TABLE IV

Change in the Nutritional Value of Cottonseed Protein on Reaction with Gossypol

Sample description	Bound gossypol	Nitrogen solubility in 0.02 N NaOH	Lysine availability [b]	Rat protein repletion value [a]		
				Av. initial wt.	Av. final wt.	Av. gain in wt. in 10 days
	%	%	%	g.	g.	g.
Original protein	0	89.0	82.9	116.0	167.0	51.0
Methanol-treated protein	0	85.5	86.0	115.8	165.2	49.4
Gossypol-protein complex	3.25	48.3	48.7	116.6	133.2	16.6

[a] Five rats in each group. [b] Three rats in each group.

Summary

A procedure is described by which bound or inactivated gossypol can be removed from cottonseed meal without the application of heat which might damage the protein. The removal of bound gossypol increased the nutritional value of the protein as determined by chick feeding tests, rat protein-repletion tests, and lysine availability tests. A procedure is described for the preparation of a gossypol-cottonseed protein complex without heating the materials. As a result of the combination of the protein with gossypol, marked reduction in nutritional value occurred. The nitrogen solubility of the complex was only about half that of the original protein. The results are in accord with the concept that the inactivation of gossypol during the processing of cottonseed meal is accomplished through the formation of an insoluble, inert gossypol-protein complex which results not only in rendering the gossypol harmless but also in the loss of part of the nutritional value of the protein.

Acknowledgment

This investigation was supported in part by grants-in-aid from the National Cottonseed Products Association and the Cotton Research Committee of the State of Texas.

The standard butanone-extracted cottonseed meal was supplied by the Southern Regional Research Laboratory of the U.S.D.A.

REFERENCES

1. Cabell, C. A., and Earle, I. P., J. Agr. and Food Chem., *2*, 787 (1954).
2. Cannon, P. R., Humphreys, E. M., Wissler, R. W., and Frazier, L. E., J. Clin. Invest., *23*, 601 (1944).
3. Castillon, Leah E., and Altschul, A. M., Proc. Soc. Exp. Biol. Med., *74*, 623 (1950).
4. Chang, W. Y., "The Chemical Characteristics of Cottonseed Meal as Related to Its Biological Utilization," a dissertation, A. and M. College of Texas, p. 93 (1955).
5. Chang, W. Y., Couch, J. R., Lyman, C. M., Hunter, W. L., Van Entwistle, P., Green, W. C., Watts, A. B., Pope, C. W., Cabell, C. A., and Earle, I. P., J. Am. Oil Chemists' Soc., *32*, 103 (1955).
6. Clark, E. P., J. Biol. Chem., *76*, 229 (1928).
7. Haddon, R., Schwartz, A. K., Williams, P. A., Thurber, F. H., Karon, M. L., Dechary, J., Guice, W., Kupperman, R., O'Connor, R. T., and Altschul, A. M., Cotton Gin and Oil Mill Press, *51*, No. 9, 18 (1950).
8. Hale, F., and Lyman, C. M., in "Cottonseed," Interscience Publishers Inc., New Lork, A. E. Bailey, ed., p. 831 (1948).
9. Hegsted, D. M., Mills, R. C., Elvehjem, C. A., and Hart, E. B., J. Biol. Chem., *138*, 459 (1941).
10. Jones, D. B., and Divine, J. P., J. Nutrition, *28*, 41 (1944).
11. Kuiken, K. A., and Lyman, C. M., J. Nutrition, *36*, 359 (1948).
12. Lyman, C. M., Chang, W. Y., and Couch, J. R., J. Nutrition, *49*, 679 (1953).
13. Lyman, C. M., Holland, B. R., and Hale, F., Ind. Eng. Chem., *36*, 188 (1944).
14. Olcott, H. S., and Fontaine, T. D., Ind. Eng. Chem., *34*, 714 (1942).
15. Pons, W. A., and Hoffpauir, C. L., J. Am. Oil Chemists' Soc., *32*, 295 (1955).
16. Richardson, L. R., and Blaylock, L. G., J. Nutrition, *40*, 169 (1950).
17. Richardson, L. R., and Blaylock, L. G., Poultry Sci., *29*, 651 (1950).
18. Schurch, A. F., Lloyd, L. E., and Crampton, E. W., J. Nutrition, *41*, 629 (1950).
19. Sherwood, R. M., and Couch, J. R., Poultry Sci., *29*, 501 (1950).
20. Zucker, T. F., and Zucker, L., Ind. Eng. Chem., *35*, 868 (1943).

[Received February 24, 1956]

Determination of Rosin Acids in Mixtures With Fatty Acids

Å. LINDER, Linder Patents, Stockholm, and V. PERSSON, Mo och Domsjö AB, Örnsköldsvik, Sweden

IN THE UNITED STATES the most widely used methods for analytical determination of rosin acids in mixture with fatty acids, especially in tall oil products, are the methods according to Wolff (1, 2, 8), McNicoll (6), and Herrlinger-Compeau (3). The first two of these give satisfactory accuracy only when the rosin content in the products is about 20–50%. The agreement between results from different analysts is also, in general, rather poor. The Herrlinger-Compeau method gives excellent results in the range of 0–15%.

The authors described in 1949 (4) a new method, applicable to all compositions. Like almost all other methods, this one was based upon the difference in esterification velocity of the fatty acids and the rosin acids. But to make the esterification of the fatty acids more complete, the water formed during the treatment was removed by azeotropic distillation.

To hold back the esterifications of the rosin acids as far as possible, benzene sulphonic acid was used as catalyst. As a small amount of catalyst was used, the esterification took quite a long time.

Olavi-Ivermark (7) have developed a method, similar to the Herrlinger-Compeau method but with corrections for all rosin acids concentrations. The method seems to give reliable results in the whole range of compositions. At high rosin acids concentrations benzene has to be added as solvent, which has an influence on the corrections. The analysis requires a lot of laborious work through repeated extractions with ethyl ether. Therefore the possibility of using potentiometric titration directly on the reaction mixture has been investigated, but the agreement was not as good as for extraction.

To eliminate the disadvantages of our original method the following changes have been made:

1. The benzene sulphonic acid has been replaced by sulphuric acid, and the concentration of acid is stronger, resulting in a decrease in the esterification time from 1–1.5 hrs. to 20 min. It was also found that the variable quality of the purchased sulphonic acid affected the result of the analysis.
2. An attempt to use another solvent mixture with less water-solubility, for example, cyclohexanol and 2-ethylhexanol, did not materially improve the esterification of the fatty acids. It was found advantageous though to increase the content of benzene in the vapors by the use of a column prior to the condensation and water separation.

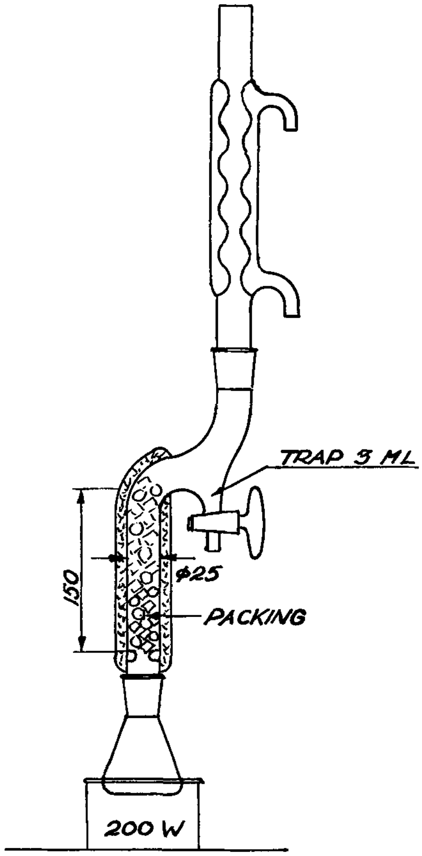

FIG. 1.

Description of the Method

Apparatus.

a) Special moisture trap, constructed according to Figure 1. All sizes in millimeters.

b) Condenser 300-mm. long with drip tip, fitted with ground joints.

c) Flask, 250-ml. Erlenmeyer, fitted with ground joint.

d) pH-meter (not always necessary).

Reagents.

a) Esterification solution.

Mix 500 ml. of n-butyl alcohol, 500 ml. of benzene, and 6 g. of concentrated sulfuric acid. Place the mixture in a flask, connect to a condenser with a moisture trap to entrap the separated water, and reflux for 30 min. to form butyl sulfuric acid. Cool and store in a glass-stoppered bottle.

b) Alcoholic KOH solution, 0.2 N.

c) Phenolphthalein indicator solution.

d) Thymol blue indicator solution (may sometimes be used).

Procedure.

Weigh accurately a 1.0- to 8.0-g. (for high rosin content [>50%] 1–2 g. and for low rosin content [<5%] 5–8 g.) sample into a 250-ml. Erlenmeyer flask with ground-glass joint.

Using a constant delivery pipette, accurately measure 50 ml. of the esterification solution into the flask and add a few boiling stones. Connect the flask to a moisture trap and condenser, place on a hot plate, heat to boiling, and reflux for 20 min.

At the end of the heating period allow the flask and contents to cool somewhat. Remove the flask, cool, and add phenolphthalein indicator solution.

Make a blank titration on the same volume of esterification solution after refluxing it in the same manner.

NOTE: The boiling must be vigorous all the time so that water formed separates rapidly. When the reaction is completed, the bottle is cooled immediately and the sample is titrated with alcoholic KOH solution. Light-colored rosin products are titrated with phenolphthalein as indicator while dark products and those containing small amounts of mineral acids or alkalis are preferably titrated potentiometrically.

Calculation. When phenolphthalein is used as indicator

$$\frac{(A - B) \times N \times 302.4 \times 1.018}{i \times 10} - 0.3 =$$

$$\frac{(A - B) \times N \times 30.78}{i} - 0.3 = \%\ \text{rosin acids}$$

where

A = ml. alcoholic KOH for the sample

B = ml. alcoholic KOH for the blank

N = normality of alcoholic KOH

i = grams of sample

302.4 = assumed molecular weight of the rosin acids (*i.e.*, abietic acid)

1.018 = experimentally-determined factor to correct for the slight esterification of rosin acids

0.3 = experimentally-determined term to correct for unesterified fatty acids.

Figure 2, curve b, shows a normal titration curve. It is to be noted that the first equivalence point B′ of the sample coincides with that of the blank and the content of rosin acids will be represented by the difference A — B.

If the sample consists of a product containing alkalis or mineral acids, for instance raw tall oil, the first equivalence point B′ may be displaced. Figure 2, curve C, shows an example of this. If B′ of the sample differs much from B′ of the blank, the base or the strong acid must be neutralized to obtain the proper amount of catalyst. The sample analyzed in curve C contained 5% alkali, which was neutralized with sulphuric acid. In such cases it is necessary to carry out the titration potentiometrically or use, for instance, thymol blue as indicator to allow the determination of both points of change.

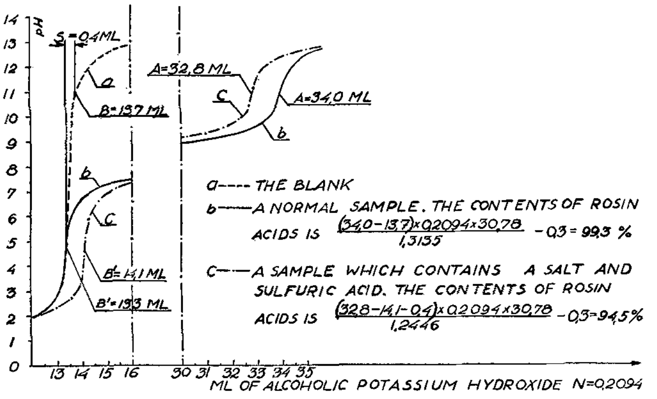

FIG. 2. Titration Curves.

Titration with thymol blue requires light-colored products and much experience as the first point of color change for the indicator does not coincide exactly with the inflection point of the titration curve. According to curves c and a in Figure 2 the content of rosin will be calculated according to the following formula when thymol blue is used or the sample is titrated potentiometrically.

$$\frac{(A - B' - S) \times N \times 30.78}{i} - 0.3 = \%\ \text{rosin acids}$$

where B′ is the amount of alkali in ml. consumed at the first color change (or inflection point) and S is the amount of alkali in ml. consumed in the blank between the first and second color change or between pH 4.5 and 10.5 when the titration is potentiometric. Other symbols are the same as before.

Determination of the Correction Factor. In order to determine the correction factor for different rosin acids content, a great number of tests were made on pure fatty acids and pure rosin acids. The unreacted portion of the fatty acids after 20 min. of reaction time was on the average of 0.3% while the amount of the pure rosin acids which was esterified during that time was 1.45%.

If the rosin acids content which is obtained after 20 min. is called H_{20}, the real rosin acids content C_{corr} and the correction k, the following correlations exist:

$$H_{corr} = H_{20} + k$$

$$k = \frac{H_{corr} \times 1.45}{100} - \frac{(100 - H_{corr}) \times 0.30}{100} \text{ and}$$

$$H_{corr} = 1.018 \times H_{20} - 0.30$$

To check calculated correction the Central Laboratory of Cellulose Industry in Stockholm has analyzed pure rosin acids produced from crystallized rosin of tall oil origin and also mixtures of this with tall oil fatty acids. At the research department of the Hercules Powder Company, Wilmington, Del., tests have been made on pure oleic acid and pure hydroabietic acid and mixtures of these compounds. All these results are presented in Figure 3. The straight line, which represents the average of the values, satisfies the equation:

$$\%\ \text{Rosin Acids added} - \%\ \text{Rosin Acids found} = \%\ \text{Rosin Acids found} \times 0.018 - 0.3$$

Thus % Rosin Acids added = H_{corr}, % Rosin Acids found = H_{20}, and $H_{corr} = H_{20} \times 1.018 - 0.3$.

The agreement is generally rather good, and the deviation from our results is about 0.1%.

Distilled tall oil products with low rosin acids content have been analyzed by Mo och Domsjö AB, using both Herrlinger-Compeau's method and the new method suggested in this paper. The results are presented in Table I.

TABLE I

Comparison of New Method with Herrlinger-Compeau Method

Test no.	Rosin content, %			
	Herrlinger-Compeau		New method	
		Average		Average
1	7.00 6.98 7.04 7.04	7.02	6.96 6.99 — —	6.98
2	2.67 2.69	2.68	2.69 —	2.69
3	3.34 3.38	3.36	3.27 3.33	3.30
4	3.91 3.93	3.92	3.91 3.99	3.95

Time Required for Analysis. The disadvantage with our original method was the long time required for an analysis. As mentioned above, the reaction time has now been cut from 1.5 hrs. to 20 min. Herrlinger-Compeau and Olavi-Ivermark have a shorter reaction time, but, because of the many extraction steps involved, the total time for an analysis will be considerably longer than in our case. This is evident from time studies which were done by Mo och Domsjö AB.

The time for analysis according to our method is about 45 min. against 60–70 min. for methods by Herrlinger-Compeau and Olavi-Ivermark.

It may happen that a larger amount of free acids is obtained after the esterification than before. This happens when products (tall oil pitch) have been exposed to high temperatures for long times and contain small amounts of fatty acids. The fatty acid content, which is expressed by the difference in acid number before and after the esterification, would then appear negative. This circumstance can be explained by the fact that certain rosin compounds of lactone or anhydride type may become hydrolyzed during the acid esterification. In such cases the rosin acids may be determined by weighing, for instance, according to method CCA 15 (5).

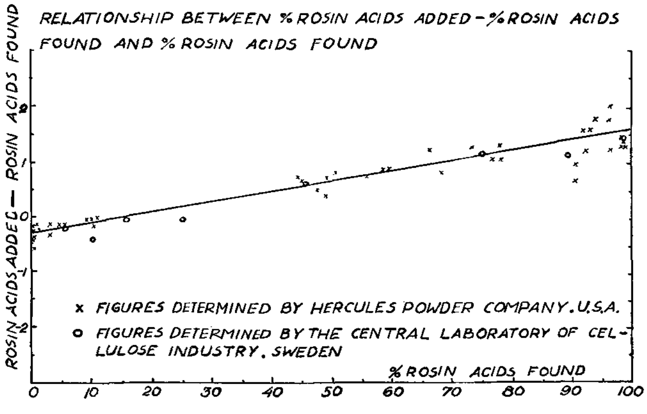

FIG. 3.

Summary

The authors describe an improvement in their analytical method for determination of the rosin-acids content in mixtures with fatty acids, especially for tall oil products.

Good agreement is obtained for the whole range of rosin acids and fatty acids compositions.

The method is faster, simpler, and less laborious than other methods that have been suggested, but seems to give as good results as these.

The method can therefore advantageously be applied both for research work, sales analysis, and as a routine control analysis.

Acknowledgment

The authors want to express their thanks to Mo och Domsjö AB, Hercules Powder Company, and Central Laboratory of the Cellulose Industry as well as their employees who have given valuable assistance in development of the method.

REFERENCES

1. Am. Soc. Testing Materials, "A.S.T.M. Methods of Testing Tall Oil" Serial Designation D-803-49T.
2. Hastings, R., and Pollak, A., Oil and Soap, *16*, 101–103 (1939).
3. Herrlinger, R., and Compeau, Gerald M., J. Am. Oil Chemists' Soc., *29*, 342–4 (1952).
4. Linder, Å., and Persson, V., Svensk Papperstidning, *52*, 331–7 (1949).
5. Method of Swedish Association of Pulp and Paper Engineers, CCA 15, Svensk Papperstidning, *49*, 97–103 (1946) (English summary, p. 105).
6. McNicoll, D. J., Soc. Chem. Ind., *40*, 124 T (1921).
7. Olavi, S., and Ivermark, R., Svensk Papperstidning, *56*, 251 (1953).
8. Wolff, H., and Scholze, E., Chem. Ztg., *38*, 369, 370, 382, 430 (1914).

[Received March 16, 1956]

Pilot Plant Development of the Alkali Cooking Process for Cottonseed Meats. I. Effect of Flake Thickness and of Time, Temperature, and Moisture Content During Cooking

W. H. KING, N. B. KNOEPFLER, C. L. HOFFPAUIR, and E. J. McCOURTNEY, Southern Regional Research Laboratory,[1] New Orleans, Louisiana

IN A PREVIOUS REPORT on the processing of cottonseed (4) it was pointed out that cottonseed meals of low free gossypol content, high nitrogen solubility, and high nutritive value (as determined by short-term chick feeding studies) could be produced by subjecting the flaked meats to a vigorous stirring action in the presence of high moisture content and alkali, followed by mild evaporative dehydration. The extracted oils were also shown to be of high quality. The basic function of this type of treatment is to rupture the pigment glands and inactivate the gossypol by causing it to bind with the meal constituents under conditions which result in high solubility of the meal nitrogen. Under the conditions employed the meats passed through a plastic and doughlike state. Reduction of the moisture content during agitation resulted in crumbling of the plastic mass to discrete particles of meal mixed with oil in such a manner that the oil is readily extracted. A planetary-type mixer, similar to a Hobart food mixer,[2] was found to be convenient for accomplishing the above procedure in the laboratory.

The unique qualities of the materials produced in the laboratory seemed to justify investigation of the process on a larger scale in an effort to determine the equipment requirements. At the same time it was considered desirable to obtain additional data on the effect of flake thickness, cooking moisture content, temperature, and time of cooking in the presence of alkali on the characteristics of the meals and oils produced. Some 27 experiments were selected in accordance with a "Latin square" design (2, 9), as shown in Table I. The combinations of conditions selected for the 27 experiments are indicated by "X" marks.

TABLE I
Experimental Rolling and Cooking Conditions Investigated

Flake thickness............		0.005"			0.009"			0.014"		
Cooking moisture %.........		18	24	31	18	24	31	18	24	31
Cooking tempera- ture °F.	Cooking time (min.)									
180	minimum	X				X				X
180	45		X				X	X		
180	60			X	X				X	
200	minimum		X				X	X		
200	45			X	X				X	
200	60	X				X				X
214	minimum			X	X				X	
214	45	X				X				X
214	60		X				X	X		

Experimental

The jacketed Banbury-type, sigma-blade mixer (Evarts G. Loomis Manufacturing Company) used permitted handling 15.4-lb. batches of flaked meats. This mixer is equipped with rotating blades, which provide thorough agitation, in a jacketed box having a capacity of approximately 2 cu. ft. Either steam or cooling water may be circulated through the jacket and blades. The cover is arranged so that compressed air may be circulated through openings into the chamber, and a thermocouple well extends down from the cover to the middle of the rotating blade area. A diagram of this mixer is shown in Figure 1.

A single lot of prime cottonseed, analytical data for which are given in Table II, was used throughout the investigation. Sufficient seed for each day's cooking operation was adjusted to a moisture content of 9 to 10% and allowed to equilibrate in a closed container for 24 hrs. These seed were hulled through the pilot plant Carver equipment in such a manner as to yield whole and cracked meats which were essen-

---

[1] One of the laboratories of the Southern Utilization Research Branch, Agricultural Research Service, U. S. Department of Agriculture.

[2] The use of trade names does not constitute an endorsement by the Department of Agriculture of the product named over similar products of other manufacturers.