

SOFTENING ENZYME ACTIVITY OF CUCUMBER FLOWERS
FROM NORTHERN PRODUCTION AREAS^a

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When pectic substances in the middle lamella of pickling varieties of cucumbers are hydrolyzed by pectic enzymes, softening of the tissue results. Previous studies (3, 5) have demonstrated that a pectin-splitting enzyme, similar in behavior to polygalacturonase, is chiefly responsible for the softening of salt-stock cucumbers cured under commercial conditions. The enzyme was found to be active in brines from numerous commercial brining stations and its activity correlated with the softening of the salt-stock. It was further shown that a purified form of the enzyme could readily deteriorate the firmness of sound salt-stock cucumbers. Additional studies (4, 7, 9) have since demonstrated that cellulolytic enzyme systems are also present in curing brines and may contribute in part to the total softening action of brined material.

It has been established (7, 8) that the pectinolytic and cellulolytic enzyme systems are introduced into the curing vats by way of the partially dried cucumber flowers that remain attached to the cucumbers, particularly the small-sized fruit (see Figure 1, right). In addition, microbiological studies strongly point to mold growth in the cucumber flowers as the actual causative agent responsible for the softening enzyme activity.

Numerous brining experiments covering several seasons in two important production areas of the South (7) appear to substantiate the basic findings on the origin of softening enzymes in cucumber brines. For example, brine samples from vats filled with small-sized cucumbers (1 to 1½ in. dia.) that had a high percentage of flowers, either retained by the cucumbers or added experimentally, were shown to have high enzyme activity and usually the cured salt-stock was either soft or definitely inferior in firmness. However, when the cucumbers were deflowered by hand, the brine samples were low in enzyme activity and the cured stock was exceptionally firm.

More recently, Bell and Etchells (1) have found that pectinolytic and cellulolytic enzymes are inhibited by a water soluble fraction of Scuppernong grape leaves (*Vitis rotundifolia*). Furthermore, cucumber brining tests have demonstrated that the pectinolytic and cellulolytic enzyme systems of cucumber flowers were effectively reduced in activity by use of a crude extract of Scup-

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pernong leaves (2). The inhibition of the softening enzymes resulted in an increased firmness of the fermented, brine-cured cucumbers.

Softening can, at times, be a serious problem in northern brining areas as well as in other sections of the country. Etchells (6), during the Fall of 1955, tested the firmness of 89 vats of commercially brined salt-stock from 4 brining stations located in Michigan and Indiana. Examination of the stock for firmness demonstrated that approximately 25% of the material as of October 17, 1955 was less than firm . . . also, of the 89 vats examined, stock from 7 had lost more than 50% in firmness and were definitely soft. Although this was the only instance of commercial softening observed first-hand during the 1955 season in northern brining areas, reports from packers have since indicated that loss of firmness of salt-stock, to a varied degree, may have been widespread in Indiana, Michigan, and Wisconsin that year.

The present investigation was undertaken chiefly as an exploratory survey to determine the nature and extent of softening enzyme activity of cucumber flowers collected in northern production areas; the study also provided the opportunity to compare the values obtained for pectinolytic and cellulolytic activity with those determined earlier for flower samples collected from southern areas (i.e., brining stations located in North Carolina and Texas). It was an interesting coincidence that the study on the softening enzyme activity of cucumber flowers from the northern production area was made during a season (1955) which provided direct evidence of enzymatic softening of commercially brined cucumbers in that area.

MATERIALS AND METHODS

Collecting flower samples and flower retention data. During August 16-25, 19 samples of retained cucumber flowers (Figure 1, Right) obtained from No. 1 size (1 to 1 $\frac{1}{8}$ in. dia.) stock were collected at 17 brining stations and 1 receiving station located in Michigan, Wisconsin, Indiana, and Ontario (Figure 2). Eight additional samples, consisting of freshly opened flowers (Figure 1, Left) were removed from cucumber ovaries in 8 commercial fields located in Michigan and Wisconsin. The brining stations and fields visited represented operations by 12 pickle companies and were selected over a wide area to give geographical coverage. Each sample was usually composed of 100 individual flowers; these were removed from the cucumbers or ovaries with forceps and put in polyethylene freezer-bags. Each sample was labelled and immediately frozen with dry ice and stored at approximately -10° C. until analyzed for enzyme activity. Additional information was gathered at each brining station as to the varieties planted and, the estimated flower retention on No. 1 size cucumbers. The latter was obtained by counting the number of retained flowers per 100 cucumbers; the final estimate was based on samples from 4 to 6 bushels of stock that had been machine graded.

Softening enzyme extraction and analysis. Each flower sample was weighed, counted, and placed in a Waring blender⁴ with 100 ml. of 2% NaCl, blended for 1 minute, then filtered and the clear flower extract dialyzed against water for 4 hours using 0.75 in. dia. cellophane tubing. Viscometric methods were used to determine the pectinolytic and cellulolytic (Cx) enzyme activities; these have been given in detail by Bell *et al.* (4) and 100 units of pectinolytic activity equals 50% loss in viscosity in 20 hours of a 1.0% sodium polypectate solution, buffered with citrate at pH 5.0, at 30° C. Cellulolytic activity units are equal to the same conditions as above except 1.0% sodium carboxymethylcellulose is used as the substrate.

⁴ It is not the policy of the Department of Agriculture to recommend the products of one company over similar products of any other company. The name supplied herewith is furnished for your convenience and information.

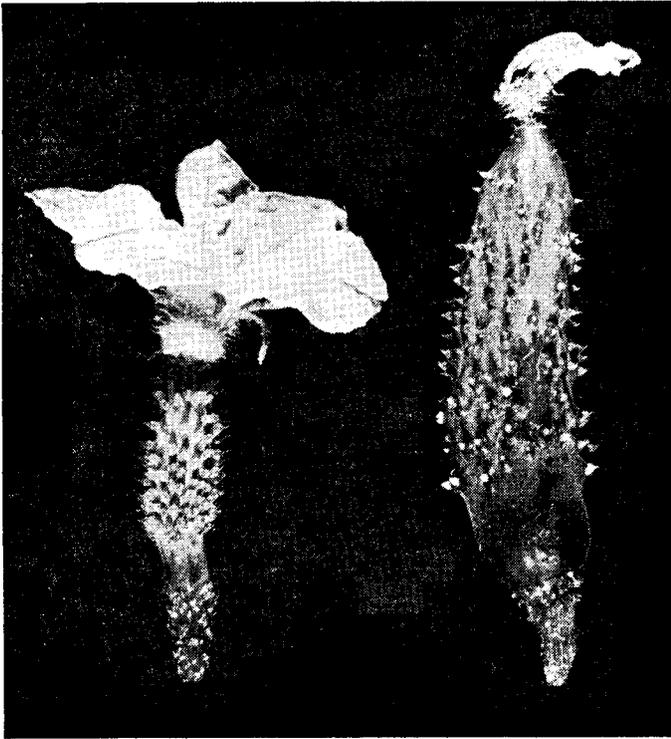


Figure 1. Left: Opened pistillate flower typical of samples collected from the growing cucumber plant in commercial fields.

Right: Partially dried flower on a small sized (No. 1) cucumber; this retained flower is typical of samples removed from 1 to $1\frac{1}{16}$ " diameter cucumbers at brining stations. About actual size.

RESULTS AND DISCUSSION

Flower retention on cucumbers. It has been observed (8) that flower retention of commercially harvested, machine graded No. 1 size cucumbers

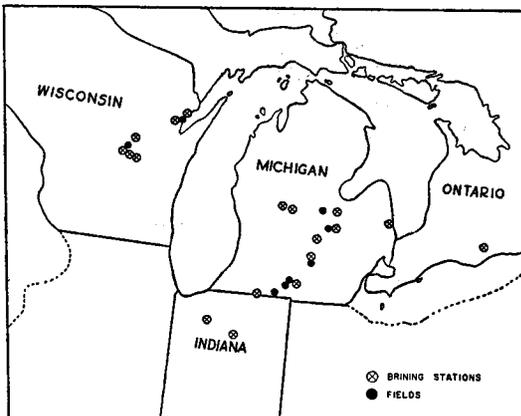


Figure 2. Geographical location of brining stations and commercial fields where cucumber flower samples were collected during 1955 harvest.

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grown in two important production areas in the South can vary extensively during the intake season. Retention was usually found to be highest (70 to 80%) during the first part of the harvest, whereas during the latter part it was negligible (5 to 10%). Furthermore, flower retention was greater on smaller fruit than on the larger.

The cucumber fields visited during the northern survey were in their third to fourth week of production and the crop conditions varied greatly; ranging from lush, vigorous vines with heavy production in certain parts of Michigan to less vigorous vines suffering from high temperatures and lack of rain in the principal growing sections of Wisconsin. Information on the percentage of flowers retained by the different cucumber varieties examined at northern brining stations is given in Table 1; also included are flower weights. The

TABLE 1

Estimated flower retention, weight, and enzyme activity of cucumber flowers from different varieties sampled at northern brining stations during Aug. 16-25, 1955

Brining station	Variety ⁴	Estimated retention of flowers	Average flower weight	Pectinolytic activity (per flower)	Cellulolytic activity (per flower)
<i>code</i>		<i>%</i>	<i>grams</i>	<i>units</i>	<i>units</i>
Michigan					
WP.....	SMR-12-12	26	0.021	97	547
LT.....	MR-25	10	0.024	98	707
MA.....	SR-6	8	0.022	122	654
LA.....	SR-6	4	0.017	33	677
NL.....	SR-6	5	0.015	59	554
SG.....	SR-6	11	0.019	55	734
ED.....	SR-6	8	0.022	33	780
LV.....	SR-6	7	0.016	165	488
CR.....	SR-6	3	0.031	37	340
Wisconsin					
AB ¹	SR-6	14	0.031	80	246
OC.....	SR-6	6	0.025	193	654
WA.....	SMR-12-12	47	0.027	240	487
SL-1.....	SMR-12-12	29	0.028	233	407
SL-2 ²	SMR-12-12	25	0.031	31	213
WU.....	SMR-12-12	40	0.027	78	507
BR.....	SR-6 and SMR-12-12	12	0.025	147	547
Indiana					
NJ ³	MR-25	12	0.029	308	2,199
MC ³	MR-17	2	0.040	201	1,421
Ontario					
WF.....	MR-17 and Chicago pickling	3	0.021	60	760
Average.....			0.0238	120	680

¹ Receiving and grading station only.

² Represents a second sample from Station SL collected September 4.

³ Material from these 2 stations was shipped to Chicago by truck on August 20 and sampled August 22.

⁴ SR (= scab resistant) and SMR (= scab and mosaic resistant) represent University of Wisconsin introductions; MR (= mosaic resistant) represents Ohio State University introductions.

latter show that the average station flower weighed 0.0238 g. with a range from 0.015 to 0.040 g.

The most widely grown cucumber varieties in the areas visited in Michigan and Wisconsin were SR-6 and SMR-12-12 respectively. Flower retention values for varieties at all stations ranged from 2 to 47% with 8 of the stations above the 10% level; also, 4 of these were above 25%. Retention estimates from the 5 stations receiving and grading variety SMR-12-12 ranged from 25 to 47% (av. 33%). In contrast, only 2 of the 9 stations with variety SR-6 gave retention values above 10% and the range for all lots of this variety examined was 3 to 14% (av. 7%). Based on this information, it appears likely that flower retention estimates for the recently introduced variety SMR-12-12 were generally much higher than those for SR-6. Four of the five stations where SMR-12-12 was observed for flower retention were in Wisconsin where hot, dry weather was prevailing. Such unfavorable growing conditions may have contributed to flower retention of this variety. However, subsequent observations by northern packers during the 1956 season on these two varieties tend to substantiate the varietal difference indicated above.*

Enzyme activity of flowers from stations. The pectinolytic and cellulolytic activities of retained cucumber flowers collected at southern brining stations in 1953 have previously been presented (7). However, summarized results based on more recent southern studies—1954 and 1955—are reported herein for comparison with the 1955 northern observations because improved technics (4) for measuring softening enzyme activity were in use for material collected in the two areas during the particular years mentioned.

The pectinolytic activity of 16 flower samples obtained from southern areas ranged from 34 to 185 units (av. 88) calculated on a per flower basis. These flowers, when added to experimental vats of No. 1 size cucumbers in quantities equal to 100% retention, reduced the firmness of the cured as much as 50 to 75% over that of deflowered controls. The pectinolytic activity values of the 19 flower samples collected from northern brining stations (Table 1) show a range from 31 to 308 units (av. 120).

The results for cellulolytic activity of flowers from southern and northern areas parallel those for pectinolytic activity. The cellulolytic activity of the 16 flower samples from southern areas ranged from 477 to 1,191 units (av. 782); the values for northern area flowers (Table 1) ranged from 213 to 2,199 units (av. 680). A rather wide variation of enzyme activity of flower samples was observed in both northern and southern production areas. It is important to mention that, based on previous experience, such variation in enzyme activity of southern flower samples from the same stations, may occur not only between seasons, but also for given periods within the same season.

These results indicate that retained cucumber flowers from northern production areas possess a potential softening enzyme activity equal to that of flowers from southern areas and which, if introduced into curing vats in sufficient amounts under suitable conditions, could reduce the firmness of brined material in a manner previously demonstrated for cucumbers brined

* Plant breeders at several State Agricultural Experiment Stations (North Carolina, Arkansas, Michigan, and Wisconsin) have expressed considerable interest in studying the factors responsible for flower retention in pickling cucumbers and are evaluating their new lines for this characteristic.

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under southern conditions. In this connection, it will be recalled that enzymatic softening of commercially brined cucumbers was observed first-hand in October 1955, at northern brining stations in the same general area visited about 2 months earlier (August) during the survey.

Enzyme activity of field flowers. The enzyme activity of 8 samples of flowers removed from cucumber ovaries in fields located in Michigan and Wisconsin are presented in Table 2 together with the flower weights. The average weight of a freshly opened flower was found to be 0.281 g.; this was about 12 times the weight of a individual, partially dried flower removed from No. 1 size stock at the brining stations. The pectinolytic activity of flowers from fields ranged from 350 to 1,737 units (av. 875) calculated on a per flower basis; the cellulolytic activity ranged from 103 to 221 units (av. 151).

TABLE 2

Weight and enzyme activity of cucumber flowers from different varieties sampled from ovaries in 8 commercial fields located in Michigan and Wisconsin

Location of field	Variety	Average flower weight	Pectinolytic activity (per flower)	Cellulolytic activity (per flower)
<i>code</i>		<i>grams</i>	<i>units</i>	<i>units</i>
Michigan				
BN.....	MR-25	0.303	1737	140
LT.....	SMR-12-12	0.200	980	124
LT.....	MR-25	0.305	956	103
MA.....	SR-6	0.294	1012	132
NL.....	SR-6	0.366	1023	221
SG.....	SR-6	0.395	350	197
Wisconsin				
OC.....	Maine No. 2 and SR-6	0.219	511	160
WA.....	SMR-12-12	0.168	429	129
Average.....		0.281	875	151

Considering all samples collected from each source (brining stations and fields) the flowers removed from the ovaries in the fields had about 7 times the pectinolytic activity as compared to those removed from the No. 1 size fruit being delivered to the brining stations. In the case of cellulolytic activity, the situation was somewhat the reverse; here, the station flowers had about 4.5 times more activity than field flowers.

SUMMARY

In general, the exploratory findings reported herein indicate that retained cucumber flowers from northern production areas—such as Michigan, Wisconsin and Indiana—possess a potential softening enzyme activity (pectinolytic and cellulolytic) equal to that of flowers from southern areas. Furthermore, it seems most likely that the softening enzyme concentration of northern flowers was such that, if sufficient amounts were introduced into curing vats under suitable conditions, they could reduce the firmness of brined material in a manner previously demonstrated for cucumbers brined under southern conditions.

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