Table IV. Precision of Method, Coumestrol Estimated as Milligrams per Kilogram of Meal

Detn.	Dried Alfalfa	Fresh Alfalfa
1	85	55
2	86	56
3	80	56
4	86	58
Mean	84	56
Std. dev.	±2.88	±1.29
Coeff. of variation %	3.4	2.3

assays. Table IV shows the precision of the fluorometric method for both fresh and dried alfalfa. The accuracy is shown from the coefficient of variation, ranging from 2.3% for the fresh alfalfa to 3.4% for the dried alfalfa. Each determination is the average coumestrol value obtained from two aliquots of an extract of a sample of meal.

Evaluation by Alternate Procedures. CHROMATOSTRIP METHOD. The preparation of the chromatostrips and details of their usage were described in an earlier paper (*8*).

Aliquots of the purified extract are applied to a large number of the chromatostrips. The strips are then developed four successive times in a mixture of ether and petroleum (7 to 3), with air drying between developments. After the final drying the strips are placed under an ultraviolet lamp and the fluorescent zone corresponding to coumestrol is carved out with a spatula. The coumestrol is eluted from the silicic acid adsorbent mixture with methanol, and after filtration the solution is read at 352 mμ in an ultraviolet spectrophotometer. The concentration of coumestrol in the sample is then calculated with absorbance of a solution of pure coumestrol as the standard. The procedure was proved to be reliable by the quantitative recovery of pure coumestrol which was similarly added to the chromatostrips. Accuracy, based upon two analyses, is estimated as about ±10%.

PAPER CHROMATOGRAPHIC–SPECTROPHOTOMETRIC ASSAY. The fluorescent zones corresponding to coumestrol on the developed paper chromatograms prepared as described previously are cut out and their absorbances measured on the ultraviolet spectrophotometer. The concentrations of coumestrol in the unknown extracts are then calculated from a standard curve prepared in the same manner from the fluorescent spots of pure coumestrol.

Acknowledgment

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Literature Cited

(1) Bailey, G. F., *Anal. Chem.* **32,** 1726 (1960).
(2) Bickoff, E. M., Booth, A. N., Livingston, A. L., Hendrickson, A. P., Lyman, R. L., *J. Animal Sci.* **18,** 1000 (1959).
(3) Bickoff, E. M., Booth, A. N., Lyman, R. L., Livingston, A. L., Thompson, C. R., Kohler, G. O., J. AGR. FOOD CHEM. **6,** 536 (1958).
(4) Bradbury, R. B., White, D. E., *J. Chem. Soc.* **1951,** 3447.
(5) Cheng, E., Story, C. D., Payne, L. C., Yoder, L., Burroughs, W., *J. Animal Sci.* **12,** 507 (1953).
(6) Kirchner, J. G., Miller, J. M., Keller, G. J., *Anal. Chem.* **23,** 420 (1951).
(7) Livingston, A. L., Bickoff, E. M., Guggolz, J., Thompson, C. R., *Ibid.*, **32,** 1602 (1960).
(8) Lyman, R. L., Bickoff, E. M., Booth, A. N., Livingston, A. L., *Arch. Biochem. Biophys.* **80,** 61 (1959).

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SUGAR—STARCH TRANSFORMATIONS IN PEAS

Relation between Changes in Glucose, Fructose, Galactose, Sucrose, and Stachyose, and the Formation of Starch in Peas

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Data on the accumulation of individual sugars in processing peas during maturation were collected in an attempt to elaborate on sugar content as related to pea sweetness and starch content. Peas may contain considerable amounts of stachyose, which explains why pea sweetness bears an uncertain relation to total sugar (sucrose) content. During starch accumulation, sucrose is inversely related to total starch and amylose content, while stachyose is directly related to pea amylopectin content. Attempts to relate pea sugars to starch content and sweetness require attention to individual starch fractions and individual sugars.

PROCESSING pea quality attributes, such as sweetness and tenderness for canning or freezing, or the rehydration rate in the case of dried peas, is related to the pea sugar and starch content, which changes as peas mature. As peas mature, the sieve size increases (*7*), and starch is formed at the expense of the sugars (*1, 2–4, 11, 12*). Pea sugars are of interest, since nonreducing sugar determinations, usually calculated as sucrose and accounting for 95% of the total pea sugar (*1*), bear uncertain relation to organoleptic ratings of pea sweetness.

The accumulation of starch is also of interest since the amylose-amylopectin ratio of starch also increases (*7*). Amylopectin and amylose have distinctly different physicochemical properties, and changes in the proportion of these starch fractions influence pea tenderness, pea dehydration, and dried pea rehydration rates, and perhaps even frozen pea texture. A study was initiated therefore in an attempt to elaborate upon the relation between starch-sugar transformations and pea processing quality. This report describes changes observed in individual sugars in peas of varying

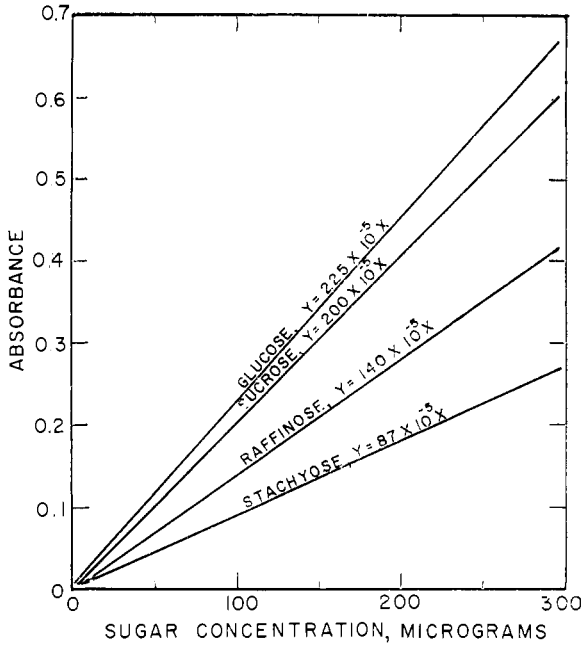

Figure 1. Standard curves of nonchromatographed glucose and chromatographed oligosaccharide used in calculation of slope of curves

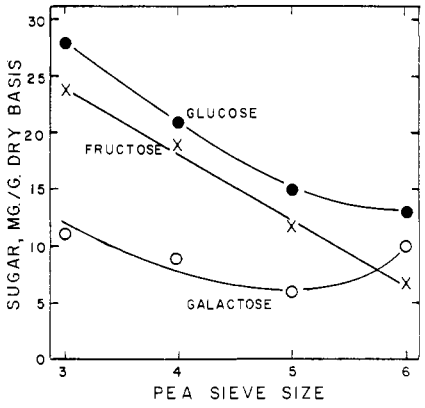

Figure 2. Changes in individual reducing sugars with increasing pea sieve size

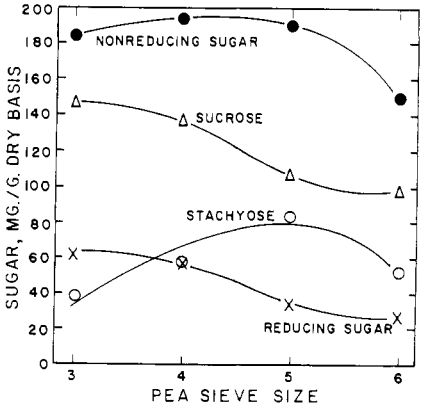

Figure 3. Changes in total nonreducing sugars (sucrose and stachyose) and total reducing sugars with increasing pea sieve size

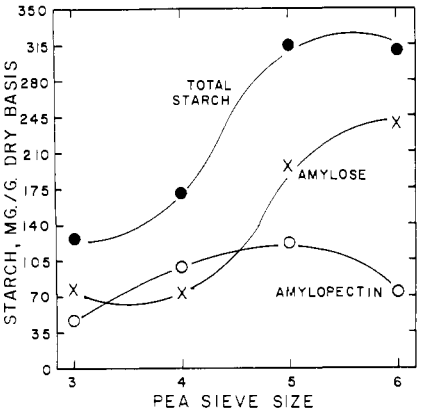

Figure 4. Changes in total starch and starch fractions with increasing pea sieve size

maturity and relates these changes to variations observed in the accumulation of the amylose and amylopectin fractions of pea starch.

Experimental

Preparation of Samples. Nine harvests of sweet peas (Perfected Freezer variety) were made over a 10-day period which encompassed a wide range in pea maturity on the basis of Tenderometer readings that ranged from 101 to 153 field run. Upon size grading, the peas were blanched in water for 1 minute at 210° F. and then frozen. When thawed, the peas were ground with an equal weight of water in a blender. Samples of the slurry were taken for dry weight (Brabender) and for starch and sugar determinations.

Determination of Starch. Five grams pea slurry were treated in a Potter-Elvehjem homogenizer for 2 minutes, and then extracted four times with 30-ml. portions of hot 80% ethyl alcohol. Total starch was determined on the sugar-free residue using the anthrone procedure of McCready *et al.* (*7*). Amylose was also determined by using the above-named method and referring to a standard amylose curve obtained from amylose prepared from Perfected Freezer peas (*8*). The following were obtained: iodine binding capacity 16% and blue value 0.20. Amylopectin was calculated by subtracting values for amylose from values obtained for total starch.

Determination of Sugars. Preliminary chromatographic studies indicated the presence of glucose, fructose, galactose, sucrose, and stachyose. Since stachyose is associated with sucrose and raffinose in plant materials (*5*), a quantitative paper chromatographic procedure (*9*) was expanded to include the routine determination of the raffinose family of oligosaccharides in mixtures.

Separation of sucrose, raffinose, and stachyose on Whatman No. 1 paper using a butyl alcohol–acetic acid–water solvent required 72 hours at 70° F., allowing the solvent to drip from the end of the paper. To achieve a routine operation, the concentration of oligosaccharide was computed by referring to a nonchromatographed glucose standard.

Sucrose, raffinose, and stachyose were spotted in concentrations of 50 to 300 γ on large sheets of filter paper. After the chromatograms had been developed, the sugars were eluted with water in blood sugar tubes, treated with invertase, and allowed to stand overnight. After the contents of the tubes were reacted with Nelson's reagents (*9*), absorbance was determined at 500 mμ and plotted against sugar concentration (Figure 1). A nonchromatographed glucose standard curve was also prepared and regression equations for each standard curve were calculated by using the method of least squares.

During the routine determination of sugars, the combined alcohol extracts of the peas were concentrated to 5 ml., and two 20-μl. aliquots were spotted on paper. Reducing sugars were determined as described previously (*9*). After elution, invertase hydrolysis, and reaction with Nelson's reagents, oligosaccharide concentration was calculated from the relation:

$$O_c = \frac{O_a \times \frac{G_b}{O_b}}{G_a} \times G_c$$

where O_c is the micrograms of oligosaccharide spotted, O_a is the oligosac-

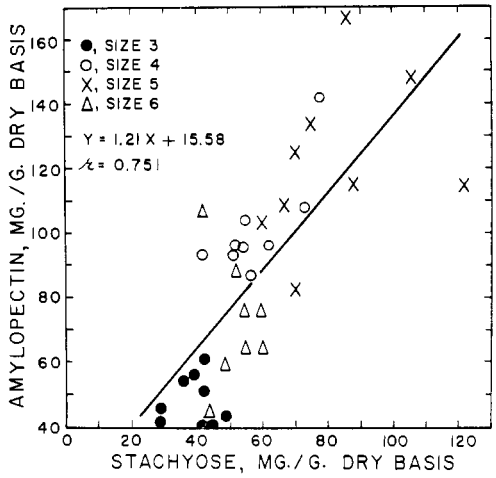

Figure 5. Relation between stachyose and the amylopectin content of pea starch

Table I. Relations between Various Pea Sugars and Pea Starch Fractions

$n = 35$

Parameters	Correlation Coefficient
Total sugars *vs.* total starch	$r = -0.559^a$
Total nonreducing sugars *vs.* total starch	$r = -0.274$
Total reducing sugars *vs.* total starch	$r = -0.7871^a$
Sucrose *vs.* total starch	$r = -0.817^a$
Stachyose *vs.* total starch	$r = +0.578^a$
Sucrose *vs.* amylose	$r = -0.828^a$
Stachyose *vs.* amylopectin	$r = +0.751^a$
Sucrose and stachyose *vs.* total starch	$R = 0.866^a$
Sucrose, stachyose, and total reducing sugars *vs.* total starch	$R = 0.881^a$

[a] Significant at 1% level.

charide absorbance corrected for filter paper present in the tube (*9*), O_t is the slope of the oligosaccharide curve (b value only in $y = bx$), G_c and G_a are the concentration and absorbance of a nonchromatographed glucose standard, and G_b is the slope of the nonchromatographed glucose standard curve.

The slope ratio factor, G_b/O_b, compensates for the decreasing proportion of reducing groups formed when oligosaccharides of increasing molecular weight are hydrolyzed at the terminal β-fructofuranoside unit by melibiase-free invertase. The ratio factor also compensates for a loss of oligosaccharide during 72 hours of chromatography, which appears to be logarithmically proportionate to the molecular weight of the sugar. The standard error of a determination was calculated to be 15γ.

Results

Over the 10-day harvest period, and within a given pea size grade, changes in individual sugars or starch fractions were slight in relation to harvest time. With the exception of a slight linear increase in amylose, these changes were not statistically significant (data not shown).

An analysis of variance indicated that the major chemical difference was between pea size grades. Therefore, the data were plotted in Figures 2 to 4 as the mean of triplicate determinations for each harvest date, and also the average of the nine harvests ($n = 27$). Appropriate free hand curves were drawn if the data tested significantly for linear, quadratic, or cubic functions.

When plotted against sieve size, glucose and fructose decreased in concentration, while galactose increased in size 6 peas. Total nonreducing sugars first increased, and then decreased. Sucrose steadily decreased, while stachyose exhibited a positive parabolic trend. In peas of sieve size 5, stachyose averaged 44% of the total nonreducing sugars. Raffinose could not be detected in the peas examined. Total starch and the starch fractions developed a pattern very similar to that reported by others (*7*). As the peas matured, starch accumulated, and the amylose-amylopectin ratio in starch increased.

Visual inspection of the data shown in Figures 2 to 4 suggests several relations between individual sugars and starch fractions. These were tested by correlation, and relevant correlation coefficients are summarized in Table I. The figures suggest that galactose accumulates when stachyose and amylopectin decrease. However, this apparent relation was not found to be significant when tested by means of correlation. On the other hand, the inverse relation between total reducing sugars and total starch was highly significant.

Total nonreducing sugars (sucrose plus stachyose) correlated poorly with total starch, but treating these sugars individually suggests why the correlation is poor. Sucrose alone correlates well with total starch, and even better with amylose. While these latter relations are inverse, stachyose is positively related to total starch content, and correlates even better with the amylopectin fraction of starch. The positive relation between stachyose and amylopectin is shown in Figure 5, utilizing the data for all sieve sizes and harvest dates. Since one nonreducing sugar, sucrose, correlates well, but inversely, with total starch, and the other, stachyose, correlates positively, multiple correlation was applied to the data. As shown in Table I, the regression of total starch on sucrose and stachyose raised the correlation from the nonsignificant -0.274 to the highly significant 0.866. Finally, regression of total starch on sucrose, stachyose, and total reducing sugars yielded a correlation coefficient of 0.881. The relation, then, between total starch and sugar accumulation in the peas studied is best expressed as

$$y = -1.82x_1 + 1.17x_2 - 1.49x_3 + 448$$

where y equals total starch, x_1 equals sucrose, x_2 equals stachyose, and x_3 equals total reducing sugars.

Discussion

The inverse relation between sucrose and total starch was expected, since, in peas, sucrose is the main transport sugar available for starch synthesis (*11*). However, the rather large amount of stachyose found was not entirely anticipated. Turner and Turner (*12*) in examining sugars in peas did not detect stachyose. On the other hand, Tanret (*10*) found that peas may contain as much as 1.7% stachyose. It would appear that treating and interpreting data on nonreducing sugars of peas as sucrose, based upon acid or invertase hydrolysis of extracts, can be misleading. In particular instances, nearly 50% of the total sugars in the peas employed in this study was found to be stachyose. It is estimated that stachyose is only about one tenth as sweet as sucrose, and it is understandable why total sugar determinations may bear uncertain relation to organoleptic evaluations of pea sweetness.

The highly significant, positive relation between stachyose and amylopectin was entirely unanticipated, and it is not clear if there are significant biochemical or physiological manifestations implied in this relation. French (*6*) has suggested that the raffinose family of oligosaccharides (of which stachyose is a member) may be related to galactomannan synthesis. The data presented here suggest a relation to starch synthesis. In any event the best expression of the relation between starch content and

sugar concentration is a multiple regression equation using the individual nonreducing sugars sucrose and stachyose, and the total reducing sugars.

Literature Cited

(1) Bisson, C. S., Jones, H. A., *Plant Physiol.* **7,** 91 (1932).
(2) Danielson, C. E., *Acta Agr. Scand.* **9,** 181 (1959).
(3) Danielson, C. E., *Physiol. Plantarum* **9,** 212 (1956).
(4) *Ibid.*, **12,** 176 (1959).
(5) deWhalley, H. C. S., Gross, D., "Methods of Biochemical Analysis," Vol. I, D. Glick, ed., Interscience, New York, 1954.
(6) French, D., *Advances in Carbohydrate Chem.* **9,** 149 (1954).
(7) McCready, R. M., Guggolz, J., Silviera, V., Owens, H. S., *Anal. Chem.* **22,** 1156 (1950).
(8) Potter, A. L., Silviera, V., McCready, R. M., Owens, H. S., *J. Am. Chem. Soc.* **75,** 1335 (1953).
(9) Shallenberger, R. S., Moores, R. G., *Anal. Chem.* **29,** 27 (1957).
(10) Tanret, G., *Compt. rend.* **155,** 1526 (1912).
(11) Turner, D. H., Turner, J. F., *Australian J. Biol. Sci.* **10,** 302 (1957).
(12) Turner, J. F., Turner, D. H., Lee, J. B., *Ibid.*, **10,** 407 (1957).

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ONION FLAVOR AND ODOR

The Volatile Flavor Components of Onions

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A number of the more important volatile flavor components of onions, *Allium cepa*, have been isolated by gas-liquid partition chromatography and identified by infrared methods and chemical derivatization. In particular, methyl disulfide, methyl trisulfide, methyl-*n*-propyl disulfide, methyl-*n*-propyl trisulfide, *n*-propyl disulfide, and *n*-propyl trisulfide were isolated and identified. Neither monosulfides nor allylic disulfides could be detected.

SEMMLER (*11*), in 1892, studied the composition of an onion oil and reported that the principal odoriferous volatile component was allyl *n*-propyl disulfide. This identification has apparently been accepted for many years without question and many textbooks and reference books (*7*) repeat this statement. Kohman (*8*), in 1947, found that propionaldehyde was an important volatile component obtained from onions, and Challenger and Greenwood (*3*), in 1949, demonstrated the presence of *n*-propyl mercaptan. More recently, Niegisch and Stahl (*9*) using the mass spectrometer for identification found hydrogen sulfide, sulfur dioxide, acetaldehyde, propionaldehyde, methyl alcohol, *n*-propyl alcohol, *n*-propyl mercaptan, and traces of *n*-propyl disulfide. No evidence for allylic disulfides could be obtained.

This paper describes the isolation and characterization of a number of the important volatile flavor components of the onion, *Allium cepa*. Both gas-liquid partition chromatography and conventional precipitation methods were used in the isolation of compounds. Two extraction methods were used, by carbon adsorption and with isopentane. As adsorption on carbon can lead to decomposition and rearrangement of the volatile components, a second milder procedure was used to confirm the results. Isopentane was chosen because of its low boiling point which should minimize thermal decomposition and its nonpolar nature which should minimize mercaptan-disulfide exchange reactions. Owing to the extreme lability of the materials responsible for the lachrymatory effect of freshly bruised onions, this effect is altered or lost during isolation and the final concentrate approaches more nearly that of a cooked onion than of a fresh onion.

Experimental

Extraction of Onions by Carbon Adsorption. Two separate batches of ca. 142 pounds each (peeled weight) of Sunspice onions, a strain of Improved Southport White Globe onions, were diced to pieces of ca. $^3/_8$ inch and each batch was treated as follows: The diced onions and 25 gallons of water were poured into a stainless steel vacuum pot of 100-gallon capacity which was exhausted through two parallel stainless steel tubes (1.5 inches I.D. and 33 inches long) to a very efficient vacuum system. Approximately 400 grams of activated carbon (Columbia AC brand, National Carbon Co.), 6 to 14 mesh, were placed in each tube. The onion slurry was then distilled for 40 hours, in vacuo at a pressure of 29 inches of mercury. Steam was bled in very slowly to agitate the thick slurry and to maintain a temperature of approximately 25° C. At the end of the distillation, the second batch of onions was treated similarly with fresh carbon in the adsorption tubes. The carbon was then dried in vacuo with an oil pump and a dry ice trap at temperatures of 25° C. and lower. The carbon was then extracted in Soxhlet extractors with peroxide-free ether for 40 to 44 hours. These extractions were performed in the dark to minimize decomposition. The combined ether extracts, 3 liters, were dried over anhydrous calcium sulfate and most of the ether was stripped off with a 15-plate Oldershaw column to give 115 ml. of concentrate. Further concentration was obtained by distilling off most of the remaining ether with a small, vacuum-jacketed column packed with glass helices. The final concentrate, 14 grams, was a pale yellow oil. It had a very slight dextrorotation $[\alpha]_D^{25} = +0.02°$(1 dm.). The oil was then distilled in vacuo at 1 mm. pressure with a bath temperature of 25° to 120° C.—most of the material distilling between 40° and 60° C. The yield of distillate was 8.7 grams, representing