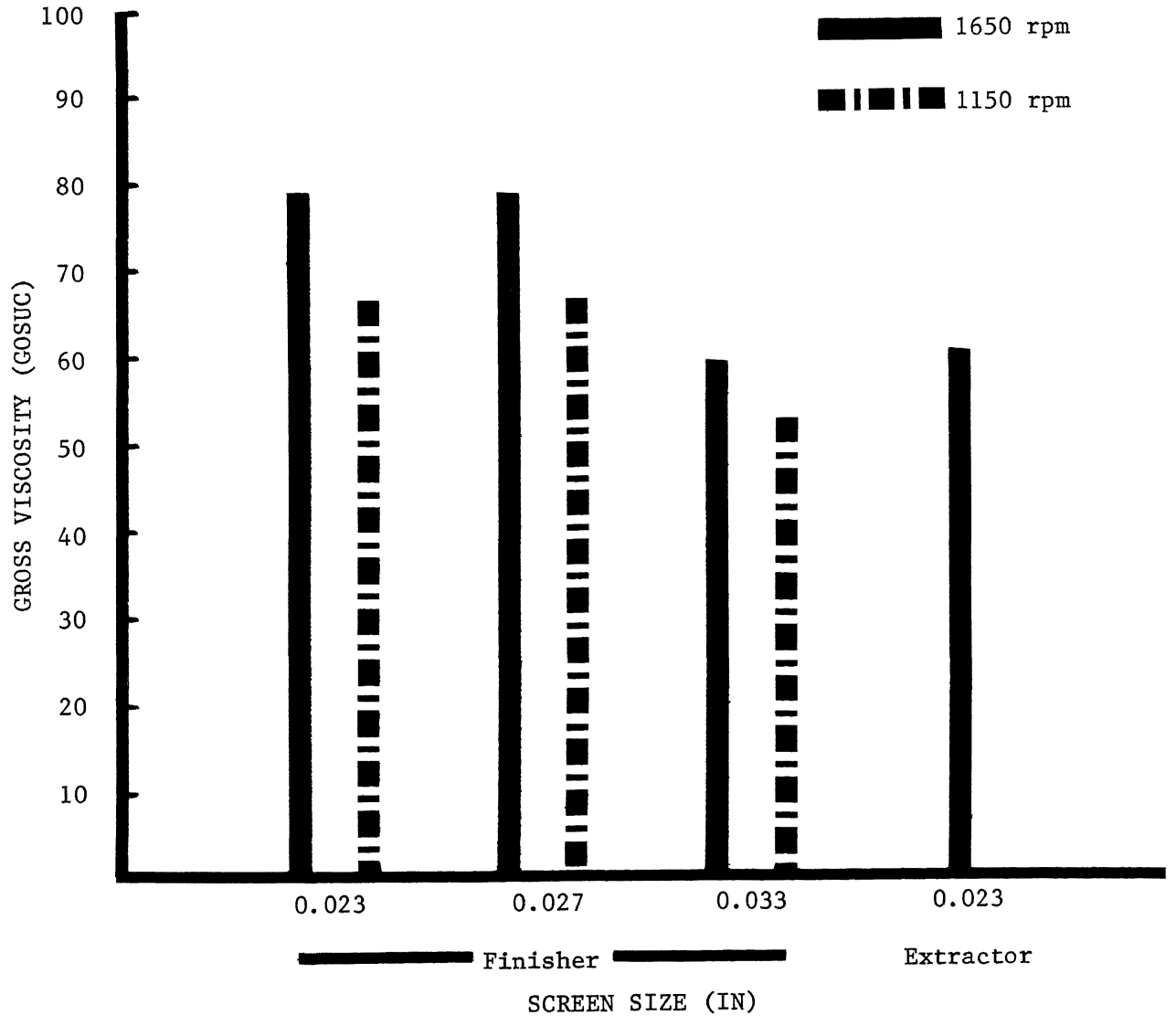

FIG. 4.—Effects of screen size and operating speed on gross viscosity of tomato juice.

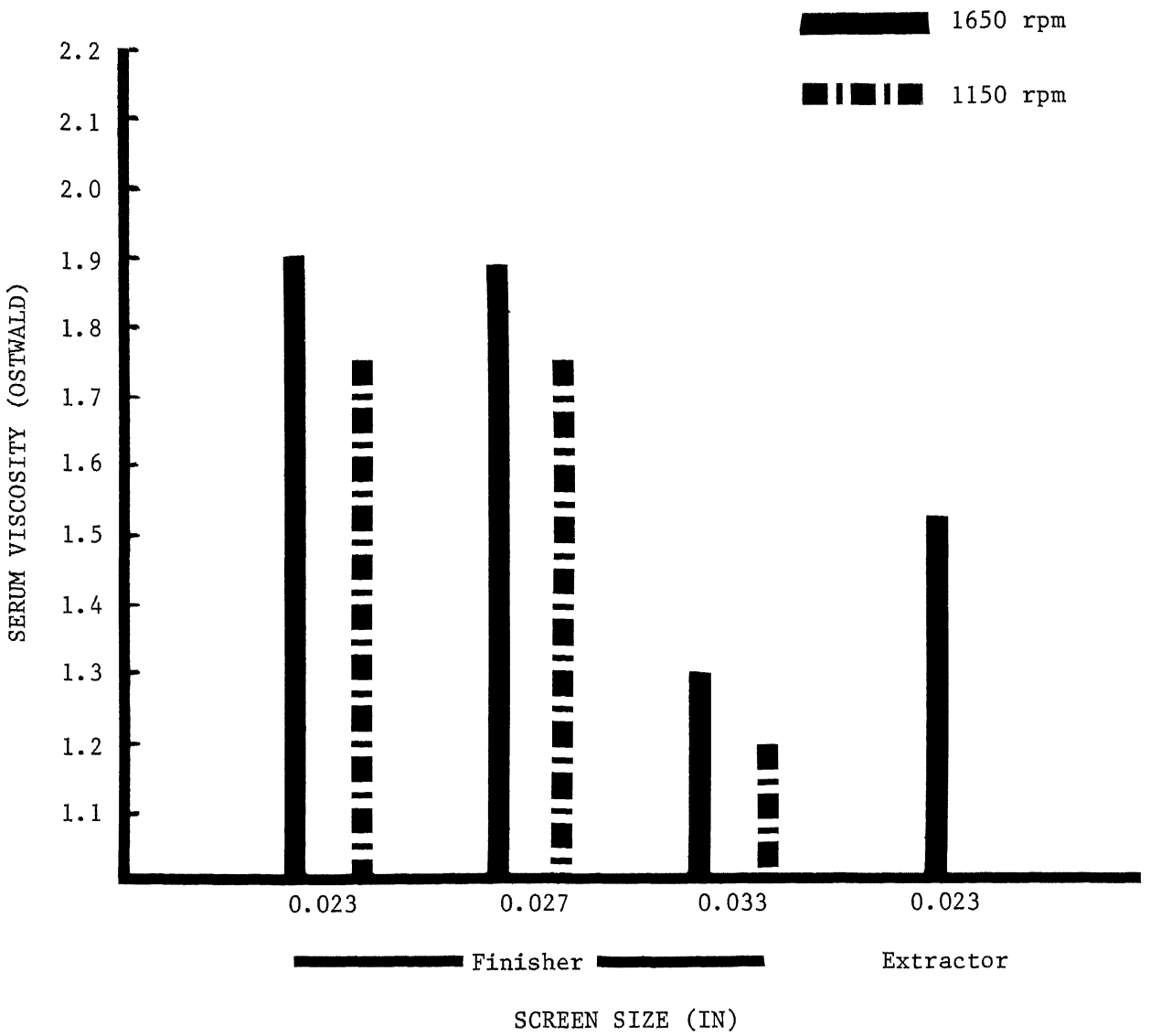

FIG. 5.—Effects of screen size and operating speed on serum viscosity of tomato juice.

A Study of Some Factors Affecting the Solid Content of Canned Tomatoes

J. R. MOUNT and W. A. GOULD[1]

INTRODUCTION

The Food and Drug Administration published a drained weight or solid content weight labeling proposal for canned fruits and vegetables in the Dec. 9, 1977, Federal Register (1). This proposal would require canned tomatoes, when packed in containers whose capacity is equal to or less than a No. 10 can, to bear on their label the net weight and a statement of the drained weight of the solid tomato content except that the solid content weight (fill weight) may be used in place of the drained weight.

The FDA also decided to evaluate the proposal during the 1977 and 1978 processing seasons by determining the variations occurring in the fill weights and drained weights within different processing plants and among different processing plants. The FDA was also going to look at the control procedures used by a packer to minimize the variation of the actual fill weight.

This study was initiated to determine the amount of variation occurring in fill weights and drained weights of 303x406 cans of whole tomatoes in Ohio tomato processing plants. The study also evaluated some of the factors affecting the variability of fill weights and drained weights of canned tomatoes.

MATERIALS AND METHODS

The study was divided between the Food Processing Pilot Plant at Ohio State University and several Ohio tomato processing plants.

During the summer of 1977, 20 cultivars of tomatoes were grown at the OARDC Northwestern Branch near Hoytville. They were mechanically harvested into bulk bins and transported to the Pilot Plant. Each tomato cultivar was processed separately within 24 hours after being delivered to the Pilot Plant. The tomatoes were initially separated in a water separator into two lots, one of red tomatoes and the other of green and overripe tomatoes. A portion of the red tomatoes was then processed as canned whole tomatoes. The tomatoes were prepared for canning by washing, lye peeling (18% caustic soda and Faspeel at 205° F for 35 seconds), and inspection for extra standard or fancy grade fruit.

The tomatoes were then filled at 9.0 to 9.5, 10.0 to 10.5, or 11.0 to 11.5 ounces into 303x406 fruit enamel tin cans. A 50-grain salt tablet containing 44.5% sodium chloride, 15% calcium sulfate, 37% citric acid, and 3.5% sodium bicarbonate was then added to each can and the tomatoes were covered with tomato juice at a temperature of 200° F. The cans were exhausted for 3½ minutes and then steam flow closed at 15 psi. The cans of tomatoes were processed at 210° F for 25 minutes, water cooled, and stored for 30 days before being opened and graded.

Eight Ohio tomato processing plants were selected on the basis that they packed whole tomatoes into 303x406 cans and each was given a code number with which to be identified. Twenty-one separate lots of canned tomatoes were evaluated from the eight plants during the 1977 processing season. Each lot of tomatoes evaluated had the following data recorded:

a) The cultivar and/or size of tomatoes being processed
b) The concentration, temperature, and time immersed in the caustic peeling solution
c) The filling method
d) The exhaust method
e) The initial temperature
f) The type, time, and temperature of the heating and cooling processes
g) The code on the cans of tomatoes
h) The grade of tomatoes being packed.

Fill weights were determined for groups of six cans at a time which were randomly selected every 10 to 20 minutes over a period of 2 to 3 hours for each lot of tomatoes. The fill weights were determined by allowing the cans of tomatoes to drain for 1 minute on a No. 2 size sieve and recorded to the nearest 0.1 ounce. Sample cans of tomatoes were obtained after they had been processed for determining the drained weights. These cans were transported to the Pilot Plant and stored for 30 days and then opened and graded.

RESULTS AND DISCUSSION

Cultivars: The cultivar of tomato processed was found to have a significant influence on the drained weight of cans of tomatoes. The average drained weights of the 20 cultivars of tomatoes processed in the Pilot Plant were found to be significantly different ($P \leq 0.01$) at each of the three fill weights at which they were packed (Table 1). The cultivars Chico III,

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TABLE 1.—Average Drained Weights of 20 Cultivars of Tomatoes Packed into 303 x 406 Cans at Three Fill Weights—9.0 to 9.5, 10.0 to 10.5, and 11.0 to 11.5 Ounces During 1977.

Cultivar	Average Drained Weights*			
	Fill Wt. (oz) 9.0-9.5	Fill Wt. (oz) 10.0-10.5	Fill Wt. (oz) 11.0-11.5	Cultivar Average
Chico III	9.24 a†	10.30 a	11.21 a	10.25 a
07668	9.10 ab	10.21 ab	10.99 abc	10.10 ab
076170	8.89 a-d	10.16 ab	11.13 ab	10.06 ab
07664	8.99 abc	9.99 abc	10.63 a-e	9.87 abc
07681	8.61 c-f	9.91 a-d	10.97 abc	9.83 abc
07678	8.58 c-f	9.78 b-e	10.76 a-d	9.70 bcd
076156	8.88 a-d	9.57 c-f	10.37 d-g	9.61 cde
076122	8.34 efg	9.40 e-h	11.20 a	9.61 cde
07630	8.61 c-f	9.51 d-g	10.56 b-f	9.56 cde
PU-74-74	8.50 def	9.62 c-f	10.54 b-f	9.55 c-f
C-37	8.50 def	9.54 c-g	10.49 c-g	9.51 c-g
076123	8.72 b-e	9.53 c-g	10.24 d-g	9.50 c-g
076121	8.63 c-f	9.37 e-i	9.98 fgh	9.33 d-h
076120	8.50 def	9.22 ghi	10.01 fgh	9.24 e-h
07635	8.29 efg	9.10 ghi	10.14 efg	9.18 fgh
Wakefield	8.03 g	9.22 ghi	10.30 d-g	9.18 fgh
076G1069	7.97 g	9.26 f-i	10.06 fg	9.10 fgh
076G1034	8.04 g	8.96 i	10.18 d-g	9.06 gh
07663	8.27 fg	9.09 ghi	9.49 h	8.95 h
07667	7.37 h	9.00 hi	9.95 gh	8.77 h
Average‡	8.50 x	9.54 y	10.48 z	

*10 cans of tomatoes were analyzed to determine each average drained weight.
†Values in a column followed by a common letter are not significantly different at $P \leq .01$.
‡Values in this row followed by a common letter are not significantly different at $P \leq .01$.

07668, and 076170 had significantly higher drained weights at all three fill weights than cultivars 07635, Wakefield, 076G1069, 076G1034, 07663, and 07667.

The size of the tomatoes being processed in the commercial processing plants varied and resulted in significant variations occurring in the average fill weights. Six lots of canned tomatoes from Plant 110 were evaluated and the four lots in which tomatoes size 6-7 tomatoes per pound were packed had an average fill weight of 10.49 ounces. The two lots in which tomatoes size 7-9 per pound were packed had an average fill weight of 11.24 ounces. The difference in fill weights resulted from more of the smaller tomatoes being packed into the cans when the same filling operations were used.

Plants 107 and 108 both had lots of canned tomatoes in which the size of the tomatoes varied in the middle of the lot. Both plants cored all of their tomatoes and hand filled them. The fill weights of the cans of tomatoes averaged 9.76 ounces when tomatoes size 5-6 tomatoes per pound were being filled. The fill weights decreased to an average of 8.70 ounces when tomatoes size 7-9 per pound were being filled. This resulted because the same number of tomatoes continued to be filled into the cans for the entire period of time; as the size of the tomatoes decreased, so did the fill weights.

Peeling Conditions: All of the tomato processing plants used approximately the same lye peeling conditions for the lots evaluated except for Plant 101. This plant increased the concentration and temperature of the caustic solution between the times when the first and second lots were evaluated. This resulted in the tomatoes in lot 2 being softer, which in turn resulted in the average fill weight for lot 2 being higher (12.04) than the fill weight for lot 1 (10.93). The percent of solid tomato material lost during processing was also affected, with the percent loss of lot 2 being 5.99% and for lot 1, 4.87%.

Filling Method: Four different methods were used to fill the cans with tomatoes in the processing plants. They were: hand filling, machine filling by volume with a hand top, machine filling by overflowing the cans with tomatoes and then a hand top, and machine filling by overflowing the cans but then vibrating the excess tomatoes out of the cans. The average fill weights obtained from the four methods were not significantly different ($P \leq 0.05$). The average fill weights from the different plants, however, were significantly different ($P \leq 0.01$). This was due to the differences in desired fill weights and to the amount of tomatoes the person or persons hand topping added to the cans. The average fill weights and drained weights for the plants are shown in Table 2.

Trimming Method: The two trimming treatments observed were coring of the tomatoes and not coring. The trimming method did not have an effect upon the fill weights but did influence the drained weights. The percent loss of solid tomato content in the cans which contained tomatoes that had not been cored was an average of 4.41% and was significantly higher ($P \leq 0.01$) in the cans which contained tomatoes that had been cored, where it was an average of 8.22%.

Thermal Process Method: The cans of tomatoes processed in the commercial plants were thermal processed in either a drag cooker or a rotary cooker. The processing methods did not significantly affect ($P \leq 0.05$) the average drained weights for the different lots of canned tomatoes.

Fill and Drained Weight Variations: The average fill weights, variances, and medians for the 21 lots of canned tomatoes evaluated during 1977 are shown in Table 3 and the average drained weights and variances are shown in Table 4. The average fill and drained weights are considerably different when all the plants are evaluated. However, when Plants 104 and 107 are disregarded since they did not attempt to pack extra standard tomatoes, the difference in fill weights is 9.89 to 12.04 ounces, a range of 2.15 ounces, and in drained weights is 9.11 to 10.99, a range of 1.88 ounces. These differences are approximately one average sized tomato and are mainly due to whether or not the person hand checking the fill of the can added an extra tomato.

TABLE 2.—Average Fill Weights and Drained Weights for 303 x 406 Cans of Tomatoes from Ohio Tomato Processing Plants.

Plant	Average Fill Wt. (oz)	Average Drained Wt. (oz)
104	13.70 a*	12.52 a*
103	11.88 b	10.79 b
101	11.56 b	10.67 b
110-f†	11.13 c	10.56 b
109	10.97 c	10.57 b
110-e‡	10.91 c	10.40 b
108	10.48 d	9.59 c
111	10.45 d	10.19 bc
110-s**	10.41 d	10.26 b
107	8.80 e	8.40 d

*Values in a column followed by a common letter are not significantly different at $P \leq 0.01$.
†Plant 110 packing fancy tomatoes.
‡Plant 110 packing extra standard tomatoes.
**Plant 110 packing standard tomatoes.

The average fill weights of the lots of tomatoes can be used to fairly accurately predict the average drained weight, as shown in Figure 1. The relationship of the two factors has a correlation coefficient of 0.961 which is significant at $P \leq 0.01$. The equation for estimating the drained weights from the fill weight is:

$$\text{drained weight} = 0.790\ (\text{fill weight}) + 1.677.$$

TABLE 3.—Average Fill Weights and the Variances of the 21 Lots of Canned Tomatoes from Ohio Tomato Processing Plants.

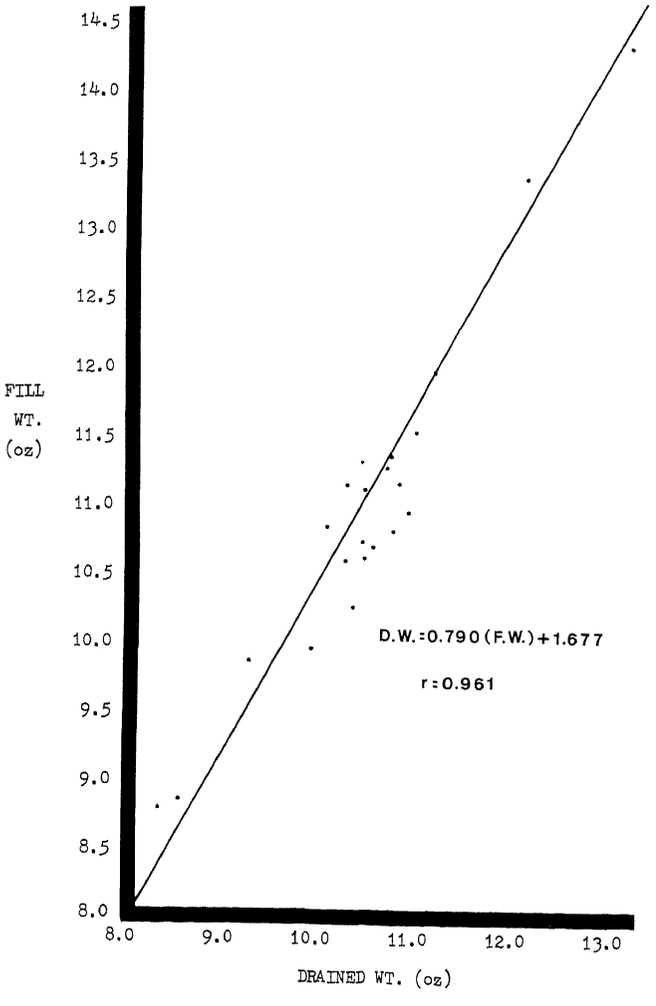
Plant	Week	Lot Average Fill Weight (oz)*	Lot Variance*	Lot Median Fill Weight (oz)
104	3	14.37 a	1.318 b-e	14.20
104	1	12.95 b	2.427 a	12.90
101	2	12.04 c	2.094 ab	12.05
103	2	11.88 c	1.479 a-e	12.20
110-e	3	11.34 d	1.363 a-e	11.30
110-f	3	11.13 de	1.134 def	10.80
108	2	11.13 de	2.405 a	11.15
109	2	11.09 de	1.301 b-e	11.15
109	3	10.97 def	0.976 ef	11.00
101	1	10.93 def	0.984 ef	11.00
109	1	10.80 efg	0.851 ef	10.70
111	1	10.74 e-h	0.582 f	10.65
110-e	2	10.65 e-h	1.315 b-e	10.70
111	2	10.57 fgh	1.238 b-e	10.50
110-e	1	10.53 fgh	0.747 ef	10.60
110-s	2	10.49 fgh	1.794 a-d	10.30
110-s	1	10.30 ghi	1.100 def	10.30
111	3	10.23 hi	1.188 c-f	10.30
108	1	9.89 i	1.999 abc	9.80
107	1	8.85 j	1.048 ef	8.60
107	2	8.81 j	1.324 b-e	8.60

*Values in a column followed by a common letter are not significantly different at $P \leq 0.01$.

TABLE 4.—Average Drained Weights and the Variances of the 21 Lots of Canned Tomatoes from Ohio Tomato Processing Plants.

Plant	Week	Lot Average Drained Weight (oz)*	Lot Variance*
104	3	13.03 a	0 456 b
104	1	12.00 b	1.626 a
101	2	10.99 c	1.470 a
103	2	10.79 cd	1.954 a
109	3	10.78 cd	0 485 b
109	1	10.61 cd	0.544 b
110-f	1	10.56 cd	0.826 ab
110-e	2	10.54 cd	0.960 ab
110-e	1	10.39 cd	0.664 ab
101	1	10.35 cd	0.936 ab
109	2	10.32 cd	0.789 ab
110-s	1	10.31 cd	0.550 ab
111	1	10.28 cd	0.487 b
110-e	3	10.28 cd	0.496 b
111	3	10.20 cd	1.520 a
110-s	2	10.20 cd	1.953 a
111	2	10.10 d	0.873 ab
108	2	10.06 d	2.239 a
108	1	9.11 e	1.539 ab
107	1	8.45 ef	0.355 b
107	2	8.19 f	0.794 ab

*Values in a column followed by a common letter are not significantly different at $P \leq 0.01$.



This indicates that the main factor influencing the drained weight of canned tomatoes is the fill weight.

The evaluation of the Ohio tomato processing plants showed that the fill weights of 303x406 cans of tomatoes are affected by tomato size, lye peeling conditions, and the filling operation. Changes in these factors will result in increased variation in the fill weights. The drained weights of 303x406 cans of tomatoes were affected by tomato cultivar, lye peeling conditions, trimming requirements, and fill weights. Changes in any of these factors will result in increased variation in drained weights.

The variation occurring in the fill and drained weights needs to be held to a minimum to be able to accurately label the cans of tomatoes with the fill or drained weight. Variations occurring due to changes in the processing factors can be kept under control by adequate auditing of the fill weights and making the necessary adjustments in the filling operations to maintain the fill weights at the declared label weight.

REFERENCE

1. Anonymous. 1977. Drained weight or solid content weight for fruits and vegetables; label statement, standards of fill of containers, and temporary labeling exemption. Fed. Register, 42(237): 66282-62305.

FIG. 1.—Regression line for comparison of average fill weights vs. average drained weights of 21 lots of commercially processed canned tomatoes.

Prediction of Ascorbic Acid Stability in Canned Apple Juice

MOHAMED I. MAHMOUD and WILBUR A. GOULD[1]

INTRODUCTION

Several researchers have proposed the fortification of apple juice with ascorbic acid since apple juice is naturally low in this vitamin and is used interchangeably in the diet with juices high in ascorbic acid (1, 2, 3, 9, 13, 21). Few studies, however, have been conducted for the purpose of predicting the retention of ascorbic acid in fortified apple juice. Strachan (21) indicated that a storage loss of at least 8 to 10 mg of added ascorbic acid is expected per 100 ml of apple juice processed under commercial methods and stored for 3 months. He pointed out that this loss occurs irrespective of the quantity of added ascorbic acid without presenting evidence on actual trials. Andreae (2) showed that 20-25% loss of ascorbic acid in fortified apple juice is expected after 4 months' storage at room temperature and predicted no additional loss would take place during further storage. Johnston (13) also predicted 30% loss of ascorbic acid in fortified apple juice after processing and 1 year storage at room temperature.

Other researchers have previously reported the loss of ascorbic acid in canned fruit and vegetable juices held in storage (10, 11, 12, 14, 15, 19). These investigators have observed varying decreases in the percentage of retained vitamin C with increased storage time and temperature. The reports of Semmelman (18) and Pope (16) suggested that ascorbic acid retention in fortified tomato juice may also be different depending on the level of fortification. Nevertheless, the most common method of reporting losses of ascorbic acid has been simply to express the vitamin content as a percentage of the initial amount present. These data do not allow the determination of kinetic parameters necessary to describe the effects of time and temperature on this nutrient.

Currently there is a dearth of information on the loss of nutritional value during storage from a kinetic standpoint. Knowledge of the reaction rate and kinetics of storage-induced losses is needed in order to predict the shelf life of important nutrients. This investigation was undertaken to study: 1) the effects of time, temperature, and fortification level on the retention of ascorbic acid; and 2) the degradation kinetics of ascorbic acid in apple juice in order to predict the retention of the vitamin in the fortified juice.

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MATERIALS AND METHODS

Materials

Grimes Golden and Jonathan apples (*Malus sylvestris,* L.) were grown and harvested at the Horticulture Farm of The Ohio State University and transported to the Food Processing Pilot Plant in the Dept. of Horticulture.

Juice Processing

The two apple cultivars were blended at the ratio of 1:1 by weight. The apples were sorted, washed, and ground in a hammer mill with a ½-inch screen to a pulp suitable for juice extraction. The juice was then extracted in a hydraulic press by the rack-and-cloth procedure. It was immediately flash pasteurized at 250° F for 30 seconds, cooled to 210° F, fortified with ascorbic acid in an 8-gallon filler bowl, and hot filled at 195° F into No. 303 cans.

Ascorbic Acid Fortification

To each 8 gallons of the juice were added 0, 50, 75, 100, or 150 ml of a solution of 90 g ascorbic acid (Merck U.S.P.) in 500 ml of the previously pasteurized and cooled apple juice. The added solution was calculated to increase the ascorbic acid concentration of the juice by 0, 30, 45, 60, or 90 mg/100 ml.

The juice was then carefully mixed and filled in No. 303 fruit enamel cans. The cans were sealed and coded to identify the fortification levels referred to hereafter as level 0, 30, 45, 60, or 90. The cans were inverted and then held for 3 minutes prior to spin cooling in water to 100° F. The cans within each fortification level were then divided into five groups and stored at 32, 50, 68, 86 and 104° F.

Sampling and Measurement

Two random samples from each fortification level were taken after cooling prior to storage. In addition, two other random samples from each fortification level were drawn at 3, 6, 9, and 12 months storage at each storage temperature. All measurements were made after the samples had been equilibrated at room temperature.

Reduced ascorbic acid content was determined colorimetrically according to the method of Strohecker and Henning (22). The absorbance of the sample, sample blank, and ascorbic acid standard solutions was measured at 570 nm on a Bausch and Lomb spectronic 20 spectrophotometer. Concentration of ascorbic acid in the sample was calculated

from a standard curve and is reported in mg/100 ml of the juice.

RESULTS AND DISCUSSION

Unfortified Juice

Ascorbic acid content of unfortified apple juice was 1.5 mg/100 ml after processing (Table 1). At all storage temperatures there was a very rapid loss of ascorbic acid in a very short time. These results are in general agreement with the reports of Bunnel (6), Esselen *et al.* (9), and Strachan (21). Strachan (21) found 0.2 mg/100 ml of unfortified canned apple juice after 2 weeks' storage at room temperature. It is evident from these data that ascorbic acid content of processed apple juice is negligible and ordinary canned apple juice is thus apparently valueless as an antiscorbutic product.

Fortified Juice

Results in Table 1 show that ascorbic acid content of fortified apple juice consistently decreased with time at all fortification levels. The retention of this vitamin was also adversely affected by higher temperature storage. The drastic effect of storage at 104° F was seen after 9 months' storage: no ascorbic acid was found in any juice even when fortified to 93 mg/100 ml. Juices stored at refrigerated and room temperatures also lost ascorbic acid, although not as rapidly as those stored at higher temperatures. These findings are consistent with the observations of several other workers. Bender (5), Guerrant *et al.* (12), and Pope (16) reported decreased ascorbic acid retention in fruit and vegetable juices with increase in storage time and temperature.

A factorial analysis of variance (20) of the results indicated that fortification level, in addition to storage time and temperature, significantly ($P < 0.01$) influenced ascorbic acid retention. As the fortification level increased, the retention of the vitamin decreased.

To illustrate the effect of fortification level, linear regressions were used to determine the relationship between ascorbic acid loss and time at each temperature, using the linear function $y = a + bx$. The results of the linear regression fit and correlation coefficients are presented in Table 2. The high correlation coefficients obtained indicate that a linear relationship between loss of ascorbic acid and time exists. Using the F value test, the relationships are all shown to be significant at the 1% level.

Results in Table 2 show that the rate of ascorbic acid loss, expressed as mg/100 ml/month, increased

TABLE 1.—Effects of Fortification Level, Storage Time, and Temperature on Concentration of Ascorbic Acid in Apple Juice.

Fortification Level	Temperature °F	Storage Time in Months				
		0	3	6	9	12
		mg. Ascorbic Acid/100 ml Juice				
0	32	1.5	1.0	0.0	0.0	0.0
	50	1.5	1.0	0.0	0.0	0.0
	68	1.5	0.0	0.0	0.0	0.0
	86	1.5	0.0	0.0	0.0	0.0
	104	1.5	0.0	0.0	0.0	0.0
32	32	28.5	28.0	27.5	26.5	24.0
	50	28.5	26.5	25.0	24.5	22.0
	68	28.5	24.0	22.5	22.0	19.5
	86	28.5	23.5	21.0	16.0	12.5
	104	28.5	22.0	9.5	0.0	0.0
45	32	47.5	46.0	43.5	42.0	39.5
	50	47.5	43.0	40.5	38.0	35.0
	68	47.5	38.5	37.5	35.5	31.0
	86	47.5	38.0	33.5	28.0	19.5
	104	47.5	32.0	14.0	0.0	0.0
60	32	66.5	65.0	59.0	60.5	54.0
	50	66.5	62.0	58.0	53.0	48.5
	68	66.5	54.5	54.0	48.5	42.5
	86	66.5	55.0	46.5	38.0	25.0
	104	66.5	44.0	15.0	0.0	0.0
90	32	93.0	84.5	82.0	78.0	73.5
	50	93.0	80.0	75.5	74.5	66.0
	68	93.0	71.5	70.5	67.0	58.5
	86	93.0	71.5	58.5	46.5	30.5
	104	93.0	57.0	18.0	0.0	0.0

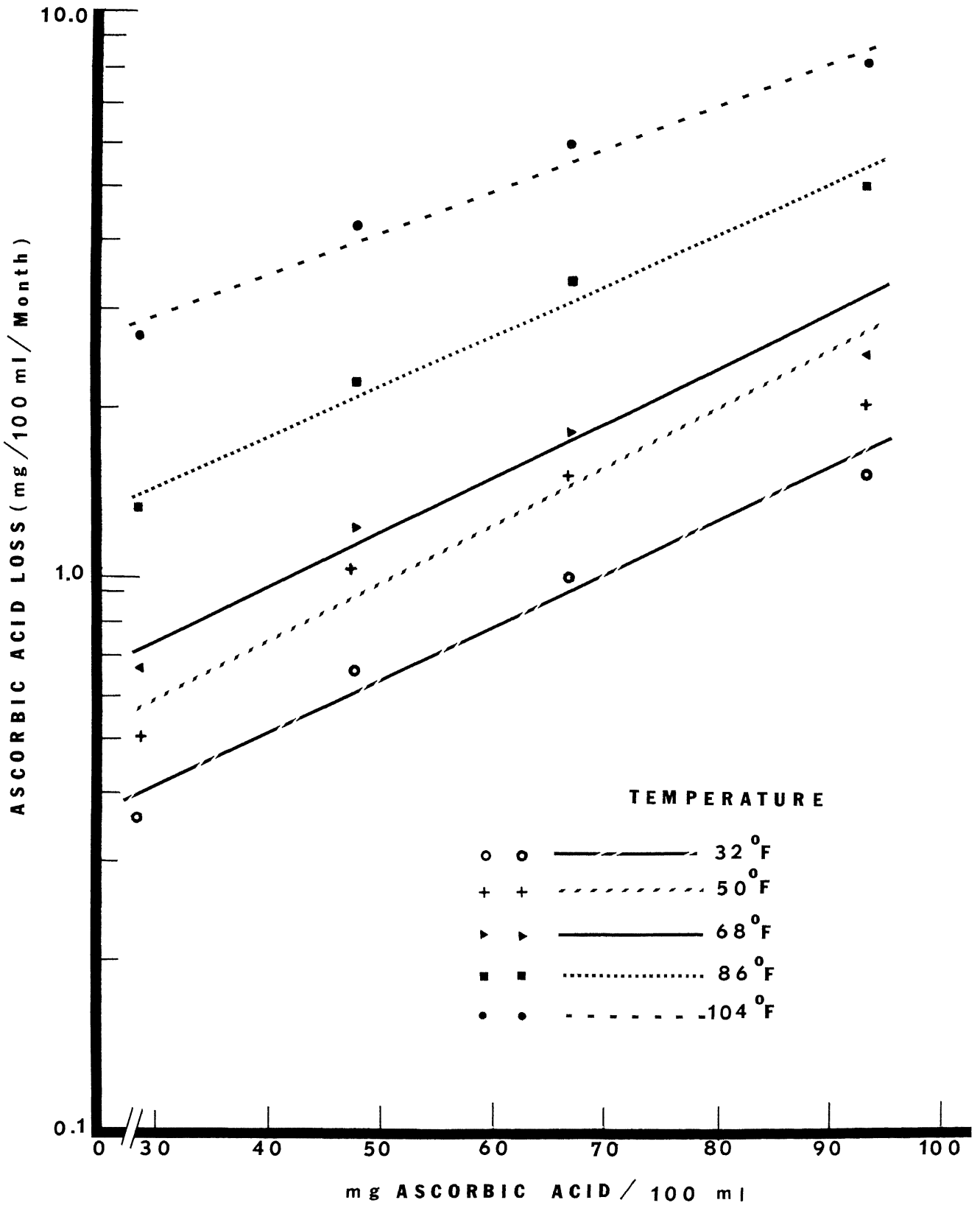

FIG. 1.—Relationship between rate of ascorbic acid loss expressed as mg/100 ml/month and initial ascorbic acid concentration in fortified apple juice.

TABLE 2.—Coefficients Describing Relationship Between Rate of Ascorbic Acid Loss and Temperature of Storage in Fortified Apple Juice.

Fortification Level	Temperature °F	a	b	r	S.E.E.	F Value
30	32	29.0	—0.350	—0.85	.07615	21.12*
	50	28.3	—0.500	—0.88	.09354	28.57*
	68	27.3	—0.667	—0.93	.09538	48.85*
	86	28.2	—1.317	—0.97	.10721	150.84*
	104	27.8	—2.633	—0.97	.24552	115.04*
45	32	47.7	—0.667	—0.89	.12388	28.95*
	50	46.8	—1.000	—0.94	.13202	57.37*
	68	45.2	—1.200	—0.92	.18690	41.22*
	86	46.5	—2.200	—0.98	.14884	218.48*
	104	44.1	—4.233	—0.96	.41020	106.50*
60	32	66.9	—0.983	—0.92	.15241	41.62*
	50	66.6	—1.500	—0.98	.10992	186.20*
	68	64.0	—1.800	—0.95	.20983	73.59*
	86	66.3	—3.342	—0.99	.11818	799.50*
	104	60.5	—5.900	—0.95	.65490	81.16*
90	32	91.3	—1.517	—0.94	.19284	61.85*
	50	89.7	—1.983	—0.93	.28019	50.10*
	68	86.8	—2.450	—0.90	.42894	32.62*
	86	90.0	—5.000	—0.99	.19983	626.08*
	104	82.2	—8.100	—0.95	.97365	69.21*

a = intercept.
b = rate of ascorbic acid loss, mg per 100 ml per month.
r = correlation coefficient.
S.E.E. = standard error of estimate.
* = significant at the 1 % level.

with increasing fortification level. By plotting the rate of ascorbic acid loss (mg/100 ml/month) on logarithmic scale vs. initial ascorbic acid concentration (Fig. 1), the results indicate that the rate of loss is also a logarithmic function of initial ascorbic acid concentration. These findings support the results of Beattie *et al.* (4) and Pope (16), who reported increased ascorbic acid loss with increasing the initial concentration of the vitamin.

Order of Ascorbic Acid Destruction

Semilogarithmic plots of ascorbic acid concentration in fortified juice vs. time yielded straight lines, indicating that the relationship could be described by a first-order reaction mechanism. The slope of the resulting line is k/2.303 according to the first-order reaction equation:

$$k = \frac{2.303}{t} \log \frac{C_o}{C}$$

where k is the rate constant of ascorbic acid destruction, C_0 is the initial ascorbic acid concentration, and C is the vitamin concentration at time t in months.

The results in Fig. 2 illustrate a typical first-order reaction between ascorbic acid concentration and time at 68° F. Plots of first-order reaction at other storage temperatures gave the same linear relationship. As shown in Fig. 2, the regression lines were nearly parallel for all fortification levels stored at the same temperature and had almost the same slopes. The rates of ascorbic acid destruction were computed from equation (1). No significant difference was observed between the rate constants of ascorbic acid destruction at all fortification levels stored at the same temperature.

The values of k (Table 3) indicate that the rate constants of ascorbic acid destruction in fortified apple juice are relatively small at refrigerated and room temperature (68° F), while the reverse is true at accelerated storage temperatures. This suggests

TABLE 3.—Destruction Rate Constants and Half-lives of Ascorbic Acid in Fortified Apple Juice at Constant Temperature.

Temperature (°F)	k (Months^{-1})	Half-life* (Months)
32	0.01588	43.6
50	0.02410	28.8
68	0.03158	21.9
86	0.07606	9.1
104	0.22877	3.0

*Half-life = 0.693/k

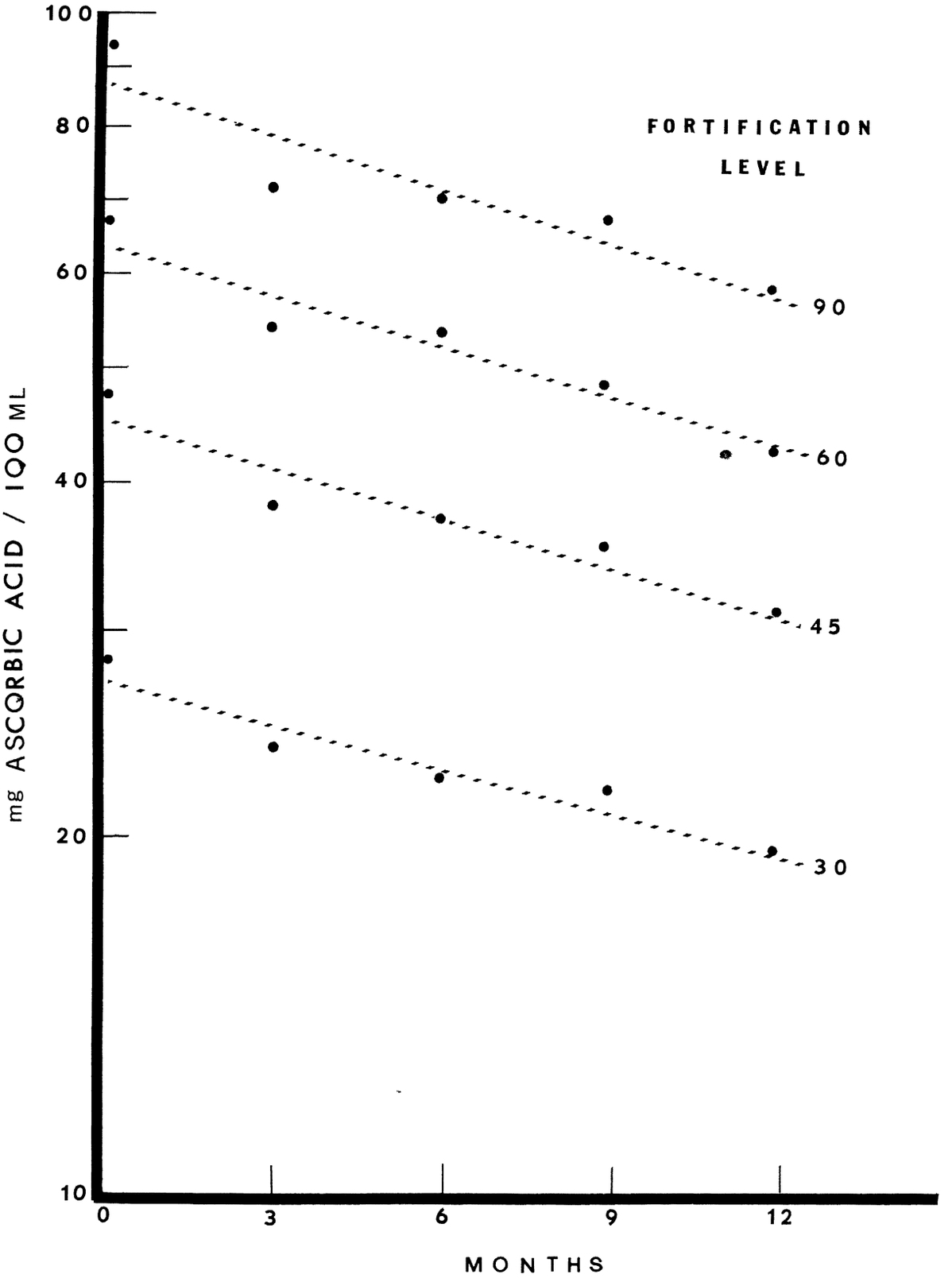

FIG. 2.—First order plots of ascorbic acid destruction in fortified apple juice stored at 68° F.

negligible loss of the vitamin at lower temperatures of storage.

Half-life of Ascorbic Acid

The half-lives (0.693/k) of ascorbic acid in fortified apple juices are presented in Table 3. These values represent the time required to destroy 50% of ascorbic acid originally present, thus yielding useful information as to the shelf life of the vitamin C in this product. The estimated half-life data clearly demonstrate the expected excellent shelf life of ascorbic acid in juice at refrigerated temperatures.

Favorable shelf life would also be anticipated when the juice is held at room temperature; slightly less than 2 years will be required to reduce the original ascorbic acid content by 50%. Projections from accelerated temperatures show a sharp decrease in the shelf life. It is interesting to note that calculated half-lives at high temperature give a shorter shelf life than observed. This gives a safety factor for the juice processor since shelf life is actually longer. For example, at 86° F the actual shelf life is about 1.7 months longer than that predicted for 50% destruction of the vitamin.

Q_{10} of the Rate of Ascorbic Acid Destruction

The temperature coefficient (Q_{10}) of the rate of ascorbic acid degradation was calculated from the relationship:

$$Q_{10} = \frac{k_{(T)}}{k_{(T-10)}}$$

where $k_{(T)}$ is the reaction rate constant at temperature T_1 and $k_{(T-10)}$ is the reaction rate constant at 10° C (18° F) below T_1.

The calculated Q_{10} values (Table 4) show that between 32° and 68° F, the rate of ascorbic acid destruction increases approximately one and a half times for each 18° F (10° C) rise in temperature. It can also be seen that by raising the storage temperature from 68° to 86° F, the rate slightly more than doubles; increasing the temperature from 86° to 104° F triples the rate at which ascorbic acid is lost from the product.

Again, the dramatic effect of increased storage temperature on the rate of ascorbic acid degradation can be readily seen. The present findings compare favorably with those reported by Bender (5) for the rate of ascorbic acid degradation in fruit squash. Ross (17), however, indicated that increasing the storage temperature from 80.5° to 98.6° F (27° to 37° C) quadrupled the rate of ascorbic acid loss (mg/ml/month) in orange juice.

Rate of Ascorbic Acid Destruction as a Function of Temperature

The calculated rate constants were plotted on an Arrhenius type plot (Fig. 3) vs. the reciprocal of absolute temperature. The corresponding activation energy (E_a) was then computed from the Arrhenius plot. The results, illustrated in Fig. 3, show that the data do not fit exactly on a straight line; two rather distinct curves are obtained. It appears that a change in the kinetics of the reaction occurs between 68° and 86° F, as evidenced by the break in the curve at approximately 75° F. Above 75° F, the activation energy is almost four times as much as that below 75° F. This demonstrates that changes in temperature above 75° F have a more significant influence on the rate at which ascorbic acid is lost from the product.

This change in observed kinetics between 68° and 86° F suggests that another mechanism, which

TABLE 4.—Initial Concentration (C_0) Required to Produce 60 mg Ascorbic Acid per 8-Fluid-Ounce Serving of Apple Juice.

Time (Months)	Temperature (°F)							
	50	55	60	65	70	75	80	85
1	61.4	61.5	61.7	61.9	62.1	62.3	63.3	64.7
2	63.0	63.1	63.5	63.8	64.2	64.6	66.7	69.7
3	64.5	64.8	65.4	65.8	66.4	67.0	70.3	75.1
4	66.1	66.4	67.2	67.9	68.7	69.6	74.2	81.0
5	67.7	68.2	69.2	70.1	71.1	72.2	78.2	87.3
6	69.3	69.9	71.2	72.3	73.6	74.9	82.5	94.1
7	71.0	71.7	73.2	74.5	76.1	77.7	86.9	101.4
8	72.8	73.6	75.4	76.9	78.8	80.7	91.7	109.3
9	74.5	75.5	77.5	79.3	81.5	83.7	96.7	117.8
10	76.3	77.4	79.8	81.8	84.3	86.9	101.9	127.0
11	78.2	79.4	82.1	84.4	87.2	90.1	107.5	136.9
12	80.1	81.5	84.5	87.0	90.2	93.5	113.2	147.6

has a less limited reaction step, may be governing the rate of change here. The finding that the mode of ascorbic acid destruction in fortified apple juice deviated from an Arrhenius relationship is similar to that observed by Charm (7) when analyzing data collected by Dietrich *et al.* (8). Charm (7) observed a change in kinetics of ascorbic acid destruction in frozen spinach betwen 59° and 68° F (15° and 20° C). Pope (16) also indicated that the rates of ascorbic acid degradation in fortified tomato juice did not sat-

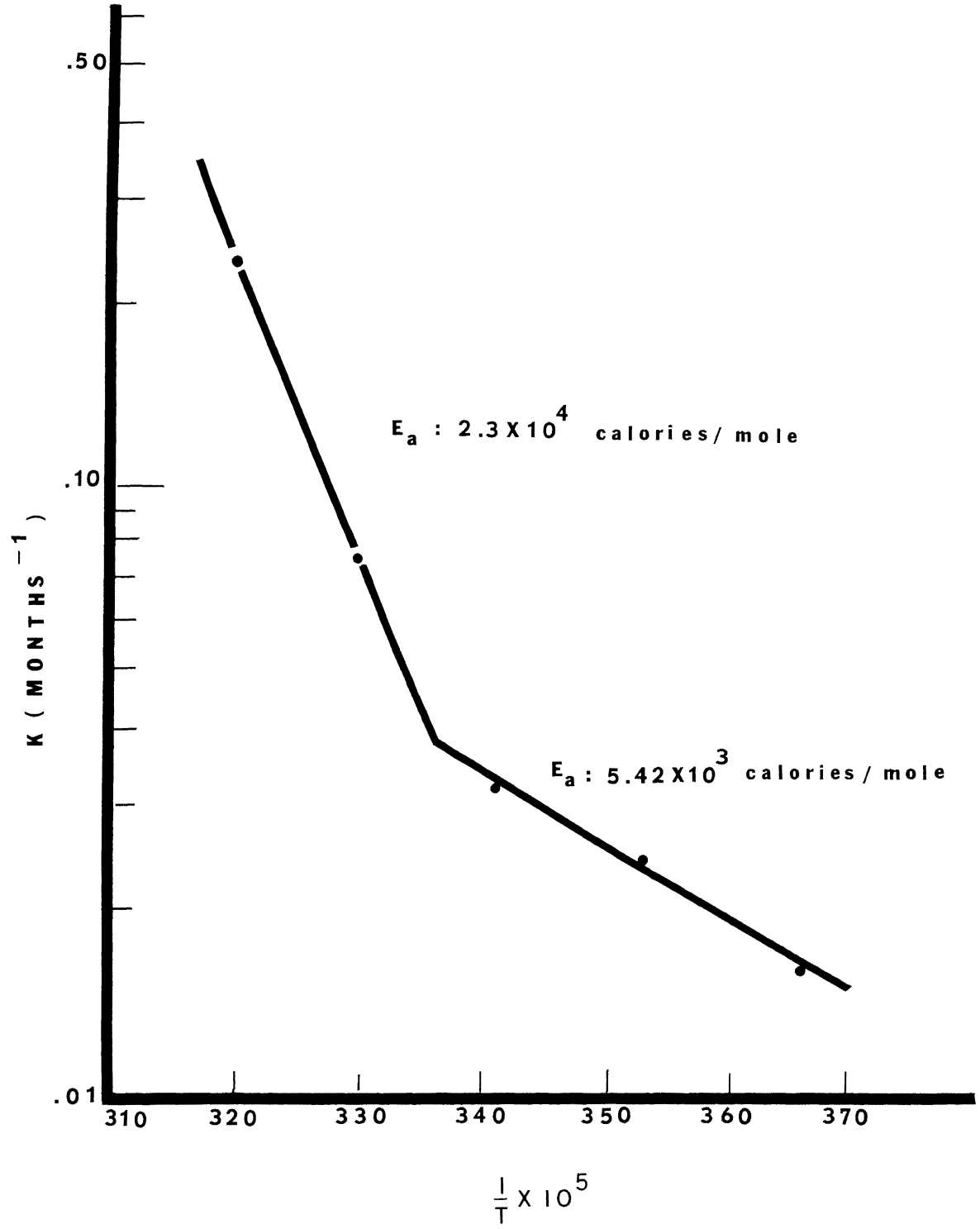

FIG. 3.—Effect of storage temperature on the rate of ascorbic acid destruction in fortified apple juice (Arrhenius plot).

isfactorily fit an Arrhenius equation.

It should be noted that fortified apple juice held at 104° F developed a dark brown color and a caramelized flavor. Therefore, k values for ascorbic acid destruction above 86° F, while valid, do not give any information about the acceptability of apple juice.

The above discussion illustrates that various kinetic parameters can be used to compare degradation of ascorbic acid under different storage conditions and that the kinetic analysis is particularly useful in predicting the shelf life of ascorbic acid in the fortified juice. By measuring the concentration of ascorbic acid over a period of time, parameters such as reaction rate constants and activation energies can be calculated. Thus, the initial concentration of ascorbic acid can be calculated knowing the desired vitamin level at the end of shelf life when storage time and temperature conditions are specified.

For example, rearranging equation (1) yields:

$$2.303 \log \frac{C_o}{C} = kt$$

$$\frac{C_o}{C} = 10^{\frac{kt}{2.303}}$$

$$C_o = C \cdot 10^{\frac{kt}{2.303}}$$

The initial concentration (C_o) required to give 100% of the recommended daily allowance (RDA) of ascorbic acid per 8-fluid-ounce serving of fortified apple juice under given storage conditions and duration have been calculated from the data obtained in this study and appear in Table 4. Similarly, an apple juice processor can determine the necessary initial concentration of ascorbic acid required to meet his label claim after specific storage time and temperature.

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Investigation of Protein Content of Ohio Grown Soybeans

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INTRODUCTION

Soybean has been the number one cash crop in the U. S., surpassing both wheat and corn for several years. This year, according to a USDA report (5), soybean plantings will set a new high, 63.9 million acres. This is also expected to set a new production record, 1.74 billion bushels as compared to 1.72 billion bushels in 1977.

Soybeans in the United States were originally mainly used as hay and silage before their value with respect to oil and protein content was realized. The good quality of oil and proteins and high quantity of unit production made the soybean itself a success in the food and feed market.

The protein content of soybeans is affected by many factors, such as variety, cultural practices, environment, etc. This study was undertaken to investigate the protein content of 30 varieties of Ohio-grown soybeans and their milk derivatives in order to provide such information to the Ohio soybean food industry and others who are interested. Soybean milk has been adopted by the food industry as a milk substitute for infants who are allergic to cow's milk, or where cow's milk is either unavailable or too expensive.

MATERIAL AND METHODS

Thirty varieties of soybeans were obtained from the Department of Agronomy, The Ohio State University (Table 1).

Soybean samples weighing about 250 g were ground in a Wiley mill. The moisture content and crude protein were determined in duplicate.

Moisture was determined by weight difference in such a way as to dry a predetermined sample in a recirculating air oven at 100 ± 2° C for 12 to 14 hours. After cooling in a desiccator, the sample was reweighed and the percentage of moisture was calculated.

Crude protein was determined by a modified Kjeldahl method (1) and multiplied by the factor 6. 25.

Soybean milk was prepared from 100 g of representative, clean, full fat soybeans. The sample was soaked in about 350 ml of tap water overnight. The water was drained off and the sample reweighed to determine the amount of water absorbed. The beans were blended for 3 minutes with 500 g of tap water in a Waring Blendor. This slurry was filtered through four layers of cheesecloth and the residue was extracted again with 400 g of tap water. The final bean:water ratio was 1:10 (w/w).

Both the residue and the milk were weighed. The soybean milk was boiled for 10 minutes to destroy antinutritional factors, and cooled to room temperature. The loss of weight during boiling was readjusted by adding water back. The moisture content and crude protein were again determined as mentioned above.

RESULTS AND DISCUSSION

The moisture content of soybeans was in the range of 8.5% to 10.7% (Table 1), with a mean value of 9.3%. This is not consistent with the findings of DeMan *et al.* (3), 4.2% to 7.8%; and Bourne *et al.* (2), 9.7% to 14.7%. This discrepancy indicates that the moisture content depends upon many factors, such as variety, growing conditions, precipitation, harvesting, handling, and storage conditions.

The moisture content of soybean milk ranged from 93.1% to 94.7% (Table 1) with a mean value of 93.6%, which reflects total solids ranging from 5.3% to 6.9%. DeMan *et al.* (3) investigated the total solids of soybean milk of 55 varieties of Ontario-grown soybeans and found they averaged 4.7% to 7.1%, which agrees with this study.

The crude protein content ranged from 36.9% to 44.1% (Table 1), which was lower than that reported by Hymowitz *et al.* (4), 33.1% to 49.2%; and DeMan *et al.* (3), 30.3% to 46.1%. This variation

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could be attributed to the fact that a large sample population was used by these investigators. But the results of this study were higher than those reported by Bourne *et al.* (2), who examined 30 Philippine-grown soybeans and found a range of protein content from 27.3% to 36.2%. The four highest protein varieties were Calland (44.1%), Wells (43.5%), FFR 223 (43.5%), and Wayne (43.5%). French 2150 (36.9%), Asgrow 2625 (37.8%), and Cutler 71 (38.8%) were the varieties with lowest crude protein levels.

Soybean milk was found to have a crude protein content, on a fresh weight basis, of 2.3% to 3.5% (Table 1). Of course, this varies with different soybean:water ratios when soybean milk is made. This value is slightly higher than that found by DeMan *et al.* (3), whose protein range was 1.8% to 3.6%; and about the same as reported by Bourne *et al.* (2), 2.3% to 3.5%. The varieties Calland (3.5%), SKB 400 (3.4%), Wells (3.4%), Wayne (3.4%), and FM 1220 (3.4%) were highest in protein, whereas French 2150 (2.3%), FFR 223 (2.7%), Asgrow 2625 (2.8%), and Clemens (2.9%) ranked lowest in protein content in the soybean milk.

The amount of water absorbed by 100 g of soybeans during an overnight soaking with tap water was 122 to 144 (Table 1). This information would eliminate an unnecessary step of weighing the soaked beans and adjusting to the right bean:water ratio in order to control the exact solids content.

Most varieties were of a light yellow color. The exceptions were Washington II, Shawnee, FM 1220, Cherokee, and Anderson, which were green or light green, and Peterson 3105, SKB 400, Peterson 3125, and Voris B 350, which were pink colored. The green and pink pigments, although soluble in water and partially removed in the soaking water, were still retained to certain degree in the soybean milk.

Except for the varieties French 2150, FFR 223, and Asgrow 2625, this study revealed that all other varieties tested were suitable for soybean milk production as far as protein content was concerned.

TABLE 1.—Protein and Moisture Content of Soybeans and Soymilk (%).

Ohio-Grown Cultivar	Soybeans		Soymilk		
	Moisture	Protein (dry wt basis)	Moisture	Protein (fresh wt basis)	Water Absorbed g/100 g sample
French 2150	10.1	36.9	94.2	2.3	135
Clemens	9.3	39.2	93.9	2.9	134
Asgrow 2625	9.1	37.8	94.0	2.8	130
FFR 223	10.2	43.5	94.7	2.7	126
Agripro 20	9.5	40.1	93.9	3.0	135
Amsoy 71	10.7	40.7	93.9	3.1	137
Shawnee	8.7	41.9	93.4	3.4	130
Corsoy	9.0	40.4	93.4	3.2	136
Wells	8.6	43.5	93.4	3.4	138
CX 215 Pfizer	8.8	40.7	93.3	3.3	130
Peterson 3105	8.8	41.3	93.3	3.3	134
SKB 400	8.5	41.6	93.1	3.4	130
Gries	8.5	39.5	93.5	3.1	136
FM 1220	8.5	42.9	93.6	3.4	131
Beeson	9.2	42.9	93.2	3.3	138
Cherokee	8.5	40.8	93.7	3.2	139
Wayne	9.3	43.5	93.5	3.4	135
SRF 307 P	9.1	40.6	93.4	3.2	132
Woodworth	9.4	41.1	93.5	3.2	132
Calland	10.3	44.1	93.3	3.5	144
101644	10.0	41.9	93.6	3.2	128
Anderson	9.4	42.3	93.5	3.3	137
Washington II	9.4	41.3	93.6	3.2	136
Funks seed	8.5	40.5	93.8	3.0	131
Ruff RB 34	9.7	42.6	93.2	3.3	126
Williams	9.9	40.4	93.6	3.2	124
Peterson 3125	9.8	41.2	93.3	3.3	122
Cutler 71	9.0	38.8	93.4	3.1	131
Mitchell	9.6	39.0	93.7	3.0	134
Voris B 350	10.0	40.8	93.6	3.2	125
Mean Value	9.3	41.1	93.6	3.2	133
Range	8.5 - 10.7	36.9 - 44.1	93.1 - 94.7	2.3 - 3.5	122 - 144

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Recovery and Functionality of Soybean Leaf Proteins

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INTRODUCTION

As the world population continues to expand almost exponentially to an anticipated 7 billion by the year 2000, the need for all nutrients, especially protein, will be acute (7). The rate of world food production is falling behind the rate of population growth in many of the developing countries. Protein-calorie malnutrition has drawn international recognition. Surveys made by the Food and Agriculture Organization (FAO), World Health Organization (WHO), and other agencies indicate that protein-calorie malnutrition prevails in developing countries. This problem has escalated manyfold and an estimated 50% of the world's population suffers from malnutrition.

Worldwide protein needs for today's population are estimated at 70 million metric tons of good quality animal protein, and by the year 2000 this need will exceed 110 million metric tons (10). Protein-calorie deficiency combined with infections of the acute and fatal protein deficiency disease, kwashiorkor, retard physical growth and development and impair learning capacity and behavior (11).

Leaf protein is the most abundant protein source. Amino acids are translocated from leaves to seeds. Both leaves and seeds are consumed by animals but a great deal of potentially usable protein is lost due to poor conversion efficiency. Although the nutritional quality of animal foods is rated high, animal protein production is intrinsically inefficient, involving a waste of about 75% of the calories and proteins supplied (1).

The protein problem cannot be solved through a single source of protein, conventional or unconventional. Interest in leaf protein concentrates for animal and human consumption has increased in recent years as a result of an expected worldwide need for alternate protein sources and a desire to derive maximum usefulness from agricultural crops.

In developing a new protein source, consideration should be given to yield, nutritive value, and functionality. A protein having higher yield with poor functionality is of little value to a food industry. Preparation of protein in soluble form is most desirable since solubility and other functional properties of undenatured proteins are interrelated and important for food application. Processing treatments, *e.g.*, extraction, precipitation, drying, inactivation of various antinutritive factors or deleterious enzymes may produce protein denaturation and subsequent insolubilization.

Soybean (*Glycine max.* (L.), Merr) is the number one cash crop in the U. S. Therefore, a study of soybean leaf proteins was undertaken to evaluate: 1) yield, 2) functionality, and 3) mineral and amino acid composition of leaf protein concentrates (LPC).

MATERIALS AND METHODS

Phase I. Extraction and Recovery of Soybean Leaf Protein Concentrates

At 45 days after planting, soybean leaves of cultivar Beeson were collected from the agronomy plots at Don Scott Field in August 1976. The leaves were stripped from the first branch down the middle axis of the plant. Approximately 40 lb of leaves were collected and processed as follows (5). In the treatment, all operations were the same as in the control except a 250 ppm SO_2 equivalent sodium metabisulfite solution was added to the fresh leaves after washing (5).

Phase II. Functional Properties

1. *Nitrogen Dispersibility.* This functional property at pH 5, 7, and 10 was studied by a modified procedure of Betschart and Kohler (3).

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2. *Water Absorption.* The water absorption ability of the concentrates was evaluated using the modified procedure of Sosulski (12).

3. *Fat Absorption.* The procedure of Betschart and Kohler (3) was modified to determine this property of the LPC using cottonseed oil and corn oil.

4. *Foaming Ability.* This experiment was performed according to the procedure of Yasumatsu *et al.* (14).

TABLE 1.—Extraction and Recovery of Soybean Leaf Protein Concentrates (%).

Sample*	Protein Yield	
	by Weight	by Kjeldahl
CA	1.09†	0.77
CH	0.89	0.76
MA	1.10†	0.78
MH	0.91	0.78

*C = control, A = acid pptd, H = heat coagd, M = metabisulfite.
†$P < 0.01$.

TABLE 2.—Nitrogen Dispersibility of Soybean Leaf Protein Concentrates (%).

Sample*	pH	
	5	7
CAF†	1.26 a	1.28 a
CAO	0.82 b	0.84 b
CHF	0.91 b	0.94 b
CHO	0.80 b	0.79 b
MAF†	1.33 a	1.40 a
MAO	0.83 b	0.78 b
MHF	0.92 b	0.97 b
MHO	0.79 b	0.81 b

*C = control, A = acid pptd, F = freeze dried, M = metabisulfite, H = heat coagd, O = oven dried.
†$P < 0.01$.
Means in columns with different letters are significantly different ($P < 0.01$).

TABLE 3.—Water Absorbed by Soybean Leaf Protein Concentrates (%).

Sample*	pH		
	5	6	7
CAF†	275.3 a	301.7 b	294.0 b
CHF†	302.7 b	324.3 d	355.7 e
MAF†	285.3 a	300.0 ab	294.3 ab
MHF†	309.0 b	337.0 d	362.3 e
CAO	93.7 c	91.0 c	94.3 c
CHO	89.0 c	90.7 c	88.3 c
MAO	91.3 c	96.0 c	94.3 c
MHO	84.3 c	90.7 c	90.0 c

*C = control, A = acid pptd, F = freeze dried, M = metabisulfite, H = heat coagd, O = oven dried.
†$P < 0.01$.
Means in both columns and rows with different letters are significantly different ($P < 0.01$).

5. *Emulsifying Ability.* The modified procedure of Yasumatsu *et al.* (14) was employed to determine the emulsifying capacity of the protein concentrates.

Phase III. Minerals and Amino Acids

1. *Mineral Determination.* Samples were ashed using a modified procedure of Chapman and Pratt (4). Elements of Ca, Mg, and Fe were determined by atomic absorption spectrophotometry, Varian Atomic Absorption Spectrophotometer, while P was determined by a modified molybdate-vanadate procedure (2).

2. *Amino Acid Composition.* The samples were hydrolyzed following the procedure of Ishino and Ortega (9) and analyzed by ion exchange chromatography on a Durrum D-500 amino acid analyzer.

RESULTS AND DISCUSSION

Phase I. Extraction and Recovery of Soybean Leaf Protein Concentrates (LPC)

Recovered and dried soybean LPC were expressed as percent on a fresh leaf weight basis. Data in Table 1 show that acid precipitation had significantly ($P < .01$) influenced protein recovery by weight, while Kjeldahl nitrogen was statistically insignificant due to the recovery method. All oven-dried samples were hard, dark, and gritty mixtures upon milling, while freeze drying produced soft, spongy, nongritty, bland, and off-white to light tan colored concentrates. Similar observations were made in alfalfa and pine LPC (3, 8). The difference in protein yield by weight was attributed to the coprecipitation of acidic polysaccharides (3).

1. *Nitrogen Dispersibility.* Data in Table 2 reveal that recovery and drying methods significantly ($P < .01$) influenced nitrogen dispersibility. All freeze dried, acid precipitated samples had higher dispersibility than the samples recovered or dried by heat. An important functional property of recovered proteins is their dispersibility at various pH values. In addition to serving as a useful indicator of protein denaturation, the dispersibility may also suggest possible uses, especially in liquid based products. Similar findings were also recorded in alfalfa LPC (3). In spite of poor dispersibility, these protein concentrates can be used by dispersing in a solid food system.

2. *Water Absorption.* This test showed that all freeze dried samples, irrespective of recovery method and pH, had a significantly ($P < .01$) higher water absorbing capacity (Table 3). All heat coagulated, freeze dried samples showed consistently higher absorption due to pH increment. Acid precipitated samples had lower absorption capacity at pH 7.0. However, heat drying reduced this property to one-third that of the freeze dried concentrates. The grit-

tiness due to heating reduced the effective surface area of the samples, while freeze drying resulted in porous concentrates having more surface area which displayed this difference among the samples. Similar results were obtained in alfalfa LPC from freeze and spray drying (3).

3. *Fat Absorption.* Data presented in Table 4 show a statistical significance ($P < .01$) in oil absorption due to recovery and drying methods. All freeze dried samples had about three to four times higher oil absorbing tendency than the oven dried concentrates. Betschart and Kohler (3) found similar behavior in alfalfa LPC. A similar explanation can be offered as described for water absorption capacity.

4. *Foaming Ability.* Data are presented graphically in Fig. 1. Analysis of variance disclosed that recovery and drying methods and pH significantly ($P < .01$) influenced foaming property. Freeze dried, acid precipitated samples displayed ability superior to the concentrates prepared by other methods. Increased foaming was associated with the alkaline pH. Such a property was noticed in alfalfa LPC (3).

5. *Emulsifying Ability.* Data in Fig. 2 reveal that recovery and drying methods and pH all significantly ($P < .01$) influenced the emulsion formation ability. Maximum emulsifying ability was obtained at all pH levels from the acid precipitated, freeze dried samples of both control and treatment. Alkaline pH exhibited increased emulsifying ability. However, oven dried samples were not affected by pH. Vananuvat and Kinsella (13) also found higher emulsion in alkali treated proteins and lower values due to heat treatment during processing of single cell proteins.

7. *Mineral Content.* Calcium, Mg, and Fe contents of the protein concentrates were significantly

TABLE 4.—Oil Absorbed by Soybean Leaf Protein Concentrates (%).

Sample*	Source of Oil	
	Cottonseed	Corn
CAF†	264.4 a	266.8 a
CHF†	236.8 b	238.2 b
MAF†	265.4 a	268.9 a
MHF†	234.8 b	235.7 b
CAO	89.8 c	86.2 c
CHO	86.6 c	87.2 c
MAO	93.3 c	92.7 c
MHO	85.5 c	84.2 c

*C = control, A = acid pptd, F = freeze dried, M = metabisulfite, H = heat coagd, O = oven dried.

†$P < 0.01$.

Means in columns with different letters are significantly different ($P < 0.01$).

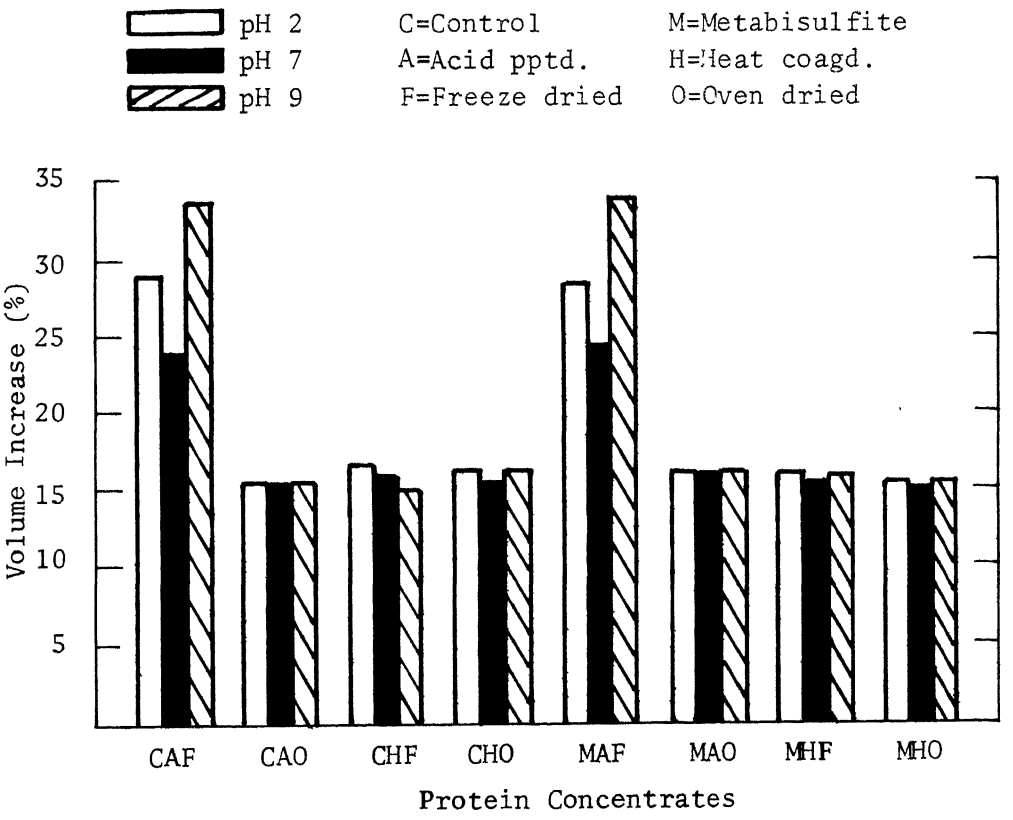

FIG. 1.—Foaming ability of soybean leaf protein concentrates.

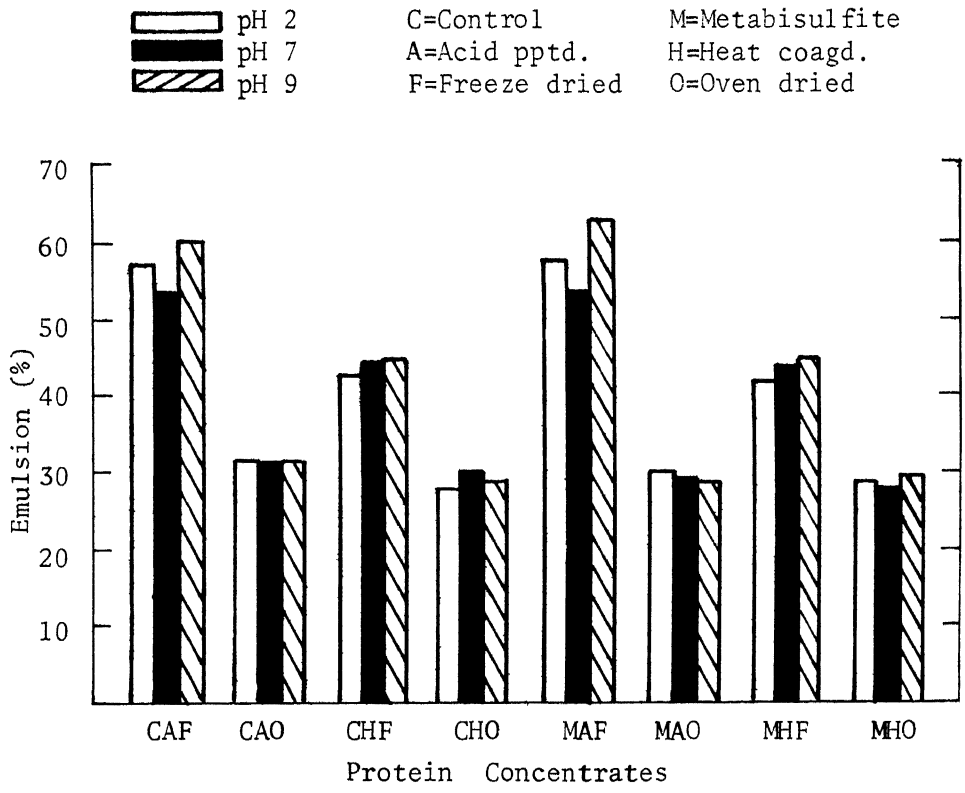

FIG. 2.—Emulsifying ability of soybean leaf protein concentrates.

higher ($P < .01$) in the heat coagulated than in the acid precipitated samples as shown in Table 5, while P occurred in a significantly ($P < .01$) greater amount in the acid precipitated samples both in control and sulfite treatments. The low mineral content in these concentrates is not a problem in food use. Minerals can be easily supplemented by some other means.

8. *Amino Acid Composition.* Essential amino acid composition was analyzed in duplicate and is presented in Table 6. Heat coagulated LPC contained less lysine and methionine compared to the acid precipitated samples. However, the essential amino acid content in both concentrates looks very promising compared to the FAO (6) recommendations, except that S-containing amino acids were low in both protein concentrates.

TABLE 5.—Mineral Content of Soybean Leaf Protein Concentrates (ppm).

Sample*	Calcium	Magnesium	Iron	Phosphorus
CAF	1.75	4.90	4.95	48.35†
CAO	1.70	4.92	4.82	48.15†
CHF	12.50†	8.00†	6.92†	12.18
CHO	12.40†	8.00†	6.80†	11.85
MAF	1.65	4.90	4.98	48.75†
MAO	1.75	4.98	4.95	47.10†
MHF	12.60†	8.00†	6.85†	11.86
MHO	12.30†	8.00†	6.80†	11.63

*C = control, M = metabisulfite, A = acid pptd, H = heat coagd, F = freeze dried, O = oven dried.
†$P < 0.01$.

CONCLUSION

The leaf protein studies showed that recovery and drying methods are important factors to obtain undenatured or reversible denatured protein concentrates which influence protein solubility. Functional properties were correlated with the solubility of the protein

TABLE 6.—Essential Amino Acid Composition of Soy Leaf Protein Concentrates and FAO Recommendation.*

Amino Acid	Heat† LPC	Acid† LPC	FAO (6) Recommendation
Threonine	4.44	5.49	4.00
Valine	5.85	7.02	5.00
Cystine	1.37	1.58	
Methionine	1.71	2.18	3.50‡
Isoleucine	4.52	5.25	4.00
Leucine	8.22	10.37	7.00
Tyrosine	4.49	5.74	
Phenylalanine	5.35	6.73	6.00**
Lysine	6.31	7.45	5.50

*Gram amino acid/100 g protein.
†Means of duplicate analyses.
‡Methionine + cystine.
**Phenylalanine + tyrosine.

samples. Finally, amino acid composition indicated LPC was a very good source of essential amino acids and thus proper utilization by supplementation or complementation will provide good nutrition.

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Carotene Content of Green Snap Beans

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INTRODUCTION

One of the most important factors for consideration in processed foods is that of nutritive value on which great emphasis is being placed today. Information relative to the nutritional evaluation of food processing has been published by Harris and Loesecke (7).

It has been estimated that vegetables and fruits supply two-thirds of the vitamin A content in the U. S. diet (1). Vitamin A is known to maintain healthy mucous membranes, to protect against infection, to be essential for light/dark adaptation of vision, to promote normal skin and tooth growth, to stimulate reproduction and lactation, and to be involved in bone protein formation (10).

Green beans are considered a good source of beta-carotene (provitamin A) (8). Although there are a number of studies concerning the effects of processing on the carotene content of vegetables (2, 4, 6, 9, 11), the recent release of new cultivars, coupled with advances in post-harvest storage conditions, have rendered many of these early data unreliable. Moreover, new analytical techniques have been developed since these were reported.

The present study was designed to investigate the effect of cultivar, maturity, processing, and storage time on the content and retention of beta-carotene in green snap beans.

MATERIALS AND METHODS

The cultivars selected for this study were: Tender Blue (Ferry Morse), GP 467 (Rogers Brothers Co.), Greenpak (Rogers Brothers Co.), Coloma (Rogers Brothers Co.), and Slim Green. These were grown under normal, commercial growing conditions in Columbus and hand harvested at maturity.

The beans were size graded into two grades depending upon the thickness of the pod, the division being made at 21/64-inch. These size grades were taken as being representative of beans of different maturities.

After grading, the beans were prepared for processing in the pilot plant of the Department of Horticulture. They were cut into sections 1 to 1¼ inches in length, and blanched at 170° F for 2½ minutes. For canning, the beans were filled into No. 303 cans, exhausted under steam flow, sealed, and processed at 240° F for 20 minutes in a still retort. After processing, the beans were cooled to 100° F and stored at room temperature until analysis. For freezing, the beans were filled into polyethylene pouches which were heat sealed. Freezing was carried out at —20° F and the frozen beans were stored at 0° F until analyzed.

Determination of carotene was done using a modification of the procedure of Bickoff *et al.* (3). A sample of beans was homogenized with acetone. This slurry was filtered and the residue further extracted with acetone. An aliquot of this extract was added to an equal volume of hexane in a separatory funnel together with a little water. After gentle swirling, the hypophase was withdrawn and discarded.

The hexane layer containing the carotenes was dried with anhydrous sodium sulfate and added to a column 25 x 7 mm composed of equal parts (w/w) of magnesium oxide and diatomaceous earth (Johns-Manville Super Cel, Fisher Scientific Company). The carotene was eluted from the column with a hexane-acetone mixture (9:1, v/v), the chlorophylls and xanthophylls being retained. The eluate containing the carotene was collected in a 25 ml volumetric flask.

The absorbance of the solution was measured at 440 nm on a Bausch and Lomb Spectronic 20 colorimeter and the carotene measured by comparison with a standard curve prepared from pure beta-carotene. Analyses were made in duplicate.

RESULTS

The results of this investigation are summarized in Table 1.

The following conclusions can be drawn from these data:

1. There was a significant difference in carotene content between cultivars. Regardless of maturity, GP 467 and Coloma were significantly higher in beta-carotene than the other cultivars.
2. The carotene content of beans decreased with increasing maturity, as measured by sieve size. This is in accordance with the observations of Hayden *et al.* (8).
3. A decrease in the carotene content with storage time was observed for both canned and frozen beans. This was more marked, however, with the frozen beans. Canned beans retained, on average, 76% of their original carotene content, while frozen beans retained 71%.

DISCUSSION

The study showed that green beans are a good source of vitamin A based on the carotene content.

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TABLE 1.—Carotene Content of Fresh and Processed Green Beans Stored for 2 Weeks, 3 Months, and 6 Months.

Sieve Size	Process	Storage	Carotene Content (mg/100 g)				
			Tender Blue	GP 467	Green Pak	Coloma	Slim Green
1-3	can	0		1.366	1.133		1.001
		2 wk		0.881*	1.047		0.897*
		3 mo		1.211*	1.177		0.728*
		6 mo		0.901*	0.960*		0.737*
1-3	freeze	0	1.293	1.366	1.133		
		2 wk	1.035*	0.693*	1.161		
		3 mo	0.951*	0.897*	1.165		
		6 mo	0.887*	0.746*	0.956*		
4-6	can	0	0.865	1.072	0.976	1.024	0.656
		2 wk	0.874	0.951	1.274*	1.092	0.733
		3 mo	0.988*	1.097	0.961	1.033	0.633
		6 mo	0.701*	0.774*	0.751*	0.687*	0.560*
4-6	freeze	0	0.865	1.072	0.976	1.024	0.656
		2 wk	0.569*	0.583*	0.817	0.851	0.587
		3 mo	0.651*	0.833*	0.901	0.911	0.646
		6 mo	0.687*	0.637*	0.687*	0.724*	0.533

*Significantly different from fresh (0-storage) at the 0.05 level.

It is likely, however, from the analytical technique used, that the vitamin A content was overestimated. Gebhardt *et al.* (5), in a critical study, have shown that not all isomers of beta-carotene measured by this procedure have vitamin A activity and they found that the vitamin A content of canned peaches was some 50% of that originally reported.

Preliminary investigations based on their procedure have shown that most of the carotene measured by the authors' procedure was indeed beta-carotene or provitamin A. A preliminary figure of between 60% and 80%, based on cultivar, has been estimated.

The fact that the carotene content of frozen beans is depleted more rapidly than that of canned beans is not really surprising. It is believed that the incomplete inactivation of oxidizing enzymes in the frozen beans is responsible. The fact that the authors were able to demonstrate the presence of peroxidase in frozen stored beans lends credence to this hypothesis.

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Effect of Reconditioning on the Ascorbic Acid Content of Potato Cultivars

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INTRODUCTION

The potato is an important food crop, not only because of its caloric content and food protein content, but also because it is an excellent source of vitamin C. It has been estimated that some 50% of the vitamin C in the European diet is derived from potatoes. Less of the U. S. vitamin C requirement comes from potatoes but, in a survey conducted in 1965, it was shown that more than 10% of the dietary vitamin C in the households surveyed came from potatoes. In the nation's food supply, as a whole, it has been estimated that potatoes contribute almost the same proportion of vitamin C as citrus fruits and nearly twice as much as tomatoes (8).

The palatability, cheapness, ready availability, and keeping quality of the potato enhance its importance in the diet. When freshly harvested, potatoes contain, on average, about 35 mg of ascorbic acid per 100 g fresh weight (6). However, there are considerable varietal differences in ascorbic acid content. Moreover, storing potato tubers has been shown to significantly deplete the ascorbic acid content (1, 2).

Storing potatoes at relatively cool temperatures has long been a standard practice. During this storage, the starches are broken down to reducing sugars, principally glucose. This can give rise to problems in subsequent processing of the potato tubers, especially when made into chips. The excessive brown color of chips made from potatoes with a high sugar content is an undesirable quality factor. To lower the sugar content, it is customary to store the potatoes for a period of time at room temperature, a process known as reconditioning.

Chemically, ascorbic acid is very similar to sugars, and it was felt that, in metabolically active tissue such as a potato tuber, changes in the ascorbic acid content were bound to occur. This study was thus undertaken to determine the changes in ascorbic acid content of potatoes during the reconditioning process.

MATERIALS AND METHODS

Potatoes of Kennebec, Shurchip, and Norchip cultivars were stored at constant temperatures (40°, 45°, 50°, and 55° F) at the Department of Horticulture facilities at Columbus After 6 months' storage time, samples were removed from storage and left at room temperature for varying periods of time to recondition. Samples were taken for analysis immediately after coming out of storage and after 1, 10, and 20 days' reconditioning time.

Cores (¼" diameter) were removed from two potatoes with a cork borer. These were then weighed and placed in a known volume of metaphosphoric acid. The samples were macerated in this solution and then filtered. An aliquot of the filtrate was taken for ascorbic acid analysis which was done titrimetrically using the 2,6-dichlorophenolindophenol method (3). Analyses were carried out in duplicate.

The results were statistically analyzed using analysis of variance (7).

RESULTS

The results of the experiment are presented in Tables 1 and 2.

The temperature of storage had no significant effect on the ascorbic acid content of the tubers. In view of the dependence of biochemical reaction on temperature, this result was somewhat unexpected. It is known, for example, that the lower the storage temperature, the greater the sugar content of the resulting tuber and, if ascorbic acid was to be metabolized in a manner analogous to other carbohydrates, it seemed reasonable to expect that temperature would have some significant effect on the ascorbic acid content of the tubers.

TABLE 1.—Effect of Storage Temperature on Ascorbic Acid Content of Potatoes.

Cultivar	Storage Temperature (°F)			
	40	45	50	55
	Ascorbic Acid (mg/100 g)			
Kennebec	6.12	5.51	5.53	5.55
Shurchip	6.19	6.48	6.39	6.43
Norchip	6.04	6.39	7.02	6.18
L.S.D. $_{05}$ = 0.626				

TABLE 2.—Effect of Reconditioning Time on Ascorbic Acid Content of Potatoes.

Cultivar	Reconditioning Time Days			
	0	1	10	20
	Ascorbic Acid (mg/100 g)			
Kennebec	5.36	5.32	5.43	6.60
Shurchip	5.05	6.30	6.64	7.51
Norchip	6.14	5.85	6.90	6.74
L.S.D. $_{05}$ = 0.626				

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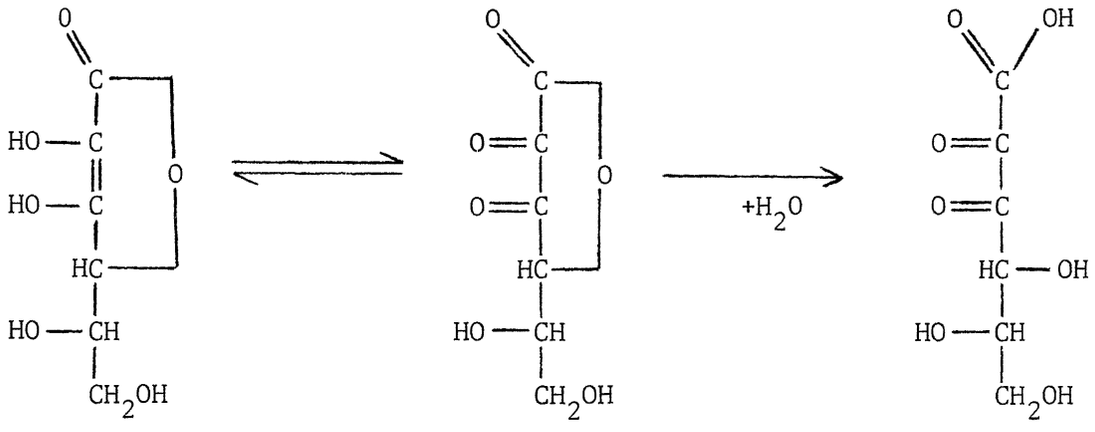

I = L-Ascorbic acid
II = L-Dehydroascorbic acid
III = 2,3-Diketo-L-gulonic acid

FIG. 1.—Breakdown of ascorbic acid.

It was noted, however, that there were significant differences among the different cultivars used in this study. Kennebec was the lowest while Norchip was the highest. This result was expected in the light of earlier work (4).

Of more interest, and of greater significance, was the fact that the ascorbic acid content of the tubers significantly increased with reconditioning time (Table 2). There was some variation among the cultivars, with Norchip increasing by 10%, Kennebec by 23%, and Shurchip by 49% over a 20-day period.

The interesting question raised by these data is, where is this 'new' ascorbic acid coming from? Two hypotheses suggest themselves. The first deals with the breakdown and disappearance of ascorbic acid during storage where it is oxidized to dehydroascorbic acid (DHA). This is an unstable compound and breaks down by hydrolysis to diketogulonic acid as shown in Figure 1.

It is possible that unhydrolyzed DHA may be reduced to ascorbic acid during reconditioning. This is of some importance since DHA also possesses vitamin C potency.

The other hypothesis is that the regeneration of the ascorbic acid occurs *de novo* by synthesis from sugars. This, too, is not unlikely since there is intense metabolic activity during the reconversion of sugars to starch and it is possible that a fraction could be synthesized to ascorbic acid (5).

One problem attending the resolution of these hypotheses is that the analytical procedure used determines only the reduced ascorbic acid. Work is proceeding on measuring the dehydroascorbic acid of the potatoes and initial results suggest that the reduction of some of this chemical may occur during reconditioning. However, the amounts of dehydroascorbic acid found do not make this hypothesis particularly likely and it would seem that some enzyme system for the *de novo* synthesis is the more likely.

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Kinetic Studies of Polyphenol Oxidase from HiDri Cabbages

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INTRODUCTION

The discoloration of many fruits and vegetables resulting from mechanical and physiological injury during harvesting, post-harvesting storage, or processing has been recognized as an oxidation of polyphenols present in the plant tissue, followed by subsequent polymerization through either enzymatic or non-enzymatic reactions. Two reactions are involved in the enzymatic browning and are catalyzed by a copper-containing enzyme, known as polyphenol oxidase (E. C. 1.10.3.1; catecholase, tyrosinase) in the presence of molecular oxygen (14). These two reactions are:

1. Hydroxylation of monophenols to 0-dihydroxyphenols.
2. Oxidation of 0-diphenols into quinones.

Activities of polyphenol oxidase (PPO), as well as other enzymes, may be associated with more than one protein but have differences in their chemical, physical, kinetic, and electrophoretic properties (isozymes). This multiplicity of enzyme system plays an important role not only in the biochemical regulation (19), but also in species differentiation (3).

PPO has been isolated and purified from a wide variety of fruits and vegetables, such as potatoes (3, 16), lettuce (12), broad bean leaves (18), peaches (21), apples (3), cherries (1), bananas (13, 15), pears (17, 20), and cranberries (2). Their physical, chemical, kinetic properties, and electrophoretic patterns were also studied.

Discoloration of cabbage on the cut surface of coleslaw may be related to enzymatic browning, reaction between PPO and phenolic substrates in the cabbage. The purpose of this study was to isolate the enzyme from three hybrids of high solid cabbages and to determine its kinetic and electrophoretic properties. The information will be applied to further studies on the factors affecting enzymic activities in the coleslaw production.

MATERIAL AND METHODS

The three HiDri cabbage lines obtained from Moreton Farm (Rochester, NY) were #1: 6428 = HD 364 x HD 328, #2: 6426 = HD 364 x HD 326, and #3: 1464 = HD 314 x HD 364.[2]

Preparation of Acetone Powder

A 100 g sample of shredded cabbage was blended with 300 ml of chilled acetone with (or without) addition of 20 ml polyethylene glycol 400 (20%) for 1 minute, filtered, the wet cake washed with chilled acetone twice or more, and then filtered to dryness. The powder was then stored at —20° C for analysis.

Preparation of Enzyme Extract

Acetone powder was mechanically stirred with 0.1 M citrate - 0.2 M phosphate buffer at pH 6.8 for 30 minutes and allowed to stand in the refrigerator overnight. The slurry was then strained and centrifuged. The clear solution was the source of crude enzyme.

The enzyme extract was further purified by precipitating with 65% saturated ammonium sulfate. This mixture was stirred for 30 minutes and centrifuged at 14,000 rpm for 30 minutes. After decanting the supernatant, the precipitate was dissolved in a minimum volume of citrate-phosphate (Ci-P) buffer at pH 6.8 and dialyzed against the same buffer for at least 24 hours. All operations were carried out in an ice bath and all reagent solutions were chilled before use.

PPO Assay Procedure

Enzyme activity was determined by measuring the increase in absorbance at 410 nm with a Beckman DU-2 spectrophotometer. The reference cuvette contained 6 ml of Ci-P buffer at pH 6.5 and 4 ml of substrate. The sample mixture consisted of 5 ml 0.1 M Ci - 0.2 M P at pH 6.5, 4 ml of 0.02 M pyrogallol freshly prepared with Ci-P buffer (at pH 7), and 1 ml of enzyme extract. The final pH of this mixture was 6.8. The experiment was carried out at room temperature and the absorbance was read every 30

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[2]HD 364, 328, 314, 326 are high dry matter inbred cabbage lines bred by the New York State Agricultural Experiment Station.

seconds. Under these conditions, linearity can be maintained within 4 minutes. The initial velocity was proportional to the concentration of the enzyme.

Protein content of each sample was determined by Lowry's method (11).

One unit of enzyme activity is defined as the change in absorbance of 0.001 per minute at 410 nm. Specific activity is the units of activity per mg of protein.

Polyacrylamide-Gel Electrophoresis

Polyacrylamide-gel electrophoresis was performed by modification of the Davis (4) method. A 12% gel was used as a spacer gel, 40 µl of enzyme solution containing 40% sucrose and one drop of 0.01% bromophenol blue was placed in the sample gel. PPO activity was detected by immersing the gel strips in Ci-P buffer (at pH 6.8) for 30 minutes before reacting with 100 ml of 0.02 M pyrogallol which contained 0.02% p-phenylenediamine.

The activity bands were detected within 1 hour, stained with Coommassie-blue, and destained with methyl alcohol-acetic acid until all the background was clear. Each gel was scanned in a scanning densitometer to detect the number and location of PPO bands.

RESULTS AND DISCUSSION

Effect of Extraction Method on PPO Activity

Several factors could affect the extraction of PPO. PEG (polyethylene glycol) and PVP (polyvinylpyrolidone) were suggested to effectively extract PPO from plant tissue by virtue of their strong potential as hydrogen acceptors (10). The addition of PEG to cold acetone during the preparation of acetone powder gave higher specific activity of PPO in Royal Anne cherries than when the chilled acetone was used alone (1), and the protein content was also doubled (2). However, PEG-acetone buffer extraction was not significant in this experiment in terms of increasing enzyme activity and protein content (Table 1).

Duration of buffer extraction is critical in obtaining higher specific activity. Twenty-four hours with continuous stirring at 4° C could double the specific activity (7). Setting overnight without continuous stirring as in this study may result in lower activity.

TABLE 1.—Effect of PEG on PPO Activity and Protein Concentration in #1 Cabbage.

Preparation	Protein (mg)	V_0 (unit)*	Km (mM)
Acetone-Buffer	0.135	7	6.1
PEG-Acetone-Buffer	0.14	9	8.05

*Changes of absorbance 0.001 per minute at pH 6.8 at room temperature = 1 unit.

Increasing the proportion of acetone powder to buffer slightly increased the V_0 (initial velocity) and V_{max} in all three hybrids (Tables 2, 3, and 4). V_0 and V_{max} are proportional to enzyme concentration; however, the enzyme concentration in the acetone powder was not estimated. The relationship between the ratio of acetone powder to buffer and enzyme activity cannot be eliminated from these results.

PPO activity can be further concentrated by precipitating with 30% to 90% saturated ammonium sulfate. It has been found that less than 10% of the PPO activity could be precipitated at 0-30% saturated ammonium sulfate, 20% of PPO activity increased at 30% precipitation, while a nine-fold increase could be reached at 90% precipitation (8). In these experiments, two to three-fold increases in specific activity (which is usually an index of purification) were shown at 65% saturated ammonium sulfate precipitation (Table 5). Difficulties in collecting the precipitates and redissolving in buffer were observed. Precipitating with acetone (13) instead of ammonium sulfate in purification of PPO is recommended in future studies.

Kinetic Studies

Km (Michaelis constant) is equal to the substrate concentration at semi-maximum rate ([S] = Km; $v = V_{max}/2$). Therefore, a high Km means a high substrate concentration is necessary to obtain semi-saturation because of its low affinity to the enzyme. Km is an absolute constant for a given substrate but depends on pH, temperature, and affectors. In this study, Km was determined by the Lineweaver-Burk method. Km obtained from cabbage lines #1 and #2 did not significantly differ from each other (Tables 2 and 3), but Km from #3 (Table 4) was comparatively lower than the others. Increasing the proportion of acetone powder in the extracting buffer didn't significantly change the Km. However, Km varied with degree of purification (9). Generally speaking, it is smaller in partially purified enzyme extract. Comparing the Km from this study to the Km obtained from broad bean leaves (2.3 mM) which was determined by oxygen consumption method (18), it can be said the PPO from cabbage has lower affinity for pyrogallol than the PPO from broad bean leaves.

Several parameters which may result in a large standard deviation should be of concern. One of these is temperature. It is well known that the rate of enzymatic reaction will usually increase 50% to 100% if the reaction temperature is raised 10° C. Electronic compartments and lamps in the spectrophotometer could raise the cuvette compartment temperature by 5-10° C over room temperature. This lack of stable accurate temperature control during

TABLE 2.—Kinetic Characteristics of PPO Extracted from #1 Hi-Dri Cabbage Line.

Source	Crude Enzyme				Partially Purified			
Acetone Powder/ ml Buffer	No. of Trials	V_0 (Unit)*	V_{max}	Km (mM)	No. of Trials	V_0 (Unit)*	V_{max}	Km (mM)
20 mg	3	8	10 6	6.51 ± 1.38	4	8 75	10.4	5.65 ± 1.4
50 mg	2	11	15.3	7.804†	2	10	12.1	4.26†
100 mg	2	14	18.3	6.107†	2	12	14.1	3.54†

*Changes of absorbance 0.001/min at pH 6.8 at room temperature = 1 unit.
†Average of two trials.

TABLE 3.—Kinetic Characteristics of PPO Extracted from #2 Hi-Dri Cabbage Line.

Source	Crude Enzyme				Partially Purified			
Acetone Powder/ ml Buffer	No. of Trials	V_0 (Unit)*	V_{max}	Km (mM)	No. of Trials	V_0 (Unit)*	V_{max}	Km (mM)
20 mg	4	8.75	11 8	6.713 ± 1.94	4	9.25	11.2	4.27 ± 1.53
50 mg	2	10	13 2	6.42†	2	8	10.3	5.33†
100 mg	2	14	18	6.11†	2	8	11.22	8.06†

*Changes of absorbance 0.001/min at pH 6.8 at room temperature = 1 unit.
†Average of two trials.

TABLE 4.—Kinetic Properties of PPO Extracted from #3 Hi-Dri Cabbage Line.

Source	Crude Enzyme				Partially Purified			
Acetone Powder/ ml Buffer	No. of Trials	V_0 (Unit)*	V_{max}	Km (mM)	No. of Trials	V_0 (Unit)*	V_{max}	Km (mM)
20 mg	3	6	7.8	5.835 ± 1.463	3	5.3	6.8	5.47 ± 2.74
50 mg	2	8	10.7	6.69†	2	2.5	10 08	4.18†
100 mg	2	10	12.1	4.26†	2	11	12.06	1.92†

*Changes of absorbance 0.001/min at pH 6.8 at room temperature = 1 unit.
†Average of two trials.

TABLE 5.—Purification Factor of PPO from Three Hi-Dri Cabbage Lines.

	Acetone Powder/ ml Buffer		V_0 (Unit)*	Protein (mg/ml)	Sp. A†	Factor‡
Line #2	20 mg	crude	8.75	0.14	62.5	1
		purified	9.25	0.05	185	2.96
	50 mg	crude	10	0.17	58.9	1
		purified	8	0.04	200	3.396
	100 mg	crude	14	0.23	61	1
		purified	8	0.08	100	1.64
Line #1	20 mg	crude	8	0.135	59.3	1
		purified	8.75	0.05	175	2.953
	50 mg	crude	11	0.16	68.8	1
		purified	10	0.055	182	2.65
	100 mg	crude	14	0.21	67	1
		purified	12	0.08	150	2.24
Line #3	20 mg	crude	6	0.15	40	1
		purified	5.3	0.055	97	2.41
	50 mg	crude	8	0.17	47	1
		purified	7.5	0.055	137	2.9
	100 mg	crude	10	0.22	46	1
		purified	11	0.07	158	3.42

*Changes of absorbance 0.001/min at pH 6.8 at room temperature = 1 unit.
†Specific activity = V_0/protein content.
‡Factor = $\frac{\text{specific activity of crude enzyme}}{\text{specific activity of partially purified enzyme.}}$

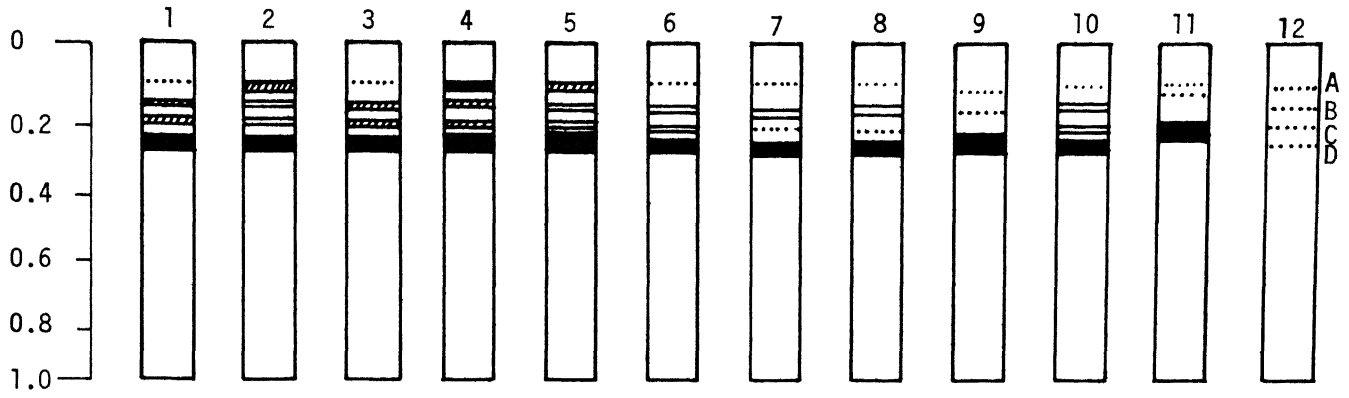

FIG. 1.—Polyacrylamide gel electrophoretic patterns of polyphenol oxidase of three Hi-Dri cabbages.
substrate: 1-5 dopamine
6-12 pyrogallol
1 and 6 = #1 cabbage
2 and 7 = #2 cabbage
3 and 8 = #3 cabbage
4 and 9 = #1 partially purified with acetone
5 and 10 = #2 partially purified with acetone
11 = #2 partially purified with 65% sat. ammonium sulfate
12 = #2 inhibited by 1 μm potassium metabisulfite

assay may lead to unreproducible data from each laboratory. Either a water bath or electronic temperature control to maintain constant reaction temperature was recommended (5).

Reaction rate data, if followed continuously by recorder, are advantageous in determining the initial velocity. Due to the limitation of the equipment, the sample readings in this study were taken at a definite time (every 30 seconds), a so-called fixed time assay which may lead to a false initial rate. Nevertheless, this preliminary study gives valuable information for future studies.

Electrophoretic Studies

Four active bands were separated in the crude enzyme extract reacting with pyrogallol, dopamine, and chlorogenic acid. Their relative mobilities are 0.06, 0.13, 0.18, and 0.23, respectively. Although the multiple form of PPO was highly characteristic for each species and variety, as was seen in mushroom, apple (3), pear (6, 17), or avocado (8), there was a similar pattern exhibited among the three cabbage hybrids for substrate specificity (Fig. 1). However, the isozymes B and C were less sensitive to pyrogallol than to dopamine and chlorogenic acid. Only three bands were observed after partially purifying with 65% ammonium sulfate, while four bands were obtained when acetone was used in purifying the enzyme extract. This might be due to the loss of activity during preparation; otherwise, ammonium sulfate might alter the electrophoretic properties of the enzyme. Gel concentration plays an important role in separating isozymes. A 10% or 12% gel was required to obtain better resolution in this study.

Inhibition of PPO by potassium metabisulfite was also studied. The gels were immersed in 10 mM and 1 mM potassium metabisulfite for 30 minutes before reacting with pyrogallol. A concentration of 10 mM completely inhibited PPO activities (no bands were observed), while 1 mM diminished the intensity of the four active bands without changing the electrophoretic pattern.

This study showed that the polyphenol oxidase extracted from cabbage is different from that of apples, potatoes, cherries, pears, and avocados in terms of substrate specifically and electrophoretic properties. The PPO system of cabbage appears to be composed of four isozymes. Further work should be done in studying the physical, chemical, and kinetic properties of these fractions.

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Refinements in Pickle Brine Recycling

T. GLAROS and J. R. GEISMAN[1]

INTRODUCTION

The volume of cucumbers for pickling in the United States has increased over the last few years. The trend in per capita consumption has also increased, with each individual consuming slightly more than 8 lb of pickles per year.

Three different kinds of pickles are available to the consumer: fresh pack, salt stock, and refrigerated. Fresh pack and refrigerated pickles do not create a major pollution problem, but the spent curing brine from salt stock does. In the United States, more than 30 million gallons of spent brine are produced annually from pickle processing. The Water Pollution Control Act of 1972 sets limits on the amount of chlorides which can be discharged from a particular plant. This limits the release of salt into the environment and therefore means that there must be a rapid and efficient method for treating or recycling brines for re-use.

Several methods have been proposed for brine recycling. These involve pH adjustment with subsequent settling times, heat treatment through specially constructed heat exchangers, and the re-use of brine with very little conditioning. Each of these techniques has particular drawbacks. The pH adjustment requires a 48 hr settling period. Heat treatment or pasteurization not only requires an expensive piece of equipment but relies on fuel which may be scarce. Re-use of brine with no conditioning is dangerous in that softening enzymes could be present which would build to a point where the entire pack could be lost.

This research was conducted to determine whether refinement of the technique developed at OARDC for brine recycling could be accomplished. There were two objectives to this study:

1. To determine the feasibility of polyelectrolyte addition and sand filtration as possible methods of recycling spent brine from the cucumber fermentation.

2. To compare the efficiency of polyelectrolyte addition, pH adjustment, and sand filtration as methods for recycling spent curing brines.

EXPERIMENTAL MATERIALS AND METHODS

The research was conducted in two phases. Phase I involved construction and use of sand filtration as a means of recycling spent curing brine. Phase II involved pH adjustment with and without the addition of organic flocculents (polyelectrolytes). Spent curing brine was obtained from the H. W. Madison Co. division of the J. M. Smucker Co.

Phase I—Sand Filtration

Construction of the Sand Filter

The sand filter was constructed of readily obtainable materials using a 55-gallon barrel for holding the material. A wooden flume was placed inside

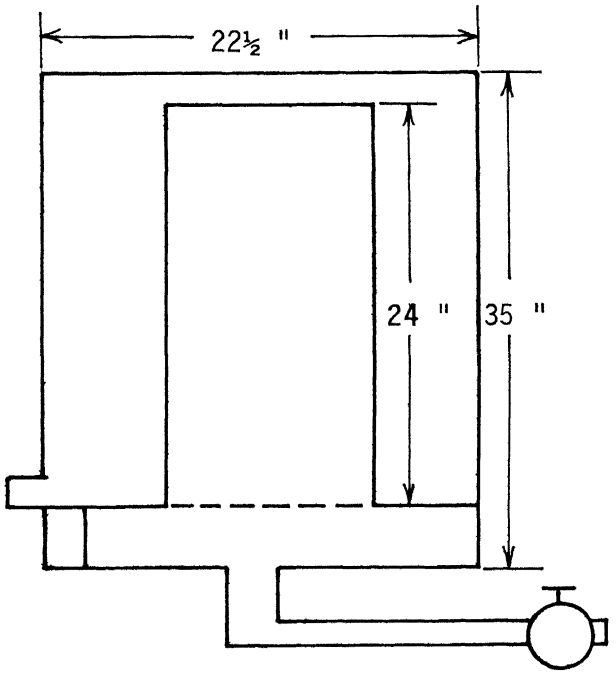

FIG. 1.—Side view of sand filter.

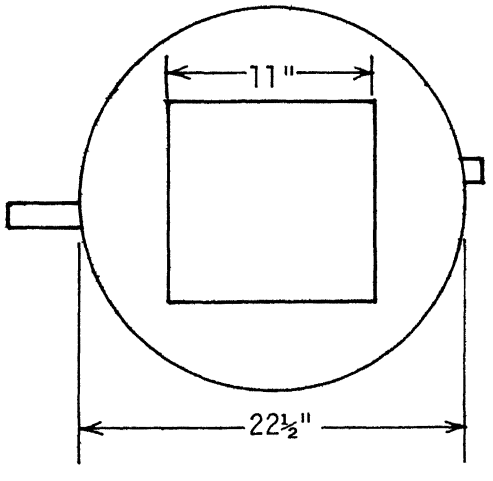

FIG. 2.—Top view of sand filter.

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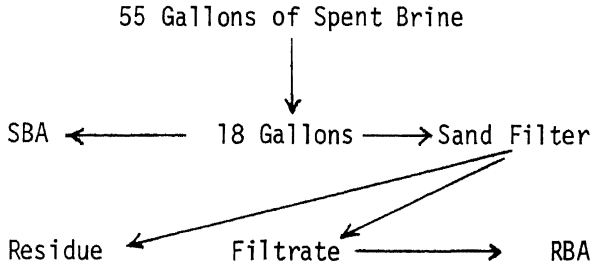
the barrel which held an 18-inch deep bed of filter sand. A false bottom was placed inside to support the flume and filter sand. The space between the false bottom and the actual bottom acted as a reservoir for the filtrate.

A drain was connected to the bottom of the barrel so the filtrate could be removed. A hole was cut on the side of the barrel just above the false bottom. This acted as a drain for backwash material which collected between the barrel and the flume. Backwashing was accomplished by forcing water up through the filtrate, then through the reservoir and through the filter sand. Construction is illustrated in Figs. 1 and 2.

Filtration Procedure

Three replications with duplicate samples for analysis were performed. Approximately 18 gallons of spent brine were used for each replicate. Before filtration, the barrel of spent brine was stirred. The filter was cleaned between replicates by backwashing with fresh water for 10 minutes.

Flow Chart



SBA = Spent Brine Analysis
RBA = Recycled Brine Analysis

Phase II: Polyelectrolyte Addition With or Without pH Adjustment

Three different polyelectrolytes manufactured by Calgon Corp. were tested for their ability to produce a floc that would rapidly settle when added to spent brine. The treatments were replicated, with duplicate samples removed for analysis. The polyelectrolytes are known by the following trade names:

Anionic Polyelectrolyte WT 2900
Cationic Polyelectrolyte WT 2870
Nonionic Polyelectrolyte WT 2690

Nonionic Polyelectrolyte WT 2690

A stock solution of WT 2690 was prepared. A magnetic stirring bar and 500 ml of distilled water were added to a clean 600 ml beaker, which was placed on a magnetic stirrer. The magnetic stirrer was set at speed 4. Exactly 1.000 g of WT 2690 was sprinkled into the agitated, distilled water and allowed to mix until completely dissolved. This solution was quantitatively transferred to a clean 1,000 ml volumetric flask and diluted to the mark. One ml of this stock solution, when added to 1 liter of spent brine, is equivalent to 1 ppm. The stock solution was stable for 2 weeks.

Twelve 1-liter bottles with rubber stoppers were cleaned, and 1 liter of spent brine was added to each bottle. The following dilutions were prepared and replicated:

0 ppm—control
0.01 ppm—1 ml of stock solution to 100 ml H_2O, 1 ml/liter
0.1 ppm—10 ml of stock solution to 100 ml H_2O, 1 ml/liter
1 ppm—1 ml of stock solution/liter
10 ppm—10 ml of stock solution/liter
100 ppm—100 ml of stock solution/liter

After the addition of the polyelectrolyte, the samples were mixed and then observed for the polyelectrolyte's ability to form a floc that would rapidly settle in comparison to the control.

Twelve more bottles with rubber stoppers were cleaned and 1 liter of spent brine was added to each. Each 1 liter of spent brine was adjusted to pH 11, using 19 N sodium hydroxide and a Beckman Expandomatic SS - 2 pH meter. The same dilutions as above were prepared and replicated with polyelectrolyte additions after pH adjustment.

The samples were mixed and then observed as previously described.

Twelve more bottles were thoroughly cleaned and 1 liter of spent brine was added to each. The same dilutions were prepared and replicated. The polyelectrolyte was added and the samples were adjusted to pH 11 as previously described. Observations were made on the rate of floc formation.

Cationic Polyelectrolyte WT 2870

A stock solution of WT 2870 was prepared in exactly the same manner as for the polyelectrolyte WT 2690. Dilutions of this stock were added to spent brine with and without pH adjustment as previously described. The rate and amount of floc information were recorded.

Anionic Polyelectrolyte WT 2900

A stock solution of WT 2900 was prepared. A magnetic stirring bar and 500 ml of distilled water were added to a clean 600 ml beaker, which was placed on a magnetic stirrer. Exactly 0.2000 g of WT 2900 was sprinkled into the agitated water. After complete solution, the mixture was quantitatively transferred to a clean 1,000 ml volumetric flask and then diluted to the mark with distilled wa-

ter. One ml of the stock solution, when added to the 1,000 ml of spent brine, equals 0.2 ppm.

Fourteen 1-liter bottles with rubber stoppers were thoroughly cleaned, and 1 liter of spent pickle brine was added to each. Each dilution including the control (no polyelectrolyte) was replicated. The following series of dilutions were made:

0 ppm—control
2 ppm—10 ml of stock solution/liter
4 ppm—20 ml of stock solution/liter
6 ppm—30 ml of stock solution/liter
8 ppm—40 ml of stock solution/liter
10 ppm—50 ml of stock solution/liter
20 ppm—100 ml of stock solution/liter
40 ppm—200 ml of stock solution/liter

After each dilution was prepared, it was observed for the polyelectrolyte's ability to produce a floc that would rapidly settle in comparison to the control.

Additional treatments were made with pH adjustment before and after polyelectrolyte addition as previously described. Observations were made on the rate of floc formation.

Analysis of Treated Brines

Only those polyelecrolyte solutions producing a floc that settled rapidly were tested for clarity and photographed to give a better indication as to the effectiveness of polyelectrolyte addition.

These treated brines were also analyzed using standard methods for:

1. Percent salt
2. Percent light transmittance (Spectronic 20 at 587 nm)
3. pH
4. Total acid (percent lactic acid)
5. Total solids and ash.

RESULTS AND DISCUSSION

Phase I: Sand Filtration

Raw pickle brine was filtered through 18 inches of filter sand in an effort to remove the suspended solids. The speed of filtration was dependent upon gravity. This filter did not utilize a pump. The sand particle size ranged from 0.25-0.55 mm.

Sand filtration alone was not an adequate method for recycling spent brine. Filtration was slow without a pump. Less than 1 liter of filtrate was collected per minute. Prior chemical pretreatment was necessary in order to produce a re-usable brine. Even though there were statistical differences in the analytical results before and after filtration, these differences were of little practical value.

1. Percent Light Transmittance at 587 nm (Spectronic 20)

Percent light transmittance at 587 nm before filtration averaged 62.08%. After filtration, the light transmittance increased to 64.73%. This was a significant increase at the 0.05 significance level.

2. Percent Salt

There was a small but significant reduction in the percent salt after filtration. The salt percentage before filtration averaged 14.66. After filtration, the salt content was reduced to 14.33%.

3. pH

The pH did not change significantly. The pH before filtration ranged from 3.51-3.55. After filtration, the pH ranged from 3.59-3.60.

4. Total Acid as Percent Lactic Acid

Total acid as percent lactic acid did not change significantly with filtration. Before filtration, total acid as lactic averaged 0.54%. After filtration, total acid as lactic averaged 0.51%.

5. Total Solids

Sand filtration reduced total solids from 16.6 to 15.26%. This was a significant difference at the 0.05 significance level. The difference between the salt percentage and the total solids percentage after filtration is approximately 1%. This difference could be attributed to organic matter such as dead bacteria, pieces of pickle, enzymes, and possibly yeast cells.

6. Percent Ash

Ash expressed as a percentage of total solids ranged from 86.88 to 90.80% before sand filtration. Percent ash before filtration averaged 89.61%. Each ash determination was performed in exactly the same manner. This variation cannot be accounted for. After filtration, ash ranged from 90.56 to 92.76% with an average value of 91.55%.

Phase II: pH Adjustment Followed by Polyelectrolyte Addition

The only polyelectrolyte capable of producing a large floc was Calgon WT 2900. The sequence of material addition was extremely important. The pH had to be adjusted to 11 before WT 2900 was added. Any other sequence with or without pH adjustment proved unsuccessful. A pH higher than 11 would inactivate the anionic polyelectrolyte. The polyelectrolyte was evaluated at different concentrations ranging from 2 to 40 ppm. Six ppm proved to be the ideal concentration. It produced a large floc which rose to the top of the container in the laboratory. Apparently during the mixing process, air was entrapped in the floc which caused it to rise instead of sink.

The anionic polyelectrolyte costs $2.50 per pound. The cost per 5,300 gallons of spent brine was less than 50 cents.

This procedure was tested at the H. W. Madison Company division of J. M. Smucker Company in Medina, Ohio. Approximately 5,300 gallons of spent brine were adjusted to pH 11. Six ppm of WT 2900 were added and mixed. Within 2 hours, a layer of sediment less than 1 foot in depth had accumulated at the bottom of the vat. The decantable supernatant was clear without any suspended solids. This process was approximately 24 times faster than pH adjustment alone.

After pH adjustment and polyelectrolyte addition, the decantable liquid was tested for percent light transmittance at 587 nm using Spectronic 20. The reconditioned brine transmitted 100% of the 587 nm light passed through the sample.

SUMMARY AND CONCLUSION

The sand filter constructed during Phase I of this project was not able to remove the suspended solids present in the spent brine that were responsible for the low light transmittance. The rate of filtration was also slow. Approximately 1 liter of filtrate per minute was collected during the filtration process.

Adjustment of the pH to 11 followed by the addition of anionic WT 2900 polyelectrolyte was effective in producing a large floc that would rise to the top of the container. Air bubbles were entrapped within the material during the dissolving process, causing the floc to rise instead of sink. The clear liquid generated as a result of this process could be re-used.

The method for recycling spent brine should be refined to pH adjustment followed by the addition of 6 ppm of the polyelectrolyte WT 2900. This is the fastest and most effective method to date. This method is 24 times faster than pH adjustment alone.

Use of Ultraviolet Light to Inhibit *Staphylococcus aureus* Growth on the Surface of Baked Pumpkin Pie

NICHOLAS N. KALLAS and WILBUR A. GOULD[1]

INTRODUCTION

Ultraviolet irradiation is a powerful bactericidal agent. But due to its poor penetrative powers, food use is limited to surface applications. Nevertheless, ultraviolet light has widespread, practical use in the food preservation industries. Several food applications include bread, cakes, aging of meats, bread slicing knives, storage and packaging cheese, air surrounding foods, and water for beverages (5, 6).

Section 128e.7 (c) (9) of the FDA's (1976) proposed GMP's for bakery foods, if approved, would have required all baked goods supporting microbial growth to be refrigerated to 40° F or below within 2 hours after baking and maintained at this temperature or below until final retail consumer purchase. Therefore, a potential alternative ultraviolet light preservation method which would allow the marketing of baked pumpkin pie without refrigeration but with safety from staphylococcal food poisoning was investigated.

This study was based on the following hypothesis. If a baked pumpkin pie is free from viable staphylococci upon leaving the oven, any postbaking staphylococcal contamination, provided the integrity of the pie's surface is maintained, would be surface oriented where treatment with ultraviolet light would effectively inhibit staphylococcal growth and/or survival. To test this hypothesis, two experimental objectives were investigated: 1) to determine if *Staphylococcus aureus* is destroyed by the baking process, and 2) to determine the effect of ultraviolet irradiation on the growth and/or survival of *Staphylococcus aureus* surface inoculated on baked pumpkin pie at various inoculum levels and stored at various temperatures.

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MATERIALS AND METHODS

Staphylococcal Strains. The S6 strain is a classical food isolate which produces enterotoxins A and B. The 836 strain is a recent food (barbecue) isolate which produces enterotoxin A.

Pie Materials: The pumpkin pie filling and pie shells were obtained frozen from a local commercial bakery and stored at —23° C until used. Prior to use, the filling was thawed at 4° C for 24-36 hours and the shells at room temperature for 20 minutes. The pumpkin filling was heated to 20° C before baking.

UV Light Apparatus: A CE-36-4 unit from the American Ultraviolet Co., Chatham, N. J., which operates with four G36T6 ultraviolet lamps was used

throughout the study. This fixture has an approximate shortwave germicidal output at 2537 A of between 10,900 and 15,000 microwatts/cm^2 at 2½ inches (6.35 cm) from the radiation source.

Inoculum Preparation: The inoculum preparation method was that of Crisley *et al.* (3). All inoculums were adjusted so that 0.1 ml cell suspensions in 0.1% peptone (Difco) water would represent the desired inoculum size. Verification of inoculum sizes was made by plating the inoculum suspension on Plate Count Agar (Difco).

The Baking Process on Staphylococcal Survival: Eight-inch diameter pies were inoculated with a 12-hour culture of 10^8 *S. aureus* S6 cells in six different localized positions (one pie-one position). The positions inoculated were on the surface of the filling both center and perimeter, in the filling both center and perimeter, and on the filling-crust interface both center and perimeter. Duplicate replications of each position were tested.

The pies were baked in a Dispatch reel oven at 190° C for 45 minutes with a fill temperature of 20° C. After baking, 50 grams of the inoculated section were blended with 450 ml of Butterfield's PO buffer and sampled using PCA spread plates and Trypticase Soy broth (TSB) (Difco) (3 tube most probable number) and incubated at 37° C for 48 hours. Positive TSB tubes were streaked for isolation on PCA and Staphylococcus 110 (Difco) media and incubated at 37° C for 48 hours.

All colonial growth was treated first by a gram stain and if gram positive cocci were found, a second and conclusive staphylococcal test would have been the determination of coagulase production. Since no gram positive cocci were isolated, coagulase tests were deemed unnecessary.

Pie Processing: Experimental conditions were designed to simulate an actual commercial operation. The 8-inch pies were baked at 190° C for 45 minutes and the 4-inch pies at 163° C for 90 minutes. The pies were open air cooled from oven temperatures of 98° C to 57° C on raised wire rack surfaces.

The pies were inoculated by pipette delivering 0.1 ml of 0.1% peptone water containing the desired inoculum size. The pies were irradiated for the desired time treatment by being passed under the UV apparatus placed on a conveyor belt. The pies were then placed in standard pie cartons which contained side vents and a "breathing" cellophane window. The pies were stored as follows: 8-inch pies at 20-22° C for 9 days and 4-inch pies at 25° C for 90 days and at 30° C for 7 days.

Sampling of Irradiated and Control Pies: Pies were sampled every 24 hours starting on the day of baking on PCA and S110 agars. For the 4-inch pies, the entire surface representing 20.0 grams was removed at a depth of approximately ⅛-inch and blended with 180 ml of PO_4 buffer. Further dilutions were made with 90 ml PO_4 dilution blanks. For the 8-inch pies, the same technique was used except only the inoculated pie surface section was removed.

Explanation of Figures: Figures 1-4 represent PCA counts only since these counts were consistently higher than S110 counts. Previous experimentation by the authors (7) with uninoculated pumpkin pies both irradiated and nonirradiated showed little if any microbial growth on PCA as a result of air-borne contamination or otherwise under these same experimental conditions. The values shown in the figures represent the average PCA counts obtained from the two pies sampled for each specific treatment.

RESULTS AND DISCUSSION

The Baking Process on Staphylococcal Survival

Since no gram positive cocci were isolated from any of the six pie positions after baking, *S. aureus* was determined to be destroyed by the baking process. This is in agreement with Cathcart *et al.* (2) and Angelotti *et al.* (1), who found staphylococci to be destroyed in custard pies when given a commercial baking process.

However, just because staphylococci are killed by the baking process, this does not mean that staphylococci will be absent indefinitely from the pie product. In automated commercial operations, the baked pies upon leaving the oven are open air cooled on moving conveyor belts. Consequently, since air is an environment which contacts the top surface of the pie during this cooling operation, it is evident that these air-exposed products may become contaminated with air-borne staphylococci, the main source of which is the food worker (8).

It also is important to note that if staphylococcal contamination, growth, and enterotoxin production occur before the baking process, the enterotoxin which is heat stable will not be completely inactivated by the baking heat treatment and foods under such conditions might cause food poisoning, although no live staphylococci can be demonstrated. Consequently, for UV light to be effective in controlling staphylococcal food poisoning, the history (raw ingredients, handling, sanitary conditions, etc.) of the product prior to baking is of vital importance in determining whether the product carries toxin.

Shelf Life Studies

With the 8-inch pies inoculated with strain S6 and irradiated at 2 inches from the pie's surface and incubated at 20-22° C, as the irradiation time increased, the lag period increased, with the 16-second irradiated pies displaying a lag period of at least 9

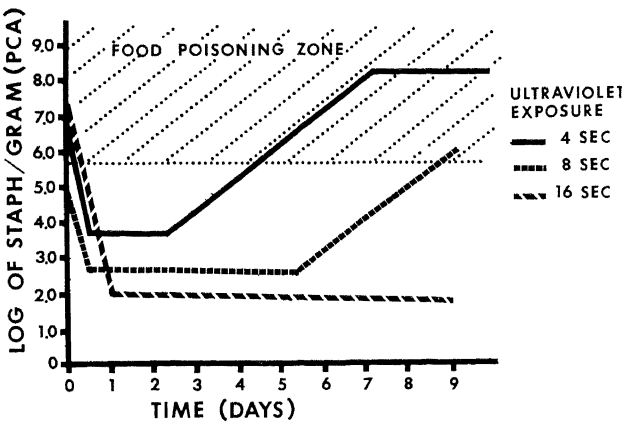

FIG. 1.—Log of average staphylococci per gram (PCA) vs. time, 8-inch pies, 20-22° C incubation; 4, 8, and 16-second irradiation at 2 inches, strain S6.

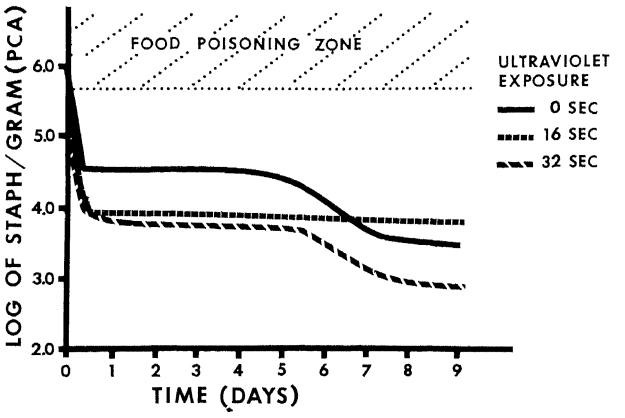

FIG. 2.—Log of average staphylococci per gram (PCA) vs. time, 4-inch pies, 25° C incubation; 0, 16, and 32-second irradiation at 2½ inches, 1.0 x 10^6 inoculum strain 836.

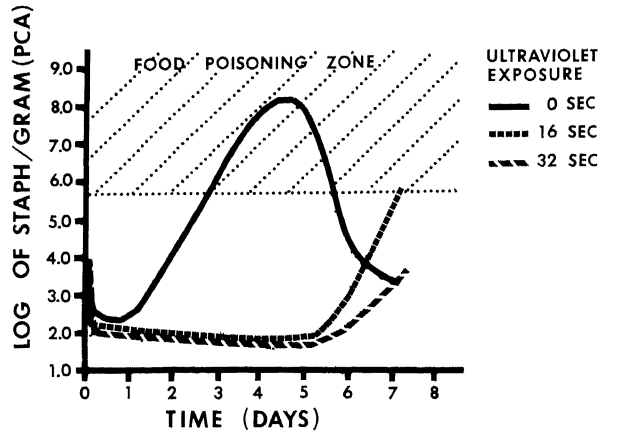

FIG. 3.—Log of average staphylococci per gram (PCA) vs. time, 4-inch pies, 30° C incubation; 0, 16, and 32-second irradiation at 2½ inches, 1.0 x 10^6 inoculum strain 836.

days. Both the 4 and the 8-second irradiated pies supported sufficient staphylococcal growth to be considered suspect food poisoning agents, whereas the 16-second irradiated pies did not support definite staphylococcal growth to be considered a suspect food poisoning vehicle (Fig. 1).

For the 4-inch pies inoculated with 1.0 x 10^6 strain 836 cells and irradiated at 2½ inches from the pie's surface and stored at 25° C, all three treatments, control, 16, and 32-second irradiated pies failed to support staphylococcal growth and displayed lag periods of at least 9 days (Fig. 2).

For the 4-inch pies inoculated with 1.2 x 10^4 strain 836 cells and irradiated at 2½ inches and incubated at 30° C (Fig. 3), the control and 16-second irradiated pies supported sufficient staphylococcal growth to be considered suspect food poisoning agents. The 32-second irradiated pies displayed a 5 to 6-day lag period and only supported slight staphylococcal growth after 6 days but remained considerably below 500,000 staphylococci per gram (generally recognized as the number of staphylococci required to cause food poisoning (5, 6)) during the entire 7-day 30° C storage period (Fig. 3).

Under the same conditions of irradiation and incubation but with a higher 1.1 x 10^6 strain 836 inoculum size (Fig. 4), the 32-second irradiated pies displayed a lag period of 7 days and at no time during the 7-day storage did the 32-second irradiated pies support definite staphylococcal multiplication, as counts decreased with time of storage.

In summary, *Staphylococcus aureus* is destroyed in pumpkin pie when given a commercial baking process. The pies were not sterilized by exposure to ultraviolet irradiation, although for a food preservation

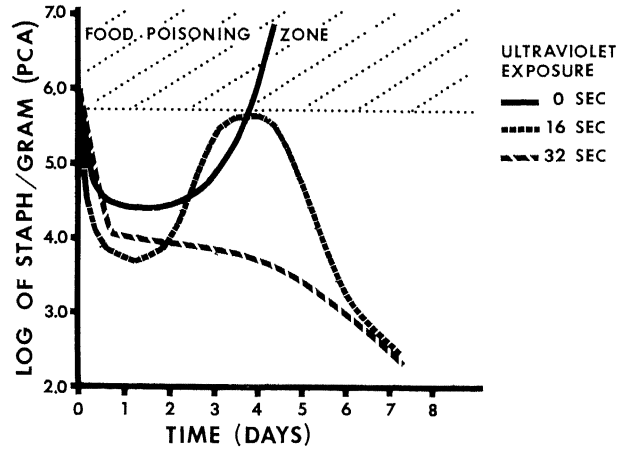

FIG. 4.—Log of average staphylococci per gram (PCA) vs. time, 4-inch pies, 30° C incubation; 0, 16, and 32-second irradiation at 2½ inches, 1.1 x 10^6 inoculum strain 836.

method to be effective it does not have to destroy or remove all microorganisms. Rather, causing a delay in the initiation of growth (lag time) is an important part of food preservation. In this study, this is exactly what preservation by ultraviolet light offers to the baking industry, as lag periods of 5 to 6 days were observed with 32-second irradiated pies inoculated with 10^4 and 10^6 staphylococcal 836 cells, respectively.

This research did show that the use of ultraviolet irradiation in the preservation of baked pumpkin pie with regard to staphylococcal food poisoning has great potential and merits serious consideration before any mandatory refrigeration regulation is promulgated.

ACKNOWLEDGMENTS

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Development of a Nutritionally Improved Corn Masa

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INTRODUCTION

Recent consumer group comments have indicated public concern about the lack of nutritional content of formulated processed foods and snack foods in particular (2, 8). Corn chips, which comprise a substantial portion of the snack industry's production, are subject to the same criticism as other snack foods.

To improve the nutritional content of corn chips, minced fish flesh was utilized in masa formulation as a suitable protein source (3) for a natural form of nutrification. The objectives of this study were: 1) to determine the applicability and acceptability of mechanically deboned minced silver bass (*Aplodinotus grunniens)* flesh as a natural fortification agent, and 2) to determine the effects of masa production procedures necessary for nutrification by addition of fish flesh.

MATERIALS AND METHODS

Masa (corn dough) was prepared with two varieties of *Zea mays* in a ratio of 70% yellow dent corn and 30% white dent corn (Fig. 1). A lye or lime process was used for removal of the corn pericarp. Lye processed corn was treated with a 20% lye solution at 190° F for 1 minute, followed immediately by washing in water.

A steam jacketed kettle was used for the cooking process, with an amount of water sufficient to cover the kernels, or approximately 90 gallons of water per 100 lb of corn. The corn, under agitation, was heated in such a manner that after initially reaching 160° F it would be heated to 210° F within 15 minutes. After reaching 210° F, the corn was immediately cooled to 160° F and allowed to soak for a minimum of 8 hours. Following the soak period, the corn was ready for masa production.

Lime processed corn was also cooked in a steam jacketed kettle with agitation. The ratio of corn to water was the same as used for lye processed corn. However, lime ($Ca(OH)_2$) was added at a rate of 12 to 16 oz per 100 lb of corn. Time and temperature conditions of cooking were identical to those used with lye processed corn. After cooking, the corn was soaked a minimum of 8 hours. For both lye and lime processed corn, the pericarp was removed from the kernels by a water spray in a reel washer.

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Fortified corn chips were prepared by mixing corn and fish flesh in a 3:1 ratio. The fish flesh was prepared from Lake Erie silver bass (*Aplodinotus grunniens*) that had been headed, gutted, split, and mechanically deboned with a Bibun SDX-16 flesh separator. The minced flesh was of a consistency similar to ground beef, which allowed for ease of mixing and subsequent homogeneous masa. To achieve a homogeneous masa, however, it was necessary to use a high speed mechanical size reduction machine. A Comitrol (Urschel Laboratories, Inc.), operated at 9,600 rpm was used to produce masas from the lye and lime processed corn and the corn/fish mixture. Stone ground (traditional method) masa was produced at the Dan Dee Co., Columbus, Ohio, and used as a control product.

Masas were extruded and fried (Fig. 1) on a commercial corn chip line by the Marion Popcorn Co., Marion, Ohio.

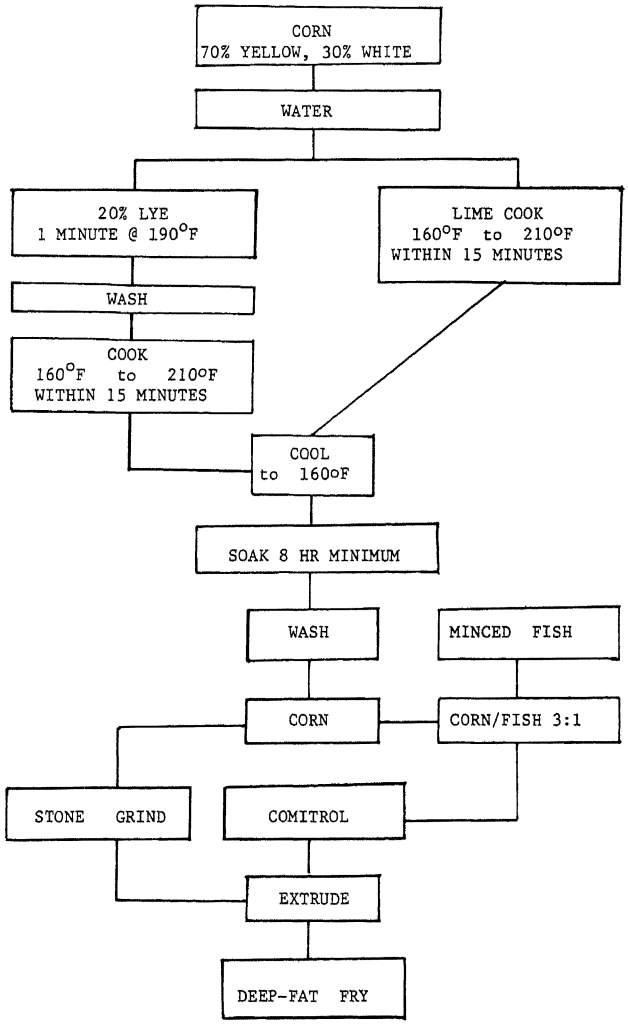

FIG. 1.—Masa and corn chip production flow chart.

Masas were analyzed for moisture before extrusion by the vacuum oven drying (7). Finished chips were analyzed for moisture using the infrared method (6). Oil content was determined using the Bailey-Walker method (6). Protein content was determined by the micro Kjeldahl method (4). Ash was determined according to the AOAC procedure (4). Caloric content was determined according to ASTM method D3286-73, using a Parr Instruments Plain Static Oxygen Bomb Colorimeter No. 1341 with a No. 1108 bomb.

The index of nutritional quality (I. N. Q.) was calculated by the following formula:

$$\text{I.N.Q.} = \frac{\text{percent nutrient requirement supplied by a quantity of food}}{\text{percent energy requirement supplied by a quantity of food}}$$

This factor is the ratio of nutrients to calories. An ideal food would have an I.N.Q. of 1.

Color of corn chips was determined with an Agtron M30-A in the red mode (R) standardized on 00 and 90 disks (6).

Chips were evaluated for color, texture, and flavor on a scale of 0 (off) to 10 (excellent), using a sensory evaluation panel consisting of 10 graduate students, staff, and/or faculty of the Department of Horticulture. Analysis of variance was used to determine significant differences between mean scores.

RESULTS AND DISCUSSION

Results of compositional analyses revealed that the addition of mechanically deboned fish flesh resulted in a 78.3% increase in protein content, 25.6% reduction in oil absorption, and an 87.5% increase in the protein index of nutritional quality (I.N.Q.). Agtron R values indicated that the color of the fortified chips was somewhat lighter than conventional chips (Table 1); however, sensory evaluation showed that panelists found the light color equally acceptable as the darker conventional chips (not significantly different $P < .01$). Caloric content of the fortified chip was reduced only slightly by the addition of fish flesh (Table 1).

Sensory evaluation of the corn chip variations showed only flavor of the salted fortified chips was significantly different ($P < .05$). No significant differences ($P < .01$) were found between mean scores of unsalted chips (Table 2). Short-term storage of the fortified chips appears feasible since panelists found the fortified chips to be equally acceptable as the conventional chips after 1 month of storage (Table 3).

These results present a possible avenue to the production of a snack food with substantially better nutritional quality through the use of underutilized spe-

TABLE 1.—Composition of Masa and Finished Corn Chips.

Type Chip	Moisture Masa %	Moisture Chip %	Oil %	Protein %	Ash %	Agtron R	Calories	Protein I.N.Q. Value
Fortified	58	0.5	29	8.2	1.0	41.5	166	0.60
Stone Gnd.	55	0.5	39	4.6	1.0	36.5	174	0.32
Comitrol	57	0.5	36	4.5	1.0	35.5	171	0 31
Lye Process	59	0.5	39	4.2	1.0	43.0	177	0.30

TABLE 2.—Acceptability of Fortified and Conventional, Salted and Unsalted Corn Chips.

	Mean Sensory Scores					
	Salted			Unsalted		
	Fortified	Comitrol	Stone Gnd.	Fortified	Comitrol	Stone Gnd.
Color	5.3	7.9	7.2	5.4	8.9	6.5
Flavor	4.8*	6.6	7.4	5.6	6.3	7.0
Texture	7.0	7.3	7.6	5.9	8.2	7.9

*Significant difference ($p < .05$).

TABLE 3.—Acceptability of Salted Fortified and Conventional Salted Corn Chips When Fresh and After Short-Term Storage.

	Mean Sensory Scores					
	Fresh			1-Month Storage		
	Fortified	Comitrol	Stone Gnd.	Fortified	Comitrol	Stone Gnd.
Color	5.3	7.9	7.2			
Flavor	4.8*	6.6	7.4	5.2	6.4	6.5
Texture	7.0	7.3	7.6	7.0	7.5	7.6

*Significant difference ($p < .01$).

cies of fish such as silver bass. The cutting waste from fish stick and portion production may also be a source of fish flesh for use in fortified corn chips, as this flesh has many of the properties of mechanically deboned fish.

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Mechanical Deboning of Lake Erie Freshwater Drum

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INTRODUCTION

Many species of fish are presently underutilized due to low yield by conventional filleting techniques or to a skeletal structure which does not allow boneless fillets. Losses in filleting and skinning freshwater drum can range from 75 to 85% of the round weight.

In an effort to recover a greater yield from such species, mechanical deboning machines or flesh separators have been used. Although the use of such machines is recent in the United States, Japan fish processors have used deboning equipment since 1950 (1). Depending upon the species of fish, yield of machine recoverable flesh can be expected to range from 28% to 66%, based on the weight of whole fish (4, 7). Mechanical deboning, however, produces definite changes in flesh color, bone content, and fat of the usable flesh (3, 5, 6, 8, 10).

The objective of this study was to determine some effects of mechanical deboning variations on freshwater drum (*Aplodinotus grunniens*) flesh.

MATERIALS AND METHODS

Freshwater drum in round form landed in the Western Basin of Lake Erie were purchased from commercial sources within 24 hours or less after landing. The drum were transported to the Food Technology Pilot Plant at OSU, boxed 60 lb/box in flake ice, and kept under 2° C refrigeration until processed.

Deboning Treatments: Drum were prepared for deboning by the following methods (Fig. 1). All preparatory operations were performed by hand. Drum were washed with cold water prior to heading and gutting and after kidney removal. A Bibun SDX-16 flesh separator with 3.0 mm cylinder perforations was used for deboning operations. All results are based on round weight lots of 54.5 kg (120 lb).

Fish Preparation Methods

HG: Round fish headed, gutted, water jet removal of kidney. No orientation of the fish to the cylinder.

HGS: Round fish headed, gutted, water jet removal of kidney, split the entire length by cutting the ribs along one side of the spine. Fish oriented with flesh against the cylinder and skin side against the belt of the separator.

HGSA: Round fish prepared same as HGS treatment. Additionally, a solution of 87.6% distilled water, 11.0% sodium tripolyphosphate, 1.3% ascorbic acid, and 0.9% citric acid (w/w) was applied to the split surface of the fish with a sprayer delivering 50 ml/minute. The sprayer was passed over the surface of the flesh twice from a distance of about 20 cm at a rate similar to that used in spray painting. Fish were oriented to the cylinder as in the HGS treatment.

HGSDT: Round fish headed, gutted, water jet removal of kidney, split as in HGS treatment, and tail removed to a point just behind the dorsal fin. Fish oriented to cylinder as in HGS treatment.

Pressure Variations: 1.5, 3.5, 6.0—numbers following treatment abbreviations correspond to relative pressures applied to the fish by the main pressure roller of the deboning machine (indicating arrow and scale device attached to the machine). Minimum pressure was 0.0 and maximum pressure was 6.0.

Mechanical deboning variations were evaluated by the following methods:

Color: Color of the raw deboned flesh was determined with a Hunter Color and Color Difference Meter D25D3A standardized with a Hunter white tile C2-4306 (L 95,00, a —0.60, b 0.40), and with an Agtron M-400A standardized on 00 and 90% relative reflectance disks in the red (R) mode.

Fat Rancidity: As indicated by 2-thiobarbituric acid values (TBA), fat rancidity was determined by the method of Tarladgis *et al.* (9), modified by adding 2 drops of Tenox II (Eastman Chemical Co.) to inhibit oxidation during blending (11). TBA values are expressed as mg malonaldehyde per 100 g fish flesh.

Bone and Scale Content: The method of Patashnik *et al.* (8) was used. Bone content reported as 3.2 mm or less, 3.2-6.4 mm, 6.4-12.8 mm, and greater. All scale and scale particles reported as the total number.

Moisture Content: Moisture content was determined by drying flesh samples on aluminum pans for 2 hours at 100° C in a convection oven, after which the samples were transferred to a vacuum oven for 5 hours at 70° C and ca. 100 mm pressure.

Fat Content: Fat content was determined by use of the Bailey-Walker apparatus with petroleum ether as the solvent. Dry 5.0 g samples were extracted in triplicate for 3 hours. Solvent was evaporated by steam bath, followed by drying for 0.5 hour in a convection oven at 100° C. Results are expressed as percent fat on a wet basis.

Yield: Results are reported as percent of round weight and as percent of prepared weight. Yield re-

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sults are based on round weight lots of approximately 54.5 kg (120 lb).

Sensory Evaluation: Samples for sensory evaluation were prepared by deep-fat frying and baking according to methods outlined by King (2), modified by preparing smaller portions, approximately 2.54 cm x 3.8 cm x 1.3 cm. Evaluation forms were hedonic/scoring, a score of 1 being lowest and 9 being highest.

RESULTS AND DISCUSSION

Rancidity (TBA): TBA values obtained from minced flesh treatments generally increased by a factor of 2 over TBA values from the round fish used in the treatments (Table 1). Pressure applied during deboning or the degree of preparation prior to deboning had little effect on the immediate increase of TBA values, but points out that the deboning process itself had the probability of rancidity development due to the tremendous increase of flesh surface areas exposed to air.

Color: Pressure applied during deboning and the degree of preparation both show effects on the color of minced drum flesh. Examination of skin and bone waste showed that at greater pressures nearly all of the red muscle tissue directly beneath the skin was removed (Table 1). Greater pressure applied generally resulted in a more highly pigmented flesh (lower R values), with the exception of the HG treatments. It is believed that some gray coloration removed from the skin during deboning of HG treatments obscured any color differences present due to pressure variations. This suggests that drum should be oriented flesh side against the cylinder to avoid discoloration of the skin. Removal of the tail of drum (HGSDT treatments), which contains a high proportion of red muscle tissue, proved to have a definite lightening effect on the color of minced drum, especially at the lowest pressure setting (Table 1).

Bone and Scale Content: Bone and scale content was directly related to pressure applied to the fish during deboning. When fish were not oriented in any particular manner as in HG 1.5-6.0 treatments, the amount of scale material increased substantially, thus indicating that splitting and orientation of the flesh side against the cylinder is advantageous. Regardless of the treatment, the majority of the bone particles were 3.2 mm or less, the type that may give a "grit" feeling in the mouth (Table 1). These results agree well with those from commercial minced cod and pollack blocks from several countries (8).

Yield: Yield, as expected, increased as pressure applied to the fish was increased (Table 1), both as a percent of the round weight and percent of the weight of the fish as prepared for deboning. Though removal of the tail (HGSDT) reduced yield, the 28% re-

TABLE 1.—Effects of Preparatory Treatment and Deboning Pressure on Several Physical and Chemical Characteristics of Mechanically Deboned Freshwater Drum Flesh.

Treatment/ Pressure	TBA No.	Agtron R	Hunter L	Yield % of Round Wt.	Yield % of Prep. Wt.	Moist. %	Fat % (wb)	Bone and Scale Content/454 g			
								3.2 mm	3.2-6.4 mm	6.4-12.8 mm	Scales
HG 1.5	0.251	35.3	40.3	26	49	77.7	4.3	27			18
HG 3.5	0.260	36.3	41.7	39	71	78.5	4.5	41			45
HG 6.0	0.248	35.9	41.8	42	75	77.1	5.3	66	9		41
HGS 1.5	0.328	44.3	42.0	27	51	78.0	4.4	18			16
HGS 3.5	0.365	36.0	40.1	38	68	79.9	4.2	61		7	9
HGS 6.0	0.322	33.3	40.2	43	80	78.7	5.3	234	5		18
HGSA 1.5	0.576	37.3	39.8	27	47	80.6	2.6	16			7
HGSA 3.5	0.433	39.9	44.4	37	67	81.1	2.6	13	16		7
HGSA 6.0	0.471	37.5	42.5	35	68	81.5	2.7	91	5		36
HGSDT 1.5	0.359	47.8	42.3	28	52	80.6	3.3	30			11
HGSDT 3.5	0.353	45.0	42.1	31	59	79.2	4.4	30			9
HGSDT 6.0	0.316	41.5	42.0	36	72	79.7	4.4	30	11		25

*TBA values from round fish used in this work ranged from 0.185 to 0.197.

covered flesh from HGSDT 1.5 treatment is better than the yield of skin-on drum fillets. The lighter flesh color resulting from tail removal is felt to offset the reduced yield.

Fat Analysis of Minced Flesh: Fat content of headed and gutted drum ranged from 4.8% to 6.1%. Results of fat analysis of minced drum showed that much of the fat on the prepared fish is removed and incorporated into the flesh (Table 1). Greater pressure resulted in higher amounts of fat being removed. It was noted that a much smaller increase between pressure settings of 1.5 and 3.5 than between 3.5 and 6.0 occurred. This range of fat in minced drum and the large surface area exposed will positively warrant the use of an effective antioxidant to prevent rapid deterioration of the fat. Fat content of fillets has been found to range from 0.5% to 2.3%, an obvious advantage of filleting although yield is somewhat reduced.

Sensory Evaluation of Minced Flesh Treatments: Samples of all minced flesh treatment/pressure variations were presented to panelists for sensory evaluation in baked and deep-fat batter fried forms. Pressure applied to the fish during deboning significantly affected the cooked flesh color. This was reflected by lower mean scores obtained for treatments of greater deboning pressure, regardless of the type of preparation fish received before deboning (Tables 2 and 3). The data support results of objective color determinations (Table 1) as to the relationship of deboning pressure to the color of the minced flesh.

Significant but minor, in that the scores were

TABLE 2.—Effects of Several Preparatory Treatments and Deboning Pressures on the Sensory Acceptance of Minced Freshwater Drum Cooked by Baking.

Treatment/ Pressure	Mean Sensory Score			
	Color	Odor	Texture	Flavor
HG 1.5	4.0 cd†	6.6 ab	7.0 a	5.3 abc
HG 3.5	4.0 cd	6.8 ab	7.1 a	5.6 abc
HG 6.0	4.8 d	7.2 ab	7.2 a	6.1 abc
HGS 1.5	5.9 abc	8.0 a	6.6 a	6.9 ab
HGS 3.5	4.3 bcde	7.1 ab	6.9 a	6.1 abc
HGS 6.0	2.5 e	6.1 b	6.3 a	5.8 abc
HGSA 1.5	5.6 abcd	7.4 ab	6.8 a	4.4 bc
HGSA 3.5	5.0 bcd	7.1 ab	6.4 a	4.2 c
HGSA 6.0	4.0 cd	6.1 b	7.3 a	4.8 c
HGSDT 1.5	6.2 ab	7.5 ab	7.5 a	7.8 a
HGSDT 3.5	5.6 abcd	7.3 ab	6.7 a	6.8 ab
HGSDT 6.0	5.8 abc	6.9 ab	6.9 a	3.8 c
Minced Fillets*	7.5 a	8.0 a	6.3 a	5.5 abc

*Skinless, boneless fillets minced in Hobart silent cutter.
†Column means with like letters not significantly different ($P < 0.01$).

TABLE 3.—Effects of Several Preparatory Treatments and Deboning Pressures on the Sensory Acceptance of Minced Freshwater Drum Cooked by Deep-Fat Frying.

Treatment/ Pressure	Mean Sensory Score			
	Color	Odor	Texture	Flavor
HG 1.5	4.5 de†	7.8 abc	6.8 ab	6.4 ab
HG 3.5	4.2 e	8.2 abc	7.0 ab	7.6 ab
HG 6.0	4.1 e	8.0 abc	7.1 ab	6.8 ab
HGS 1.5	6.6 ab	8.3 ab	7.5 a	7.4 ab
HGS 3.5	5.5 cde	7.4 bc	6.7 ab	6.6 ab
HGS 6.0	4.1 e	7.1 c	6.4 ab	6.1 b
HGSA 1.5	7.1 abc	7.9 abc	5.7 b	7.2 ab
HGSA 3.5	5.9 bcd	7.8 abc	6.5 ab	6.6 ab
HGSA 6.0	4.5 de	7.9 abc	7.1 ab	6.2 ab
HGSDT 1.5	7.4 ab	8.2 abc	7.0 ab	7.8 ab
HGSDT 3.5	7.4 ab	8.2 abc	7.4 a	7.9 a
HGSDT 6.0	6.9 abc	8.0 abc	7.1 ab	6.5 ab
Minced Fillets*	8.0 a	8.7 a	7.8 a	7.7 ab

*Skinless, boneless fillets minced in Hobart silent cutter.
†Column means with like letters not significantly different ($P < .01$).

relatively high, differences in odor were observed in minced flesh treatments, although the deep-fat fried samples generally scored higher for this attribute. Neither pressure nor treatment variation had substantial effect upon texture. Significant differences observed in deep-fat fried samples are believed to be the result of frying variation, in that baked samples did not show similar differences.

Flavor results were varied. Significant differences were observed in baked samples and deep-fat fried samples of minced drum, although differences were not as prominent between deep-fat fried samples. The poor flavor of baked samples of HGSDT 6.0 and HGSA 1.5 may be attributable to an earthy flavor present in some drum used in these two deboning variations. The effect of earthiness in drum on flavor emphasizes the need to remove these fish from any processing operation. The generally higher mean scores of deep-fat fried samples indicate that some masking of undesirable flavors is accomplished through the batter and deep-fat frying process.

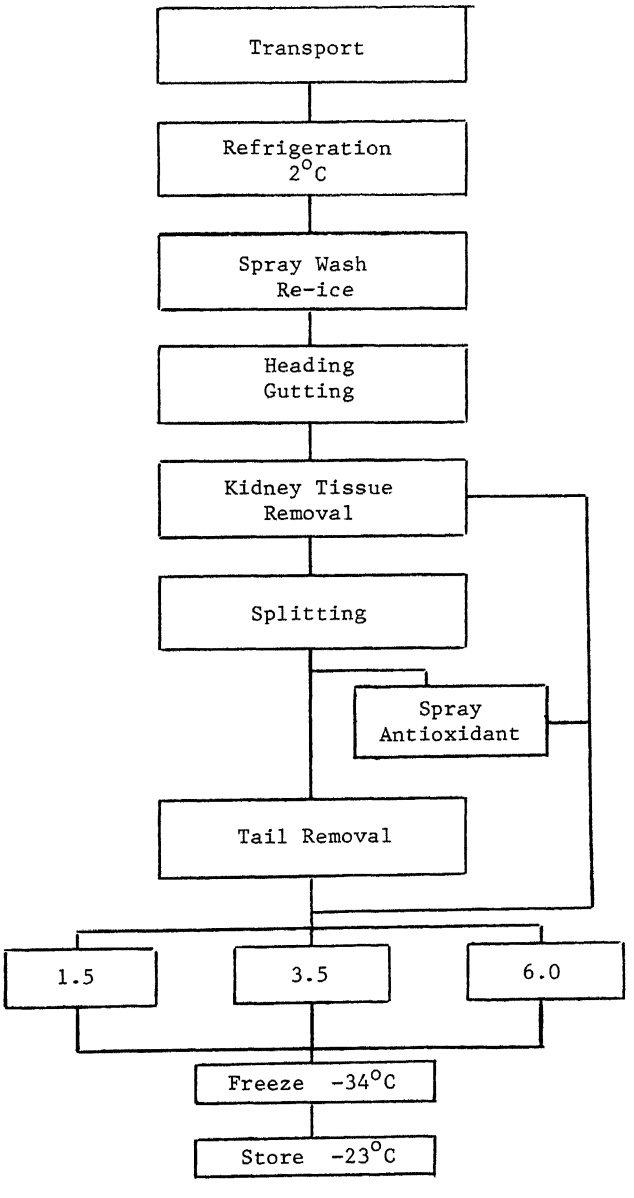

FIG. 1.—Minced fish processing.

In summary, the data suggest that moderate deboning pressures be used to avoid excessive pigmentation of the flesh obtained and prevent large increase of bone content. Orientation of fish to the separation cylinder and tail removal also appear to be beneficial in respect to obtaining lighter color flesh. Additionally, improvement in flavor was evident from scores of deep-fat fried samples, suggesting use of mechanically deboned fish in deep-fat fried products. Thus, the use of mechanically deboned Lake Erie freshwater drum flesh appears not only feasible but has been found to be suitable for products where a natural flakiness is not important.

ACKNOWLEDGMENTS

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Lake Erie Freshwater Drum Fillet Processing, Storage, and Evaluation

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INTRODUCTION

There is presently an estimated 7 to 10 million lb annual sustainable yield of freshwater drum (*Aplodinotus grunniens*) in Lake Erie (10). But commercial landings account for only 10% to 14% of this yield. Freshwater drum, like many other underutilized species, has not attained the popularity of other species as a food fish. However, as seafood prices continue to rise, the potential food use importance of these species will increase.

During 1976, frozen seafoods showed a 54% gain in dollar value over 1975. The biggest single contribution to this increase was frozen shrimp, increasing 82.8% in dollar value (6). An average price of $4.65/lb for frozen shrimp during 1976 will exclude shrimp from many household budgets and force the consumer to choose alternatives. It was also indicated (6) that the highly exploited species are experiencing large dollar value gains.

Although problems of storage and texture variations have been reported (1), Lake Erie freshwater drum and other underutilized species may become increasingly important. Therefore, a specific objective of a broader project was to develop and evaluate processing methods which would allow extended storage of Lake Erie freshwater drum fillets without serious loss of quality.

MATERIALS AND METHODS

Freshwater drum in round form landed in the Western Basin of Lake Erie were purchased each month, April through October 1976, from commercial sources within 24 hours or less after landing. Drum, 60 lb/box in flake ice were transported to the Food Technology Pilot Plant at OSU and kept under 2° C refrigeration until processed.

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Prior to filleting, drum were sprayed with cold water to remove slime and extraneous material. Filleting and skinning of drum were performed by hand prior to the following treatments:

Control (CON): No treatment to boneless, skinless fillets.

Blanch (BLH): Fillets blanched in steam followed by a 30 sec cold water spray. Fillets less than 20.3 cm in length were blanched 20 sec, fillets longer than 20.3 cm were blanched 30 sec. Unsized fillets were blanched for 25 sec.

Dipped (DIP): Fillets were dipped for 1 min in a solution of 87.6% distilled water (2° C), 11.0% sodium tripolyphosphate, 1.3% ascorbic acid, and 0.09% citric acid.

Glazed (GLZ): Fillets were frozen individually at —34° C and glazed by dipping the frozen fillets in a solution of 99.4% distilled water (2° C), 0.4% ascorbic acid, and 0.2% citric acid.

Fillets of April used for blanching and fillets of August used for all treatments were sized 20.3 cm or less (SML), and greater than 20.3 cm in length (LGE).

Packaging of processed fillets with the exception of GLZ fillets was in 0.038 mm polyethylene bags with positive locking seals. GLZ fillets were packaged bulk in large plastic bags. All fillets were frozen at —34° C and stored at —18° C.

Fillet treatments were evaluated over a 10-month storage period at approximately monthly intervals by the following methods.

Fat Rancidity: The method of Tarladgis *et al.* (11), modified by adding 2 drops of Tenox II (Eastman Chemical Co.) during blending of samples (14). TBA values are expressed as mg malonaldehyde/1000 g fish flesh.

Thaw Drip: Frozen samples of 60 to 100 g were placed in polyethylene bags measuring 11.0 x 22.0 cm. The bags were suspended in circulating water main-

tained at 18 to 21° C for 30 min. The bags were then drained of free liquid and reweighed for calculation of percent free thaw drip.

Color: Flesh color was measured with a Hunter Color and Color Difference Meter D25D3A, standardized on a Hunter white tile C2-4306 (L 95.00, a —0.60, b 0.40), after being ground through a plate having 3.2 mm holes.

Sensory Evaluation: Sensory evaluation panels were conducted with 7 to 10 members consisting of graduate students, staff, and faculty. Color, odor, texture, and flavor were judged on a hedonic/scoring scale of 1 (lowest score) to 9 (highest score). Samples for sensory evaluation were prepared according to King (4).

RESULTS AND DISCUSSION

Treating fillets with an antioxidant/antidrip solution prior to freezing (DIP) reduced thaw drip by at least 50% regardless of the month in which the fillets were processed (Fig. 1). BLH fillets released the greatest amount of drip, while CON fillets released an amount intermediate of the other treatments. Thaw drip in GLZ fillets could not be determined due to the inability to separate or distinguish actual thaw drip from muscle tissue from glaze melt. Size of fillets did not have a great effect on thaw drip release, although size has been suggested as one of the many factors influencing drip loss (8). Thaw drip decreased in fillets processed progressively later in the year, suggesting seasonal effects on thaw drip losses.

Ten months of storage had little effect upon flesh color as measured objectively or by sensory evaluation methods, regardless of the treatment the fish received. DIP and GLZ treatments provided protection to the myoglobin of red muscle tissue by retarding its oxidation to the metmyoglobin form (brown in color). Although BLH fillets displayed a whiter appearance when raw, panelists did not indicate any color prefer-

TABLE 1.—Effects of Harvest Time and Treatment on the Acceptability of Freshwater Drum Fillets as Shown by Overall Mean Scores.

Color		Odor		Texture		Flavor	
Month/Treatment/Size	Overall Mean	Month/Treatment/Size	Overall Mean	Month/Treatment/Size	Overall Mean	Month/Treatment/Size	Overall Mean
AUG/BLH/LGE	7.90 a*	AUG/BLH/LGE	7.78 a	SEP/DIP	7.39 a	SEP/DIP	7.03 a
AUG/DIP/LGE	7.80 ab	JUL/DIP	7.67 ab	SEP/GLZ	7.16 ab	AUG/BLH/LGE	6.97 ab
AUG/GLZ/LGE	7.68 abc	SEP/BLH	7.57 abc	AUG/DIP/SML	7.12 ab	AUG/DIP/SML	6.80 abc
SEP/GLZ	7.66 abcd	SEP/DIP	7.56 abc	AUG/GLZ/LGE	7.11 abc	AUG/DIP/LGE	6.76 abcd
SEP/DIP	7.60 abcde	JUN/DIP	7.56 abc	AUG/CON/LGE	7.05 abcd	JUL/DIP	6.68 abcd
SEP/BLH	7.53 abcde	AUG/DIP/LGE	7.53 abc	AUG/CON/SML	7.01 abcd	OCT/DIP	6 67 abcd
AUG/BLH/SML	7.53 abcde	JUL/CON	7.52 abc	JUL/DIP	6.98 abcd	SEP/GLZ	6.64 abcde
AUG/CON/LGE	7.51 abcdef	AUG/GLZ/SML	7.50 abc	AUG/DIP/LGE	6.98 abcd	SEP/CON	6.59 abcde
AUG/DIP/SML	7.44 abcdef	AUG/CON/SML	7.49 abc	AUG/GLZ/SML	6.90 abcd	OCT/GLZ	6.47 abcdef
JUL/DIP	7.34 abcdef	AUG/DIP/SML	7.44 abcd	SEP/CON	6.79 abcd	SEP/BLH	6.45 abcdef
AUG/CON/SML	7.36 abcdef	SEP/CON	7.43 abcd	AUG/BLH/SML	6.78 abcd	AUG/CON/SML	6.42 abcdefg
JUL/CON	7.29 abcdefg	SEP/GLZ	7.41 abcd	OCT/DIP	6.69 abcd	AUG/BLH/SML	6.40 abcdefg
OCT/BLH	7.28 abcdefg	JUL/GLZ	7.36 abcd	JUL/GLZ	6 67 abcd	OCT/CON	6.33 abcdefgh
JUN/GLZ	7.21 abcdefg	JUN/GLZ	7.36 abcd	JUL/CON	6.52 abcde	JUL/CON	6.31 abcdefgh
AUG/GLZ/SML	7.12 abcdefghi	AUG/BLH/SML	7.28 abcd	JUN/DIP	6.38 abcdef	AUG/GLZ/SML	6.21 bcdefghi
SEP/CON	7.11 abcdefghi	JUL/BLH	7.28 abcd	AUG/GLZ/LGE	6.34 abcdefg	JUN/GLZ	6.05 bcdefghi
OCT/DIP	6.99 abcdefghij	AUG/GLZ/LGE	7.27 abcd	OCT/GLZ	6 30 abcdefg	AUG/GLZ/LGE	6.03 bcdefghi
APR/CON	6.98 abcdefghij	APR/GLZ	7.25 abcd	JUL/BLH	6.28 abcdefg	AUG/CON/LGE	5.99 cdefghi
JUL/GLZ	6.84 bcdefghij	OCT/DIP	7.24 abcd	SEP/BLH	6.25 abcdefgh	JUL/BLH	5.98 cdefghi
OCT/GLZ	6.82 bcdefghij	AUG/CON/LGE	7.20 abcd	JUN/GLZ	6.12 abcdefghi	JUL/GLZ	5.95 cdefghi
JUN/DIP	6.77 cdefghij	APR/DIP	7.18 abcd	OCT/CON	6.04 bcdefghi	JUN/DIP	5.85 cdefghi
OCT/CON	6.75 cdefghij	JUN/BLH	7.17 abcd	JUN/BLH	6.03 bcdefghi	OCT/BLH	5.81 defghi
JUN/BLH	6.67 defghij	JUN/CON	7.13 abcde	MAY/BLH	5.91 bcdefghi	MAY/CON	5.70 efghij
APR/DIP	6.66 defghij	OCT/GLZ	7.10 abcde	MAY/CON	5.83 cdefghi	APR/GLZ	5.70 efghij
JUN/CON	6.63 efghij	OCT/CON	7.04 bcde	MAY/DIP	5.79 defghi	MAY/BLH	5.60 fghij
APR/BLH/LGE	6.53 fghijk	OCT/BLH	7.00 bcde	APR/CON	5.33 efghi	APR/DIP	5.53 fghij
JUN/BLH	6.32 ghijk	APR/CON	6.89 cdef	JUN/CON	5.23 efghi	APR/CON	5.52 fghij
APR/GLZ	6.26 hijk	APR/BLH	6.87 cdef	APR/BLH/LGE	5.20 efghi	APR/BLH/SML	5.49 ghij
MAY/DIP	6.24 ijk	MAY/CON	6.75 def	MAY/GLZ	5.19 efghi	JUN/BLH	5.44 hij
APR/BLH/SML	6.14 jk	APR/BLH/LGE	6.75 def	APR/BLH/SML	5.18 fghi	APR/BLH/LGE	5.30 ij
MAY/CON	6.08 jk	MAY/BLH	6.55 ef	OCT/BLH	5.13 ghi	JUN/CON	4.90 j
MAY/BLH	5.72 kl	MAY/GLZ	6.48 ef	APR/DIP	5.05 hi	MAY/DIP	4.07 k
MAY/GLZ	5.08 l	MAY/DIP	6.34 f	APR/GLZ	5.01 i	MAY/GLZ	3.52 k

*Column means with like letters not significantly different ($p < .01$).

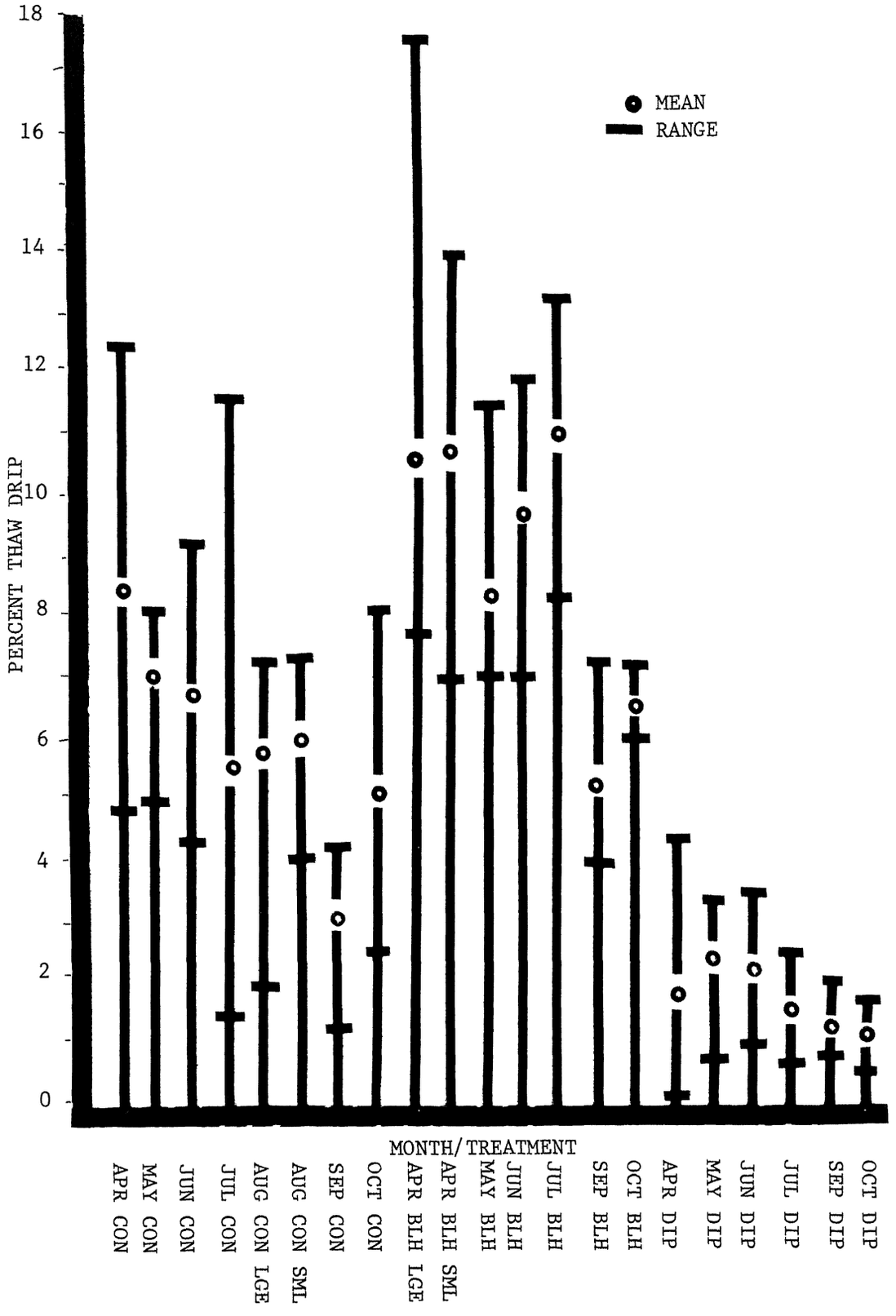

FIG. 1.—Effects of month of harvest and treatment on thaw drip in freshwater drum fillets.

ence for these fillets when cooked. Fillets processed July through October were generally scored higher regardless of treatment, again suggesting that season of harvest has definite influence on acceptance (Table 1).

Sensory evaluation of fillets throughout storage revealed that odor was the least variable of the organoleptic characteristics evaluated. Analysis of variance of fillet treatment periodic evaluation means showed no trend of decreasing scores, but only an occasional less acceptable sample.

Seasonal effects on the acceptability of freshwater drum fillets was also evident from the higher mean scores obtained for drum processed progressively later in the year. Compositional analyses reported by Krzeczkowski (5) indicated that fat and protein increased in drum during late summer months. Thus the better physiological condition of drum during this period may have significant influence on acceptability.

Definite effect of season of harvest and processing was shown by sensory flavor scores (Table 1). In addition to later month drum treatments generally being more acceptable, May DIP and May GLZ fillets were consistently scored low throughout storage. Noticeable "earthy or musty" flavors were present in these fish. May DIP and May GLZ treatments were processed from a lot of drum purchased May 25, 1976, approximately 2 weeks after drum used for May CON and May BLH fillets. Earthy flavors were also encountered in drum purchased during the same time period of the following year, 1977.

Although judged to be in equally good condition, by visual examination, as fish not exhibiting these flavors, considerable amounts of the algae *Oscillitori* sp. were found in the intestinal tract. It is believed that a compound called geosmin released by this organism is responsible for the muddy or earthy flavor in drum of this period. The problem of muddy taints, odors, and flavors in fish is well recognized (2, 12, 15). Freshly filleted drum of May 1977 (CON) and drum stored 1 year (May GLZ 1976) were found to be equally poor in flavor, and not significantly different ($p > 0.01$) (Table 2). When the same fresh May 1977 drum were compared to 1976 treatments known not to have earthy flavors, the fresh May 1977 drum were found to be significantly ($p < .01$) less acceptable (Table 3).

Results of TBA determinations were not conclusive. Poor correlation of TBA values and sensory flavor scores were found for most treatments; however, this has been reported by other researchers (3, 9, 13) and cannot be explained fully. Many treatments nevertheless showed significant ($p < .05$) or highly significant ($p < .01$) correlation of increasing TBA values with storage time of fillets (Fig. 2). While

TABLE 2.—Sensory Comparison of May GLZ (1976) Fillets with May CON (1977) Fillets.

Month/ Treatment/Year	Mean Sensory Scores			
	Color	Odor	Texture	Flavor
May GLZ 1976	6.6 a*	6.5 a	5.5 a	4.0 a
May CON 1977	5.9 a	6.7 a	5.8 a	4.3 a

*Column means with like letters not significantly different ($p < .01$).

TABLE 3.—Sensory Comparison of May CON (1977), Jun DIP (1976) and Aug CON (1976) Fillets.

Month/ Treatment/Year	Mean Sensory Scores			
	Color	Odor	Texture	Flavor
May CON 1977	6.4 b*	5 8 b	5.7 b	2.8 b
Jun DIP 1976	7.6 a	7.7 a	6.7 a	6.7 a
Aug CON 1976 (deep skinned)	8.2 a	7.6 a	5.6 a	5.7 a

*Column means with like letters not significantly different ($p < .01$).

blanching apparently promoted fat oxidation resulting in the greatest rise in TBA values, dipping (DIP) offered the best protection against fat oxidation and red muscle pigment discoloration.

In summary, the data suggest that the season of harvest, both in regard to water conditions and the physiological conditions of the fish, may be the single most important factor in freshwater drum acceptability. Treatment of drum fillets with an antioxidant/antidrip solution was shown to retard development of rancidity and substantially reduce thaw drip (thus reducing weight loss at the time of secondary use), and is suggested as the best method for preserving drum quality during extended storage.

ACKNOWLEDGMENTS

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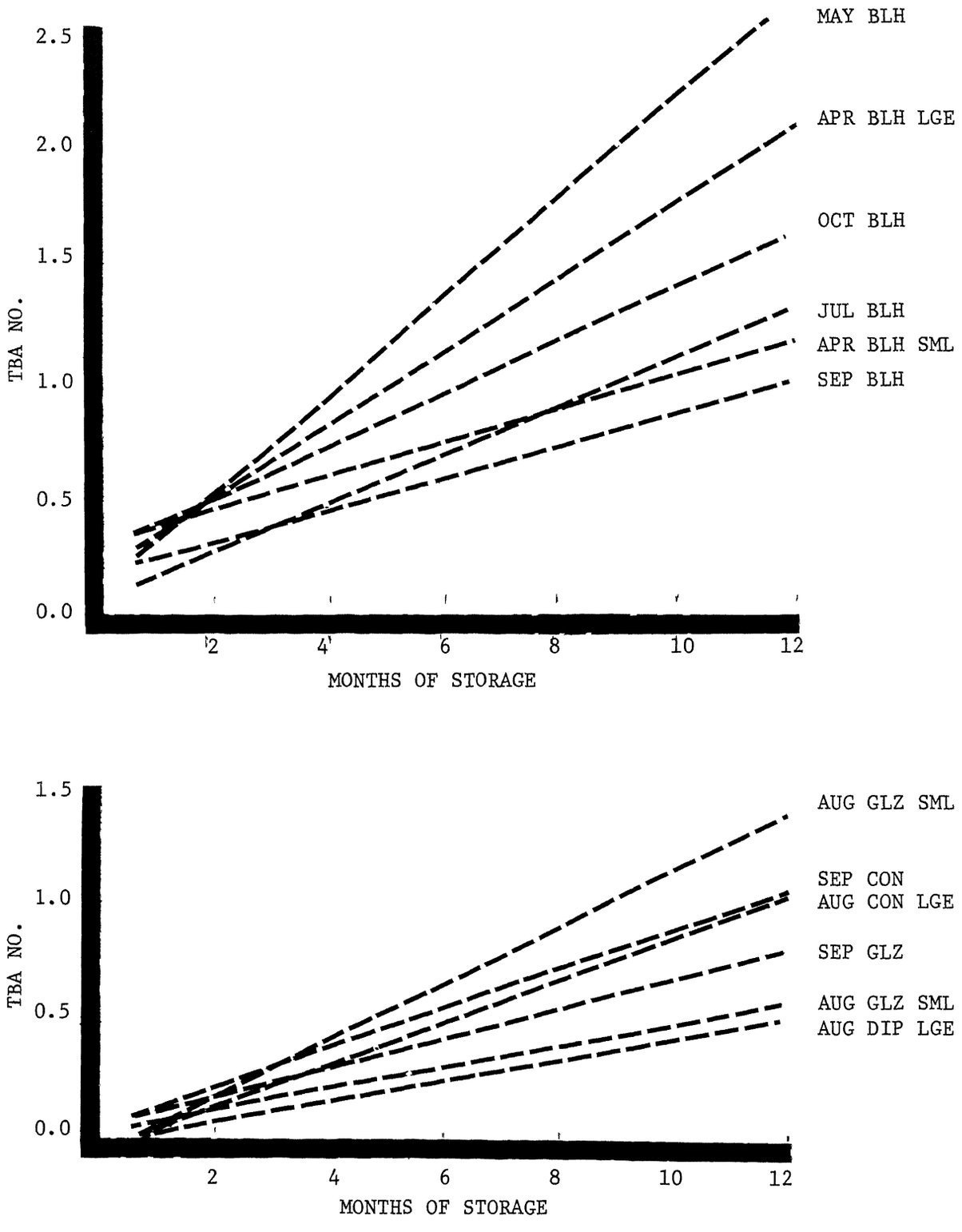

FIG. 2.—Effects of storage time on rancidity development (TBA) in selected freshwater drum fillet treatments.

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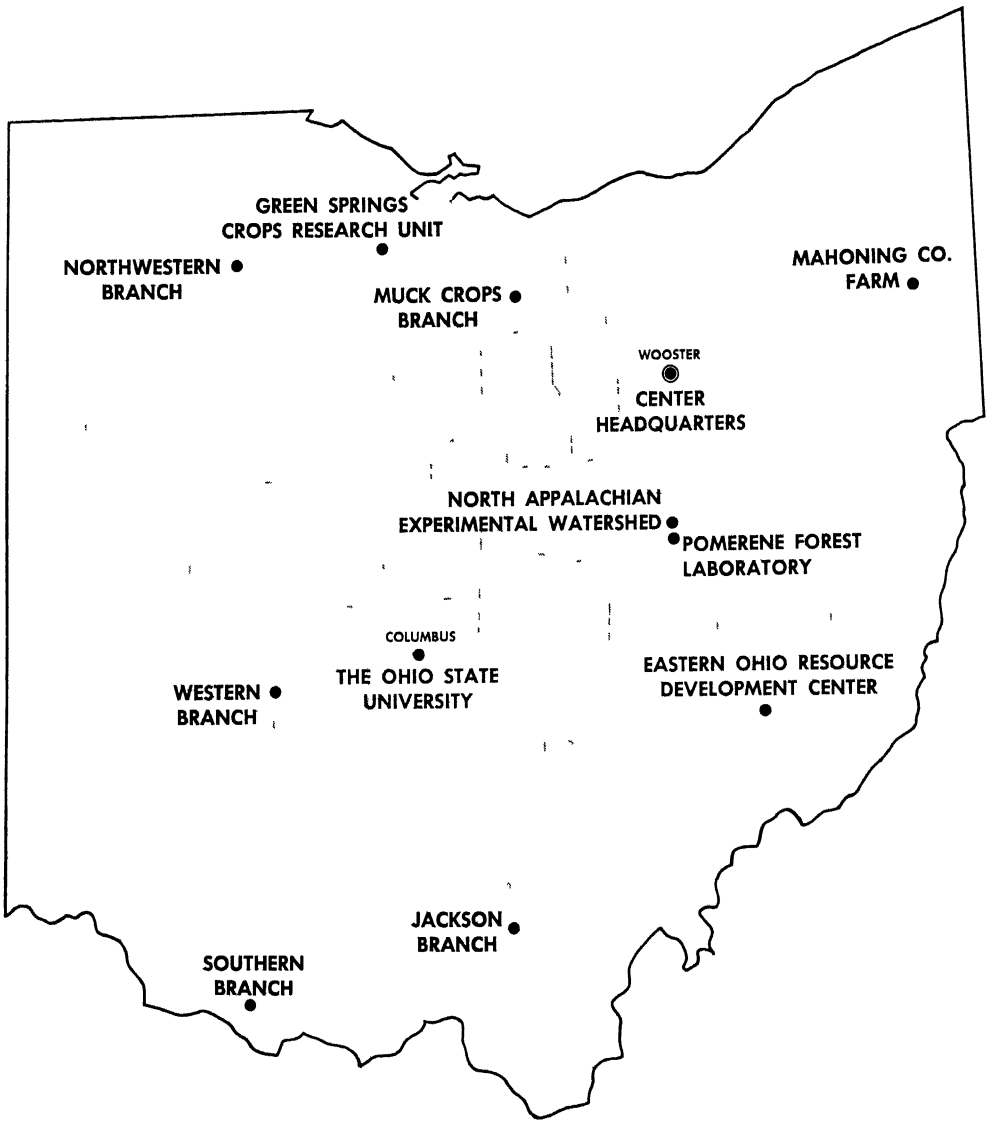

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