highly significant. The higher values by the Kjeldahl - Wilfarth - Gunning method can be undoubtedly attributed to other nitrogen-containing compounds reportedly present in oleoresins of black pepper, such as chavicine, an uncrystallizable isomer of piperine (*2*, *7*).

Figure 3 shows the effect of light on dilute solutions of piperine and oleoresins of black pepper. It is apparent from these data that piperine is photosensitive in dilute solutions of chloroform; therefore, spectrophotometric measurements should be made immediately for accurate results.

Several advantages may be cited for the spectrophotometric method as applied to oleoresins of black pepper. It is both simple and rapid, requiring no time-consuming digestion, as in the case of the total nitrogen method. The spectrophotometric method appears to be specific for piperine. In contrast, the estimation of piperine by total nitrogen would be subject to error due to the presence of other nitrogen compounds in oleoresins of black pepper.

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SUGAR DETERMINATION

Rapid Sugar Extraction Procedure for Analysis of Candied Fruits, Jams, and Fresh Fruits

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A rapid sugar extraction procedure based on use of a Waring Blendor was adapted to the analysis of candied fruits, jams, strawberries, and stone fruits. Total and reducing sugar contents as determined by this method were compared with results by a modified AOAC method for fruit and fruit products. Statistical analyses show that the methods are equivalent in precision and accuracy when applied to analysis of candied fruits, fresh strawberries, apricots, and prunes. In analysis of jams, the rapid method gives results 0.24% higher for total sugars but shows no significant difference for reducing sugars. A complete determination of total and reducing sugars may be made inexpensively in less than 1 hour by the rapid method, whereas the modified AOAC method requires 2.5 hours.

IN PREPARING JAMS AND CANDIED FRUITS using sucrose and invert sugar, it is essential that a careful control be maintained on the total sugar concentration as well as on the ratio of sucrose to invert sugar. The latter relationship is necessary to prevent crystallization. In the manufacture of candied fruits by the continuous evaporation process of Atkinson and others (*4*), and in jam production (*13*), analyses of sirup and fruit at regular intervals assist in maintaining uniformity and stability in the finished product. There is a definite need, particularly under factory conditions, for an accurate, rapid, inexpensive procedure for sugar determination to replace the present standard methods for both processed and fresh fruit. A simple plant control method developed by two of the writers (*17*) for candied fruit operations proved so successful that it was investigated further with a view to increasing its accuracy and adapting it to other products.

The standard procedure employed by the authors for accurate determination of sugar in fruits and fruit products involves two minor modifications of the Association of Official Agricultural Chemists method for fruits and fruit products (*1–3*). Prior to extraction by boiling (*1*) sufficient 1*N* sodium hydroxide solution is added (with vigorous stirring) to neutralize the natural acid. This minimizes the inversion of sucrose and consequent high values for reducing sugars. The solution should have a pH of 6 to 7 at the end of the boiling extraction period. For inversion of sucrose in the determination of total sugars as invert, citric acid is substituted for hydrochloric acid (1 + 1). Five grams of citric acid crystals are added to a 100-ml. aliquot and the solution is boiled gently for 10 minutes, cooled, and neutralized (*8*). This procedure has been found to give results comparable to those of the AOAC method (*16*).

Elimination of Clarification. Clarification of plant extracts with excess neutral lead acetate followed by precipitation of excess lead with sodium oxalate has been adopted by the Association of Official Agricultural Chemists (*2*) as an official method for fruit and fruit products. Data presented by Morris and Welton (*12*), McDonald (*10*), Williams and others (*19*), and Williams and Bevenue (*18*) indicated that in many cases aqueous or alcoholic extracts of plant materials require no clarification prior to sugar analysis. Several of these authors reported that lead acetate precipitation does not remove all nonsugar reducing substances.

Elimination of Neutralization. The necessity for neutralization prior to boiling water extraction of sugars from acid products was noted by McRoberts (*11*) and Roy (*15*). Bates and others (*5*), however, presented data to indicate that inversion at room temperature or lower is negligible in the time interval required for performing a sugar extraction.

Blender Extraction. Although elimination of clarification and neutraliza-

Table I. Total and Reducing Sugars in Candied Zucca Melon Determined by Modified AOAC and Rapid Extraction Procedures

(10 replicate determinations by each method on blended sample)

Replication No.	Total Sugars as Invert, %		Reducing Sugars as Invert, %	
	AOAC	Rapid	AOAC	Rapid
1	62.7	62.4	26.0	26.1
2	62.6	62.6	26.0	26.1
3	62.6	62.2	26.0	26.1
4	62.7	62.4	26.2	26.1
5	62.5	62.2	26.2	26.0
6	63.1	62.2	26.4	26.1
7	62.6	62.3	26.1	26.0
8	62.6	62.6	26.4	26.0
9	62.6	62.1	26.3	26.1
10	62.6	62.6	26.0	26.0
s, %[a]	0.052	0.060	0.125	0.052
$F_{9\&9\ df}$	1.33 *NS*		5.83 *S*	

[a] s, standard deviation.
F, variance ratio.
df, degrees of freedom.
NS, not significant.
S, significant at 0.05 level.

Table II. Total and Reducing Sugars in Candied Zucca Melon as Determined by Modified AOAC and Rapid Extraction Procedures

(10 different samples)

Sample No.	Total Sugars as Invert, %		Reducing Sugars as Invert, %	
	AOAC	Rapid	AOAC	Rapid
1	64.8	63.6	38.0	37.8
2	59.6	59.2	29.0	28.9
3	62.5	62.8	33.8	34.3
4	59.2	59.5	22.9	23.2
5	59.2	59.6	25.2	25.1
6	62.2	62.5	30.0	30.2
7	63.1	63.1	23.1	23.1
8	67.1	66.3	22.9	22.3
9	73.6	73.5	18.9	18.9
10	72.8	72.7	43.2	43.0
$r_{8\,df}$	0.995 *HS*[a]		0.999 *HS*	
Av. diff. (AOAC − rapid), %	+0.13		+0.02	
$t_{9\,df}$	+0.61 *NS*		+0.06 *NS*	

[a] *HS*, significant at the 0.01 level.

tion effects a slight saving of time, real economy will be effected by eliminating the 1-hour boiling extraction of the sample. A number of authors suggest the possibility of using high speed blending devices for more rapid extraction of sugars. Use of the Waring Blendor in preparation of aqueous extracts of plant materials was first reported by Davis (*6*, *7*). Its application to the preparation of alcoholic extracts of apple tissue by Leonard, Meade, and Dustman (*9*) resulted in a time saving of more than 2 hours per analysis without loss of accuracy. Reifer and Melville (*14*) described a procedure using a blender for preparing aqueous extracts of grasses and roots, in which extraction temperature is kept below 8° C. by addition of ice.

Method

Preliminary experiments showed that satisfactory aqueous extraction of sugars from jams and candied fruits was possible with a Waring Blendor. This led to adoption of the Reifer and Melville (*14*) technique with two modifications. Ether was not used during blending. Clarification was omitted and the extract was mixed with asbestos and filtered under vacuum through a sintered-glass filter.

Modified Reifer and Melville Method. A 50-gram aliquot of ground, mixed sample was transferred to a 1-quart borosilicate glass Waring Blendor jar with 250 ml. of ice-cold distilled water. The jar had been cooled in a refrigerator and, prior to use, was wetted with a small volume of distilled water to prevent sticking of the sample. Approximately 60 to 70 grams of distilled water ice (two small cubes) and 2 to 3 drops of capryl alcohol were also placed in the jar. The contents of the jar were blended for 2.5 minutes with the lid held firmly in place. An additional 60 to 70 grams of ice were placed in the jar, the lid and sides were washed down with a few milliliters of water, and blending was continued for 2.5 minutes more.

The contents were then transferred to a 500-ml. volumetric flask. The flask was shaken vigorously to remove entrapped air and made to volume at 20° C. The blender jar was rinsed twice with small portions of the macerate from the volumetric flask and the washings were discarded. Finally the remainder of the contents of the volumetric flask were poured into the blender jar and blended for 1 to 2 seconds with sufficient long-fiber, acid-washed asbestos to fill a 100-ml. beaker. The resultant slurry was filtered through a 9-cm. coarse-porosity, sintered-glass filter using vacuum when necessary. The procedure to this point required 15 minutes, and subsequent dilution, and titration could be completed in 40 minutes more.

Suitable aliquots of the filtrate (20 to 100 ml.) were diluted to 500 ml. for determination of reducing sugars by the Lane and Eynon method (*3*). The sucrose in similar aliquots was inverted by boiling for 10 minutes with 5% citric acid preparatory to determination of total sugars as invert by the Lane and Eynon method.

The rapid method described was compared with the modification of the AOAC procedure for fruits and fruit products previously outlined.

Results and Discussion

Successful results in preliminary tests led to design of a series of experiments to test the hypothesis that the concentrations of total and reducing sugars in candied fruits and jams, as determined by the rapid extraction procedure, are not significantly different from the same values determined by the modified AOAC method.

The hypothesis was first tested in the analysis of candied zucca melon. Results of chemical analyses of 10 replicate aliquots by both methods are shown in Table I.

Statistical analysis of data in Table I shows that there is no significant difference in precision of the methods for determination of total sugars in candied zucca melon, and that the rapid extraction procedure is more precise than the modified AOAC method for determination of reducing sugars in this product.

In order to estimate the correlation between the two methods, total and reducing sugars were determined in 10

Table III. Total and Reducing Sugars in Commercial Jam Samples as Determined by Modified AOAC and Rapid Extraction Procedures

Type of Jam	Total Sugars as Invert, %		Reducing Sugars as Invert, %	
	AOAC	Rapid	AOAC	Rapid
Apricot	70.9	71.2	27.2	27.1
	70.2	70.2	22.6	22.2
	69.0	68.9	39.1	39.6
Blackberry	69.1	69.9	50.2	50.1
	70.6	71.2	46.5	45.7
	67.5	68.3	35.2	35.2
	70.4	70.2	41.6	41.4
Black currant	69.2	68.9	46.2	45.7
	67.2	67.2	59.8	61.4
	67.7	68.7	46.3	46.5
	68.3	68.9	34.4	34.6
Loganberry	67.7	67.5	49.8	49.7
	68.4	69.0	54.4	55.1
	66.5	66.4	55.2	51.2
	66.7	66.8	49.1	49.6
Cherry	70.7	70.4	28.1	28.4
	68.4	68.9	29.2	28.8
Peach	70.5	70.4	24.6	24.7
	68.6	68.8	29.5	28.9
	66.5	67.2	23.6	23.8
	66.1	67.7	35.3	35.6
Plum	68.5	69.4	48.5	48.8
	70.8	70.5	39.2	38.5
	69.3	68.9	31.1	31.2
	67.7	66.9	47.6	47.8
Raspberry	72.2	71.8	37.1	36.5
	67.8	68.4	53.0	53.7
	68.9	68.5	43.8	43.7
	67.1	66.7	44.4	43.5
Strawberry	70.4	70.8	42.2	42.5
	69.9	70.1	37.6	38.0
	67.7	68.1	31.1	30.9
	67.1	67.5	44.5	44.0
Orange marmalade	70.6	70.8	24.1	23.8
	66.1	67.4	37.6	37.5
	68.2	68.1	49.2	48.5
	68.9	69.3	39.5	38.9
G. L. O. marmalade	72.4	72.7	17.7	17.4
	70.0	70.4	35.4	35.6
	71.3	71.8	36.1	35.7
$r_{38\ df}$	0.952 *HS*		0.997 *HS*	
Av. diff. (AOAC − rapid), %	−0.24		0.17	
$t_{39\ df}$	3.00 *HS*		1.44 *NS*	

different candied zucca melon samples by each method (Table II). In addition to the correlation coefficient, r, the average difference of paired values, was calculated and a Student's t test made to determine if the average difference of the paired values was significantly different from zero. Values of r and t are given at the foot of Table II.

Data presented in Table II indicate that correlation between the methods is significant at the 99% significance level for determination of both total and reducing sugars. In both determinations the mean difference of the paired values was shown to be not significantly different from zero—i.e., the accuracy of the rapid method was equal to that of the official method.

Further experiments of identical design were made to compare the rapid method with the modified AOAC procedure in analysis of 80 samples of assorted candied fruits and jams. It was found that the methods are equivalent in precision and accuracy when applied to analysis of candied fruits. However, as shown in Table III, the rapid method gave 0.24% higher total sugars in jams than the modified AOAC procedure. No significant difference was found between the methods in the determination of reducing sugars in jams.

Application of the rapid method to analysis of fresh-frozen strawberries, with no added sugar, was satisfactory because, under the conditions of the test, no significant difference in accuracy or precision of the methods was noted. For fresh-frozen apricots and prunes the rapid extraction method had a significantly higher precision than the modified AOAC procedure, while the accuracy of the methods was equal.

The rapid method has the important advantage of requiring less time and involving fewer manipulations than the modified AOAC extraction procedure. Extraction requires only 15 minutes compared with 90 minutes by the conventional procedure of neutralization, boiling extraction, and clarification. A complete determination of total and reducing sugars may be made in less than 1 hour by the rapid method, as compared with 2.5 hours required for the same operation by the modified AOAC method.

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COTTONSEED MEAL IN POULTRY FEED

Inactivation of Gossypol by Treatment with Phloroglucinol

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NUMEROUS ATTEMPTS have been made to destroy the gossypol present in raw cottonseed kernels, or make it unavailable to animals. Most of the methods involve cooking in the presence of water to rupture the pigment glands that contain the gossypol, and to enhance the reaction of the gossypol with various meal components. One process utilizes reaction with organic amines to decrease the available gossypol (*6*). Altschul (*1*) and Curtin (*2*) have ably summarized this subject.

The amount of a characteristic yellow component [believed to be gossypol-cephalin (*9*)] present in the eggs of hens fed gossypol is an extremely sensitive measure of the availability of dietary gossypol (*3*). The exact relationship between the yolk discoloration observed during egg storage and the amount of the yellow component have not been established, but it is known that gossypol is the principal constituent of cottonseed meals that is responsible for such discoloration (*8*). Thus, any process that reduces available gossypol to a very low level should prevent yolk discoloration during storage.

Storherr and Holley (*7*) have proposed phloroglucinol as a reagent for determining gossypol content of mixed feeds containing cottonseed meal. In the present work, this reaction has been studied as a means of decreasing the level of available gossypol in a cottonseed meal.

Methods and Results

In preliminary experiments, a diet containing a commercial cottonseed meal known to contain free gossypol was compared with others to which 0.1 or 1.0% phloroglucinol was added, and with control diets containing phloroglucinol without cottonseed meal. Egg production with all of these diets was normal, and gossypol availability (estimated by the ammonia test) was not affected by the added phloroglucinol; all the eggs were discolored in ammonia.