in which a fish sample has been stored when attempts are made to determine the content of compounds having high volatilities, such as amines.

The sablefish, which was considered good and edible but yet below usual commercial quality, was void of DMA, and had less than 1 μg DEA/g of wet tissue. Compared to the good flesh, the level of TMA increased nearly eightfold and MA decreased nearly sevenfold for sablefish flesh (200 g of excised portion) that was stored in aluminum foil in a 6 ± 1 °C refrigerator for 28 days. The spoiled flesh was also void of DMA, which is interesting considering the evidence that has accumulated to show that the content of DMA in fish increases with storage time (Beatty and Collins, 1940; Tokunaga, 1970; Castell *et al.*, 1971).

Sato *et al.* (1963) reported that a spoiled fish was free of DMA, but that the three ethylamines and isobutylamine were detected. Also, DMA has been reported absent from the flesh of herring (Hughes, 1958), and from fillets of flounder, halibut, wolffish, and redfish (Castell *et al.*, 1971).

In addition to the simple aliphatic amines in fish, free purines and pyrimidines do occur and increase during storage (Jones, 1960; Spinelli *et al.*, 1964). To determine the latter classes of amines in fish, isolation methods other than with steam distillation are necessary. Also, in order to analyze compounds such as 6-hydroxypurine (hypoxanthine) by glc, the procedure would require preparations of stable, volatile derivatives. The isolation of all amines from fish tissues can rely on the basicity of the class of compounds, their ability to form cations, and their solubility in organic solvents, such as in the present case.

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Sucrose Accumulation in Sweet Corn Kernels: Effects of Chelators

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Application of ethylenediaminetetraacetic acid to sweet corn, as a spray on the plants or injected into the ears, increases the sucrose content of the kernels at harvest by 67%, while the amount of reducing sugar is doubled. Ethylenediaminetetraacetic acid appears to have no effect on the conversion of sucrose to polysaccharides in the endosperm during the postharvest period. Preharvest injection of pyrophosphate into the ears, however, is quite effective in retarding this process. The results indicate an effect of ethylenediaminetetraacetic acid and other chelators on sugar transport from the leaves to the ears, perhaps by altering permeability of cell membrane. We suggest that the chelator enhances the translocation of sucrose into the kernels through a barrier of cells between the vascular system and the endosperm tissue.

In a previous publication (Amir *et al.*, 1971) it was shown that the conversion of sucrose to starch in sweet corn kernels can be regulated by preharvest pyrophosphate (PP_i). The effect of PP_i on the system is associated with the inhibition of ADP–glucose synthesis, the main glucosyl donor for starch synthesis. The enzyme ADP–glucose pyrophosphorylase, the enzyme which catalyzes ADP–glucose synthesis, has an absolute requirement for Mg^{2+}. Thus, studies on the effect of ethylenediamine tetraacetic acid (EDTA) were initially undertaken to help elucidate the possible chelation of Mg^{2+} in the endosperm by PP_i as a possible mode of action. In these studies, it was found that EDTA had no effect on the conversion of sucrose to polysaccharides in detached ears.

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Table I. The Effect of EDTA and Pyrophosphate on the Accumulation of Sucrose and Reducing Sugar in Kernels of Sweet Corn[a]

Treatment	At harvest mg/g dry weight[b]		24 hr storage mg/g dry weight		36 hr storage mg/g dry weight	
	Reducing sugar	Sucrose	Reducing sugar	Sucrose	Reducing sugar	Sucrose
Control	64	197	42	71	36	60
Control H_2O	67	193	53	62	42	57
Control mannitol, 50 m*M*	55	193	47	72	41	58
PP_i, 1 m*M*	75	236	48	71	33	51
PP_i, 10 m*M*	87	262	58	195[c]	50	61
PP_i, 50 m*M*	65	226	43	87	37	54
EDTA, 1 m*M*	91	284	64	134	54	74
EDTA, 10 m*M*	137	338[c]	78	137	64	92
EDTA, 50 m*M*	135	352[c]	71	189[c]	73	71

[a] Attached ears were injected with 10 ml of a solution containing the chemical 48 hr before harvest. Corn ears were stored at 25°C. [b] Dry weight of the whole kernel. [c] Significant at the 1% level.

Table II. The Effect of EDTA on Sucrose Transportation from the Parenchyma to the Endosperm Tissues of Detached Ears[a]

Treatment	No sucrose (mg sucrose/g dry weight)			Plus sucrose[b] (mg sucrose/g dry weight)		
	Initial 0 hr	After 24 hr	Decrease, %	Initial 0 hr	After 24 hr	Decrease, %
Control	49.8	24.7	50	52.3	30.3	42
Control H_2O	69.1	34.4	50	49.9	32.4	35
PP_i, 5 m*M*	42.9	34.5	19	42.0	38.9	7
PP_i, 10 m*M*	58.7	48.2	17	48.6	42.3	12
EDTA, 5 m*M*	46.2	29.2	36	51.3	47.5	7
EDTA, 10 m*M*	58.4	32.1	45	55.6	50.3	9

[a] Ears were obtained from commercial sources. [b] Sucrose at a concentration of 0.3 *M* was injected (5 ml) 5 hr after the introduction of the chemical treatments.

However, when EDTA was injected into the ear or sprayed on intact plants, a very dramatic influence on the sucrose concentration of the endosperm was noted. These findings are summarized in this paper.

MATERIALS AND METHODS

Except for ears obtained from commercial sources, all experimental material was obtained from hand-pollinated plants (16 to 18 days from pollination). Sugar extraction from kernels and measurement were performed as described in a previous publication (Amir *et al.*, 1971). The chemicals were introduced at the top of the ear *via* the central parenchyma tissue with a hypodermic needle (containing a wire to prevent clogging). A 10-ml solution was introduced into the ear 48 hr before harvest. In other experiments involving detached ears, 5 ml of the chemical was injected twice (following 8-hr intervals) into the cob 24 hr before placing the ears in storage at room temperature (0 time). A rubber tube containing 10 ml of the same solution was attached to the base of the ear to prevent loss of the solution and to ensure adequate supply of the chemical. The J. W. Tukey test was used for comparing the means (Steel and Torrie, 1960).

RESULTS AND DISCUSSION

The results of the field experiments are summarized in Table I. The apparent increase in sucrose production in the ears under PP_i treatment (10 and 50 ppm) was statistically significant at the 1% level. EDTA treatment was found to increase the sucrose content by 67%. Furthermore, it was also noted that EDTA enhanced the amount of reducing sugar by twofold. After 24 hr of storage at room temperature (25°C) the amount of sucrose in control tissue decreased by 65%, while the same trend was observed in the EDTA-treated ears. Nevertheless, in absolute terms, the EDTA-treated ears still contained twice as much sucrose as the control.

Table III. The Effect of Foliar Applications of EDTA on Accumulation of Sucrose and Reducing Sugar in Kernels of Sweet Corn[a]

Treatment	Concentratoin, m*M*	Reducing sugar, mg/g dry weight	Sucrose, mg/g dry weight
Control		62 ± 3	131 ± 12
EDTA	10	44 ± 3	160 ± 14
EDTA	60	66 ± 3	205 ± 17[b]

[a] Foliar spray of EDTA to greenhouse grown plants 48 hr before harvest. The amount of solution was sprayed onto the plants until "run-off," usually 100–150 ml/plant. The results are expressed as the mean of five replications (± standard error). [b] Significant at the 1% level.

The experiments performed indicate that PP_i acts on sucrose–polysaccharide conversion differently than does EDTA. Pyrophosphate (10 m*M*) was the only chemical tested that retarded sucrose conversion to polysaccharides as previously noted (Amir *et al.*, 1971). Based on previous information on the role of cations, especially Ca^{2+}, on the functional integrity of the plant cell membrane (Foute and Hanson, 1964; Findlay, 1970), the EDTA effect on sucrose accumulation is thought to be mediated through changes in the cell membrane permeability. In corn kernels several layers of cells separate the vascular bundle from the endosperm tissue at the edge of the kernel (Esau, 1965). EDTA

might increase the membrane permeability of those cells in the separation layer and thereby increase the translocation of sucrose through this barrier. Such an effect might possibly explain the lack of an effect of EDTA on detached ears (Amir *et al.*, 1971) where no sucrose is available for translocation. If this assumption were true, exogenously supplied sucrose (by injection) should be transported to the endosperm more rapidly in the presence of EDTA. Results of such an experiment show that EDTA treatment significantly increased sucrose uptake, in comparison to the same treatment without exogenously supplied sucrose (Table II). It is suggested from these data that EDTA enchances sucrose translocation and accumulation in corn kernels, probably by changes in cell membrane permeability in the separation layer. In this regard it is of interest that PP_i increased sucrose uptake to a small extent, as well as inhibiting the metabolic loss of sucrose.

Spraying EDTA on the plant foliage has the same general effect on sucrose accumulation as injection into the ear (Table III).

To determine whether the EDTA response was a result of nonspecific chelation, other chelating agents were tested at the same concentration. At least two other chelators (nitrilotriacetic acid and iminodiacetic acid) are as effective as EDTA in this respect (data not shown).

Our results show that various chelators can be used under practical conditions to greatly augment sucrose accumulation in sweet corn. Furthermore, it appears likely that a comparable amount of both EDTA and PP_i treatment would have a combined effect on the sucrose level. It is thought that accumulation of sucrose in leaves may inhibit photosynthesis (Neales and Incall, 1968; Eastin *et al.*, 1969). Therefore, it is possible that the application of various chelators to cereal crops, as well as to other agronomic plants, might greatly increase carbohydrate yield.

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Clarification of Fruit Juice by Pectin *trans*-Eliminase

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One milligram of purified pectin *trans*-eliminase from *Aspergillus sojae*, having 76.5 units of activity, was capable of clarifying 30–40 l. of apple juice within 1 hr at 40°C, while only one-fifth of the efficiency was shown in the case of grape juice under the same conditions. Optimal conditions for clarifying fruit juice by the enzyme existed in the range of pH 3–4, and 45–50°C. At the point when apple juice was completely clarified, one-half of the pectin in the juice was converted to a soluble form in 75% ethanol. A remarkable distinction between pectin *trans*-eliminase and hydrolytic pectinases was observed in the formation of carboxyl groups and methanol during clarification. Pectin *trans*-eliminase does not produce methanol but the ordinary hydrolytic pectinases do.

Pectin in fruit juice may suspend other materials in a colloidal system. For the clarification of fruit juice, therefore, pectolytic enzymes produced by molds have long been used (Joslyn *et al.*, 1952; Neubeck, 1959).

Pectin is composed mainly of α-1,4-linked polygalacturonide in which carboxyl groups of the galacturonic acid are mostly esterified with methanol (Deuel and Stutz, 1958; Pilnik and Voragen, 1970). Generally, there are supposedly three ways in which enzymatic degradation of α-1,4-linkages can occur in pectin, as shown in Figure 1 (Neukom, 1969): combined action of pectin esterase (PE) and polygalacturonase (PG); hydrolytic polymethylgalacturonase (PMG) reaction (Seegmiller and Jansen, 1952); and pectin *trans*-eliminase (PTE) (EC 4.2.99) reaction. Most of the ordinary pectinases used for clarifying fruit juice belong to the first case. In this case methanol is produced from pectin by the action of PE. The existence of PMG has never been clearly proved (Edstrom and Phaff, 1964). Unlike hydrolytic pectinases, PTE forms methylgalacturonides with unsaturated bonds between carbon atoms 4 and 5 in the anhydromethyl-galacturonosyl residues of nonreducing ends (Albersheim *et al.*, 1960). In addition to pectin *trans*-eliminase, similar enzymes (pectic acid *trans*-eliminase) which are specific for polygalacturonic acid have been reported in bacterial cultures (Nagel and Vaughn, 1962; Macmillan and Vaughn, 1964; Nasuno and Starr, 1967; Nagel and Wilson, 1970). However, these enzymes will not be useful for the clarification of fruit juice because they show the activity in an alkaline side.

Endo (1965a) ascribed apple juice clarifying activity in the crude enzyme of *Coniothyrium diplodiella* to the combination of PG's and PE when he mixed all purified enzymes in the same ratio as in the crude enzyme. Yamasaki *et al.* (1967) also reported that a purified preparation of *endo*-PG from

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