nitrile or 1-cyano-2-hydroxy-3-butene occurs in either seed when autolysis is carried out in the wetted seed meal with no pH adjustment; and in both seeds the hydrolysis of the thioglucosides to give products other than benzylisothiocyanate or (R)-goitrin is extremely rapid. The reaction products from the crambe thioglucoside differ in that no evidence for the formation of organic thiocyanates has been found.

Mild handling—such as storage of crambe seed under ambient conditions or even dilution of a slurry with water—causes an increase in goitrin formation and a decrease in the formation of the cyano-containing compounds. If nitrile formation is enzymatic, the enzyme must be extremely labile. Other labile substances present in the seed which might react with the initial enzymatic product(s) could also explain this behavior. We have no evidence that either goitrin or the group of cyano compounds is a precursor of the other product. The variation in product with pH is similar to that shown by white mustard myrosinase, although in the latter case the transition from nitrile to goitrin is from pH 3 to pH 7 (*4*). The possible enzymatic formation of nitrile is not to be confused with a two-enzyme system, reported for myrosinase in which a thioglucosidase and sulfatase are separated (*6*). The pathway of enzymatic breakdown of *epi*-progoitrin is not apparent. Clarification of the mechanism is an objective of current research.

Minor differences in autolysis conditions and treatment of the seed before autolysis affect the kind and amount of end products from the thioglucoside aglycon. Each end product may give a different physiological effect when fed to animals. Similar effects would also be produced by seed of different age or drying treatments even when the thioglucosides were not hydrolyzed. If the endogenous enzymes were still present, rapid hydrolysis (see Figure 4) would be expected during the initial stages of digestion by the animal.

In the absence of thioglucoside-hydrolyzing enzymes, progoitrin can be hydrolyzed to give goitrin by microorganisms commonly found in the nonruminant intestinal tract (*7*). It would be interesting to know whether under these conditions of thioglucoside hydrolysis the final products from the aglycon of *epi*-progoitrin and progoitrin consist also of 1-cyano-2-hydroxy-3-butene and other cyano-containing products.

Feeding experiments with rapeseed, for the most part unidentified as to species, show a wide variation in animal response (*1*, *8*). Variation of feeding value of rapeseed meals may be caused by variation in the nature of the thioglucosides present in the particular species of seed, as well as variation in the nature of enzymatic hydrolysis products because of processing conditions.

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RESIDUES IN SPINACH

Conversion of DDT to TDE in Canned Spinach

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OWING TO Federal regulations governing the registration of pesticide chemicals, large quantities of data have been accumulated on the persistence of residues on raw agricultural products. Tolerances established by the Food and Drug Administration also apply to the raw crops. The behavior of pesticide residues during the interval between the harvest of food crops and their arrival on the plate of the consumer has received little attention.

For a significant portion of the fruits and vegetables consumed in the United States, commercial canning operations intervene between harvest and use, and many foods reach the plate of the consumer after processing and storage in enameled or plain-bodied tin cans or in glass jars. The effect of commercial canning operations on pesticide residues has not been studied by modern methods. Some data on the effect of processing on DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane] in various fruits and vegetables were obtained during the period between 1947 and 1950 (*1*, *2*, *13*, *14*, *20*). The analytical procedures available to these workers would not distinguish conveniently among the various isomers and analogs of DDT that might be present initially or formed during the processing operation.

The need for information on the behavior of pesticide compounds in all parts of the environment has been recognized,

The observation of occasional traces of TDE in canned products led to the suspicion that they might be resulting from permissible DDT residues during processing or storage. Infrared spectrophotometry, gas and thin-layer chromatographic methods confirmed that DDT converts in part to TDE during canning at 250° F. (121° C.). Experimental techniques eliminated the possibility that the TDE was a gas chromatographic artifact. The conversion has been observed in spinach processed in plain tinplate, enameled tinplate, and glass containers. The TDE is evidently further decomposed to products not registered by the analytical methods ordinarily employed for chlorinated hydrocarbon pesticides.

and monitoring programs are now in progress in several government agencies. A complete understanding of the fate of these compounds requires detailed information on their reactions throughout all parts of the food chain. As a result, attention has again been focused on the fate of pesticide residues during the operations attendant upon commercial processing and home preparation. Work published recently reflects growing interest in the effect of food preparative steps on pesticide residues (*5–11*).

During the past several years in which chromatographic procedures have been utilized in the authors' laboratory for the analysis of pesticide residues in various canned foods, the occasional observation of traces of TDE, DDD [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane] residues led to the suspicion that this compound might be resulting from DDT during processing or storage of canned products in which DDT could be present as a legal residue. The TDE residues were sometimes observed when there were no records of its application to the raw product. In two instances, fortified check samples prepared in other laboratories for DDT analysis contained TDE when the canned samples reached our laboratories.

The dehydrohalogeination of DDT to DDE [1,1 - dichloro - 2,2 - bis(*p* - chlorophenyl)ethylene] is easily achieved by alkaline hydrolysis. It has been observed in nature as a result of enzymatic conversion (*15*) and on gas chromatographic columns by Burke and Giuffrida (*3*) and a number of others. The dechlorination of DDT to TDE has also been observed under a variety of conditions, which have been reviewed by Ott and Gunther (*17*). The transformation to TDE during the processing of canned foods, however, has not been reported.

Preliminary Experiments

Six cans of spinach were purchased at a local market and thoroughly homogenized in a large Waring Blendor. Residue analysis on the blended material indicated that it contained 0.2 p.p.m. of *p,p'*-TDE. Technical DDT in a small volume of acetone solution was added to the spinach in an amount equal to 10 p.p.m. The homogenized product was recanned in plain 303 × 406 cans, a popular commercial size. The designation 303 × 406 refers to a can $3^{3}/_{16}$ inches in o.d. by $4^{6}/_{16}$ inches in height.

These cans were processed for three different time periods: 50 minutes, 100 minutes, and 150 minutes, each at 250° F. (121° C.). A fourth series was frozen without heat treatment. The first process, 50 minutes at 250° F., approximates a normal cook for the product in this container.

Samples of the frozen and canned spinach were extracted with acetonitrile and portions of these extracts were cleaned up by vacuum sublimation (*4*) and by chromatography on Florisil (*16*). Residue analysis by gas and thin-layer chromatography under conditions described below provided qualitative and semiquantitative confirmation of the fact that *p,p'*-DDT is converted to *p,p'*-TDE during the process.

These experiments were repeated using somewhat smaller containers. In the second series, both plain and fully enameled cans were employed. The results indicated that the conversion of *p,p'*-DDT to *p,p'*-TDE occurred in about the same amounts in both the plain and fully enameled cans. A series of experiments in glass test tubes indicated that the conversion also takes place in glass containers.

In some of the above experiments, extraction difficulties were encountered, and recoveries were variable and sometimes less than quantitative. When fortified spinach was extracted within a few hours after fortification, reasonably quantitative (80 to 100%) recoveries were obtained. However, if fortified samples were allowed to stand for a period of 24 hours or longer, recoveries were sometimes as low as 50%. Although acetonitrile is a widely used and generally satisfactory solvent for such extractions, the indications were that it may not be the choice solvent for the removal of DDT from canned spinach.

Preparation of Fortified Samples

Qualitative identification procedures to confirm the validity of the conversion of *p,p'*-DDT to *p,p'*-TDE were conducted on a series of fortified spinach samples prepared as follows. Cans of spinach, purchased from a local market, were thoroughly homogenized in a large Waring Blendor. Pesticide residue determinations conducted on the puréed product established that it contained less than 0.1 p.p.m. of *p,p'*-TDE. The spinach was fortified at a level of 10 p.p.m. with an acetone solution of pure *p,p'*-DDT. A level exceeding the legal tolerance was used to facilitate recovery of conversion products. The crystalline material was a reference standard obtained from Nutritional Biochemicals Corporation that had been in use in the laboratory for several years. The material has been used frequently to prepare standards for gas and thin-layer chromatography, and indications of the presence of isomers or analogs other than *p,p'*-DDT have not been observed. The puréed product was filled into 202 × 214 fully enameled cans, sealed, and processed for 50 minutes at 240° F. (116° C.).

Qualitative Identification

Several cans of the fortified spinach described in the preceding section were analyzed for chlorinated hydrocarbons by electron-capture gas chromatography and by thin-layer chromatography (*4*). No *p,p'*-DDT was found by either procedure. The R_f values obtained by thin-layer chromatography, and the elution times obtained by gas chromatography corresponded to *p,p'*-TDE.

To provide sufficient quantities for infrared spectophotometric confirmation of the identity of the *p,p'*-TDE, 100-gram portions of the fortified spinach were extracted with acetonitrile and partitioned from dilute aqueous acetonitrile into petroleum ether. The entire sample was purified by vacuum sublimation (*4*), followed by further clean-up in the usual manner on a Florisil column.

Sample aliquots representing between 50 and 250 μg. of pesticide were injected on $^{1}/_{4}$-inch gas chromatographic columns packed with 10% DC-200 on Anakrom ABS at 200° C. with a nitrogen flow rate of 120 ml. per minute. A 50-to-1 stream splitter was used to proportion the effluent between a collection device and a hydrogen flame detector. The effluents were collected on potassium bromide which was compressed into micropellets for presentation to the infrared spectrophotometer.

The infrared spectra are presented in Figure 1. The uppermost curve in the illustration was obtained using a potassium bromide pellet of crystalline *p,p'*-DDT. To eliminate any possibility that conversion of *p,p'*-DDT to *p,p'*-TDE was occurring on the gas chromatographic column, as has been recently reported by several workers (*17*), an aliquot of the same stock solution of *p,p'*-DDT used to fortify the spinach was chromatographed under the conditions described above, and the effluent was collected and used for the preparation of the potassium bromide pellet whose spectrum appears in the second curve. The third curve was obtained from crystalline *p,p'*-TDE. The fourth spectrum was obtained from the spinach extract purified and chromatographed as described above.

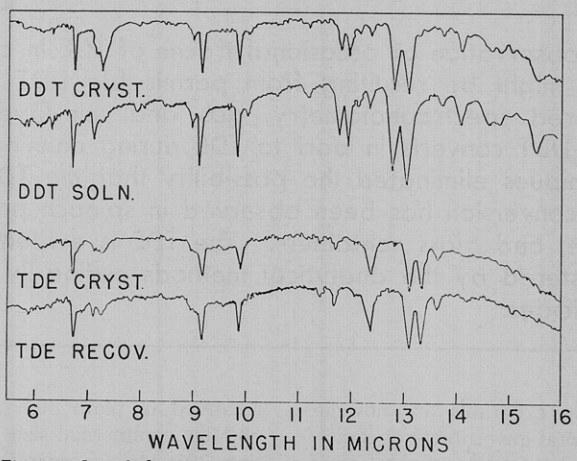

Figure 1. Infrared spectra for KBr micropellets of: crystalline *p,p'*-DDT, aliquot of fortifying DDT solution collected from gas chromatograph, crystalline *p,p'*-TDE, TDE recovered from fortified samples

Conversion and Destruction as a Function of Heat Treatment

To render canned foods commercially sterile, the heat treatment or process is designed to destroy spoilage organisms at the slowest heating point in the container. Portions of food adjacent to the outer surfaces of the can are "over processed" to a degree, depending upon the container size and the rate of heat penetration. There is considerable variation in the rate of heat penetration from one type of food product to another. In any study of the effect of processing on pesticide residues, it is, therefore, necessary to investigate a range of heat treatments that will include several multiples of the "normal" process.

Such studies can be conveniently carried out in thermal death time retorts. This equipment is used in canning industry laboratories to determine the heat treatment necessary to destroy organisms that might represent spoilage hazards in commercially canned foods. The form of apparatus available in this laboratory is shown in Figure 2. It provides a series of small, individual retorts or pressure cookers, each of which can be separately controlled. A different heat treatment can be conducted in each retort so that an entire series of processes can be investigated conveniently with a single homogeneous batch of food material. Thin cans specially designed to minimize heat penetration time are ordinarily used in this apparatus. Retorts and containers are shown in the photograph.

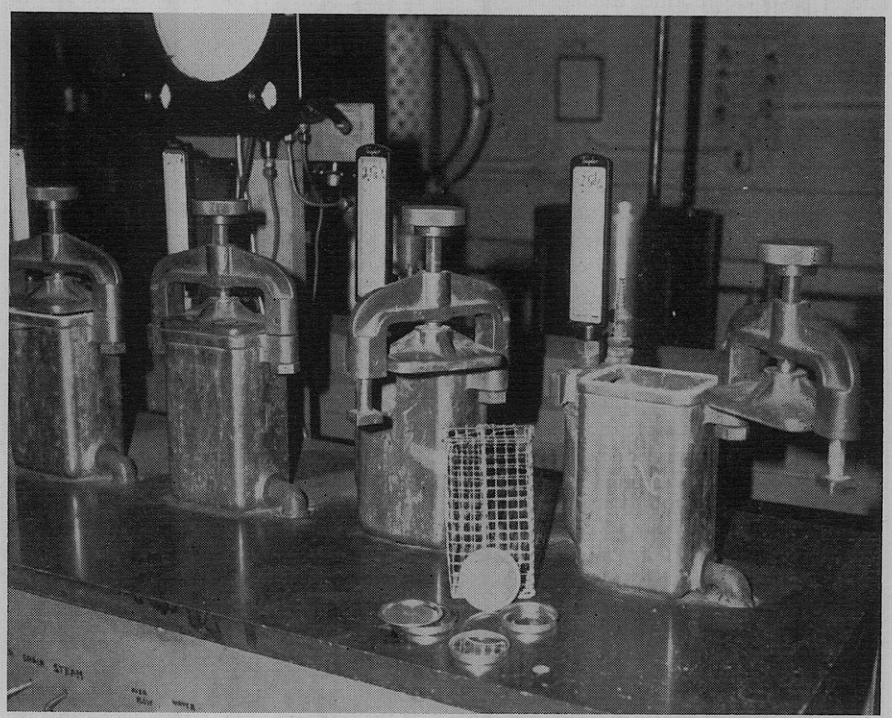

Figure 2. Thermal death time retorts and cans

Heat treatments can be individually controlled in each retort

The food to be investigated is puréed, transferred to the thermal death time cans, and the containers are sealed under vacuum in the normal manner. They are then processed in the individual retorts. At the end of each process, the cans are immediately cooled in water.

To investigate the extent of conversion of *p,p'*-DDT to *p,p'*-TDE at various process times, commercially canned spinach was purchased at a local market and fortified at a level of 10 p.p.m. with *p,p'*-DDT as described above. Pesticide residue determinations on the homogenized spinach prior to fortification indicated that DDE was present at a level of 0.4 p.p.m. and *p,p'*-TDE at a level of 0.5 p.p.m. The fortified puréed product was transferred to thermal death time cans and processed at 250° F. (121° C.) for time intervals ranging from 0 to 16 minutes. For this product, the "normal" process ordinarily recommended in commercial practice is approximately equivalent to a heat treatment of 4 minutes at 250° F. in the thermal death time cans. These times are corrected for the time interval (15 seconds) required for the entire content of the thermal death time can to reach the process temperature. The heat treatments investigated range from multiples of $^1/_4$ the normal process to 4 times the normal spinach process. All containers were frozen immediately after the process and stored in a frozen condition until analyzed.

The puréed spinach was extracted with petroleum ether and alcohol, cleaned up on Florisil, and analyzed for chlorinated hydrocarbons by thin-layer chromatography (*12*) and by electron-capture gas chromatography on a 5-foot by $^1/_3$-inch column packed with a 1 to 1 mixture of 15% QF-1 on 80- to 90-mesh Anakrom ABS and 10% DC-200 on Anakrom ABS. A thin-layer plate is shown in Figure 3 and four typical gas chromatograms are reproduced in Figure 4.

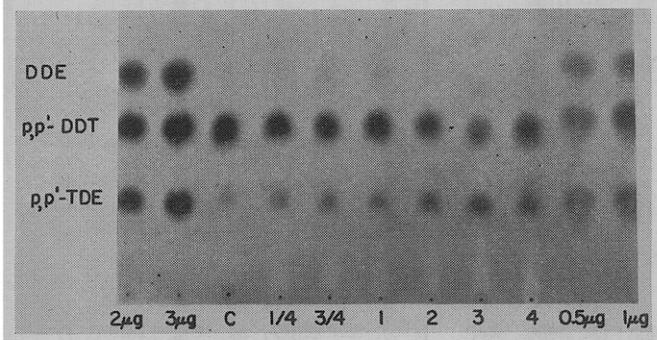

Figure 3. Effect of processing on *p,p'*-DDT 10 p.p.m. in spinach

Multiples of "normal" process at 250° F. (121° C.)

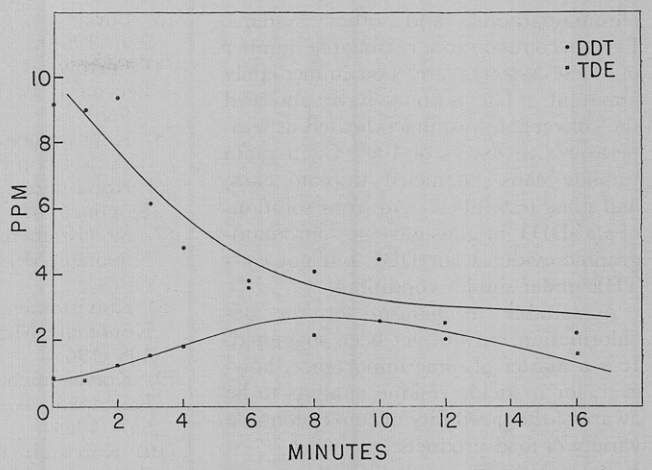

Figure 5. Conversion of *p,p'*-DDT to *p,p'*-TDE during heat processing of spinach

In this series of experiments, both *p,p'*-DDT and *p,p'*-TDE were present in all samples including those receiving the most severe heat treatment. In Figure 3, 0.3-gram aliquots of the unprocessed control and six fractions or multiples of the normal process have been spotted with 0.5-, 1-, 2-, and 3-μg. quantities of *p,p'*-TDE, *p,p'*-DDT, and DDE. Traces of DDE, as identified by gas chromatographic retention time, were also present in all of the cans analyzed. This trace was present in the spinach prior to fortification and reprocessing, and it did not increase during the heat treatments applied in this experiment. The amount was too small for convenient detection by thin-layer chromatography. Quantitative comparisons among the curves illustrated in Figure 4 should not be attempted owing to differences in the size of sample aliquots.

A small shoulder on the DDE peak (Figure 4) was observed in all chromatograms. This was present in the unfortified spinach and no change was observed in any of the subsequent heat treatments. The elution time corresponds closely with that of *o,p'*-TDE, a conversion product that could be expected to result from *o,p'*-DDT, a constituent of the technical DDT evidently applied to the growing spinach.

The results of these pesticide determinations are collected in Table I and are illustrated in Figure 5.

In contrast to most of the preliminary experiments, appreciable *p,p'*-DDT remained even in the four-times-normal process. The level of *p,p'*-DDT declined in a more or less regular pattern as the severity of the heat treatment increased. The *p,p'*-TDE level increased gradually, reaching a maximum value in the samples processed for 6 minutes at 250° F. (121° C.). The combined total of the *p,p'*-TDE and *p,p'*-DDT concentrations decreased gradually as the heat treatment increased.

Table I. Conversion and Destruction as Function of Heat Treatment

Corrected Heating Time, Minutes	Multiple of Normal Process	Parts per Million		
		p,p'-DDT	TDE	Total
C	...	8.7	0.9	9.6
0[a]	0[a]	9.2	0.9	10.1
1	1/4	9.0	0.9	9.9
2	1/2	9.3	1.2	10.5
3	3/4	6.1	1.5	7.6
4	1	4.8	1.7	6.5
6	1 1/2	3.8	3.5	7.3
8	2	4.1	2.1	6.2
10	2 1/2	4.4	2.6	7.0
12	3	2.0	2.5	4.5
16	4	2.2	1.7	3.9

[a] Sufficient heat (15 seconds) to achieve uniform temperature of 250° F. (121° C.) throughout can.

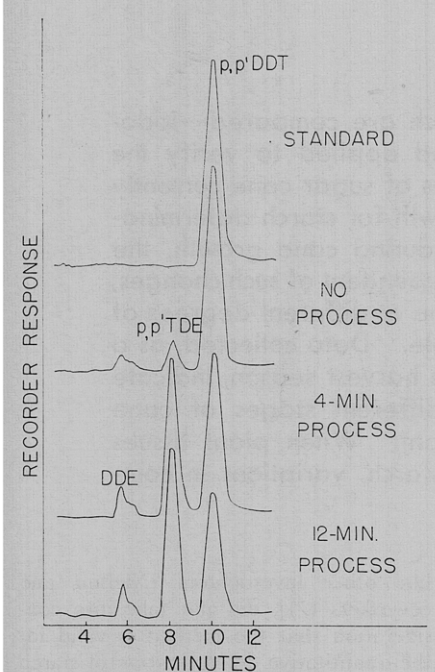

Figure 4. Representative chromatograms of standard *p,p'*-DDT and extracts of *p,p'*-DDT fortified spinach after various heat treatments

Discussion

Evidently *p,p'*-DDT is converted into *p,p'*-TDE during the heat treatment, and the latter compound undergoes further change into breakdown products which do not register on electron-capture detectors. Time was not available during the present study to investigate the thermal breakdown of *p,p'*-TDE under these conditions. The limited data available in the early literature suggest that *p,p'*-TDE does break down during canning (*2*).

Using the colorimetric method of Schechter and Haller (*18*), Tressler (*20*) investigated the losses of DDT during processing in tin and in glass, in water and in solutions buffered to pH 4. He observed no losses during processing in glass. Water and buffered solutions of DDT processed in tin gave losses ranging from 10 to 20% by the analytical method in use.

In similar experiments (*20*) in which *p,p'*-DDT was added to tomato juice and processed, up to 69% destruction was reported. Although no ortho-para isomers were added, some apparent ortho-para isomers were formed during the processing. It is not unlikely that *p,p'*-TDE could be reported as the ortho-para isomer when the colorimetric procedure is relied upon.

Brittin and Fairing (*2*) report one experiment in which the effect of processing on TDE in green beans was investigated. They observed a 51% loss during processing, as measured by the colorimetric procedure of Schechter *et al.* (*19*).

The results of early studies are consistent with those obtained by the more discriminating analytical procedures now available. Residues of *p,p'*-DDT in spinach and in related products are converted to TDE during commercial processing. *p,p'*-TDE undergoes further decomposition during processing to products that are either not registered on electron-capture detectors or are not eluted from Florisil columns by the usual solvents.

The review of Ott and Gunther (*17*) listing a number of systems in which *p,p'*-DDT is converted to *p,p'*-TDE appeared after the work just described was in manuscript. This very complete review lists a number of instances in which the conversion has been observed in gas

chromatographic and other systems. Factors common to a reasonable number of these systems are not immediately apparent. The authors have observed the conversion in spinach heated at temperatures in excess of 100° C. in plain tinplate cans, enameled tinplate cans, and glass test tubes. Aqueous solutions of *p,p'*-DDT in glass gave gas chromatographic evidence for DDE but not *p,p'*-TDE under similar conditions.

A suitable mechanism for the dechlorination has not yet been suggested. It is a matter of some importance, however, for pesticide residue analysts to be aware of the possibility of conversion in a variety of food products.

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SUGAR CANE STARCH

Comparison of Methods for Determination of Starch in Sugar Cane Juice

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Two colorimetric methods for determination of sugar cane starch are compared—iodometric and anthrone. A referee method is also described and applied to verify the results obtained by the other methods. Six commercial varieties of sugar cane currently being grown in Louisiana were taken at different stages of growth for starch determination. Because of the changing chemical nature of the starch during cane growth, the anthrone method, which facilitates total starch determinations independent of such changes, was used in analyzing a series of samples representing sugar cane at different degrees of maturity. Amylose was determined iodometrically in each sample. Data collected, as a result of a systematic investigation representative of a Louisiana harvest season, indicate that starch composition (amylose-amylopectin) varies during different stages of cane development in response to the metabolic processes of the plant. When plant tissues representing different sugar cane varieties are analyzed for starch, variations in composition are observed.

THE work reported was undertaken as part of a broader program based on the investigation of the physicochemical properties of sugar cane starch in relation to filtration problems encountered by the processors of refined sugar.

Wood (*10*), upon using the iodometric method for the determination of sugar cane starch, observed that equally concentrated solutions of starch from different plant sources give different color intensities with iodine. He established a factor for the conversion of what he termed relative values to absolute values, since he was using as a standard a starch from another parentage. However, he, like other investigators [Nielson and coworkers (*7*)], did not take into consideration that the method is valid for the quantitative determination of starch only after it is established that the ratio of amylose to amylopectin remains constant at different degrees of plant maturity, under various growing conditions,