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COTTONSEED MEAL IN POULTRY FEED

A Distinctive Yolk Component in the Fresh Eggs of Hens Fed Gossypol

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Cottonseed meal is a desirable protein concentrate, but it is not now used in ratios for laying hens because the gossypol it contains has an adverse effect on egg quality, particularly of stored eggs. In this study, an attempt was made to fractionate yolks of normal and "gossypol" eggs to discover differences between them. It was found that yolks of hens fed gossypol contain a yellow component not extractable by acetone, but soluble in 3 to 1 hexane–acetone. Although the amount of this component, estimated by its absorbance at 400 mμ, was related directly to the level of gossypol fed, its absorption spectrum was different from that of gossypol. Quantitative estimation of the egg yolk component will serve as a convenient measure of biologically active gossypol. This work is one step toward obtaining sufficient knowledge to allow use of cottonseed meal in diets of laying hens.

CAREFULLY PROCESSED COTTONSEED MEAL is recognized as a good source of amino acids for chickens, turkeys, swine, and ruminants (*4*), but it is not at present recommended for use in the rations of laying hens because it may contain enough available gossypol to cause marked discoloration of the egg yolks, especially when the eggs are kept in cold storage. The yolks of such eggs may become reddish or olive colored, and may show considerable mottling. The yolk membrane appears to be thickened, and the yolk material is often gelatinous (*7*).

Schaible, Moore, and Moore (*11*) reported in 1934 that the yolks of broken-out eggs from hens fed cottonseed meal became chocolate brown in color when kept in an atmosphere of ammonia for an hour or less. It has been suggested that this discoloration is related to that which develops during several months of cold storage. Swensen, Fieger, and Upp (*13*) concluded that the discoloration resulted from breakdown, during storage, of tightly bound iron-protein complexes, followed by combination of the iron with gossypol. Despite the plausibility of this theory, little actual evidence has been found to support it. Gossypol has never been isolated from eggs, and its metabolism in the body is not known.

Conflicting reports exist on the advisability of using cottonseed meal in laying rations. One sample of screw-pressed meal was found to be satisfactory at a 20% level, even when the eggs were stored (*12*). Heywang (*5*), however, found that discoloration occurred at free gossypol levels lower than 0.003% of the diet. Individual hens exhibit wide variation in yolk discoloration (*5, 10*).

Before cottonseed meal can be recom-

mended without reservation for use in rations for laying hens, the danger of discoloration must be eliminated. Working toward this end, the authors have studied the relationships between ingested gossypol and components of fresh egg yolks. This has led to the development of a quantitative method of determining the amount of biologically available gossypol present in cottonseed meal. This will supplement and partially replace the subjectively scored tests heretofore in use—time-consuming storage tests and the ammonia test.

Methods and Results

For most of the exploratory work, hens were fed a normal, 16% protein stock diet diluted with a source of gossypol. The stock diet, which was composed of natural feedstuffs, served as the control. Gossypol was furnished by 1% of hexane-extracted, raw cottonseed meats, or by 20% of a commercially produced, screw-pressed 41% protein meal. The first diet contained 0.0075% free gossypol and 0.0095% total gossypol, as calculated from analyses of the meats by the methods of Pons and Guthrie (*8*) and Pons, Hoffpauir, and O'Connor (*9*). The second diet contained 0.0096% free and 0.142% total gossypol. Isolated gossypol was fed in one experiment. The gossypol of uncooked meats is present in an available form; during the cooking and screw-pressing operations of commercial manufacture, most of the gossypol reacts with meal fractions to produce a nondigestible, bound form (*2*).

Many attempts were made to fractionate egg yolks, in order to differentiate normal eggs from eggs laid by hens fed gossypol (hereinafter termed gossypol eggs). One of the first procedures used was exhaustive extraction at room temperature for several days with a 3 to 1 ethyl alcohol (95%)–ethyl ether mixture. Attempts to identify gossypol in these extracts by formation of a colored reaction product with *p*-anisidine (*8*) were unsuccessful. The extracted residues from gossypol eggs were a pale, lemon-yellow color, while the residues from normal eggs were white. In the light produced by a theatrical ultraviolet lamp, the residues from gossypol eggs appeared to be deep red in color, while similar residues from normal eggs were light red. The lamp was a 250-watt Purple-X, manufactured by the General Electric Co. A similar combination of long and short wave lengths is passed by Corning filter No. 5970. Ultraviolet light alone produces only a weak green fluorescence.

Spectrophotometry of various extracts of yolks was tried as a possible means of differentiating normal from gossypol eggs. The characteristic absorption spectrum of gossypol (peak at 365 mμ in chloroform) (*1*) might be expected to be useful for detecting gossypol in eggs, since the carotenoids normally found in eggs, especially lutein, do not absorb strongly in this region (*3*). If the gossypol exists in free form in egg yolk, as postulated by Swensen, Fieger, and Upp (*13*), its detection in extracts should be relatively simple.

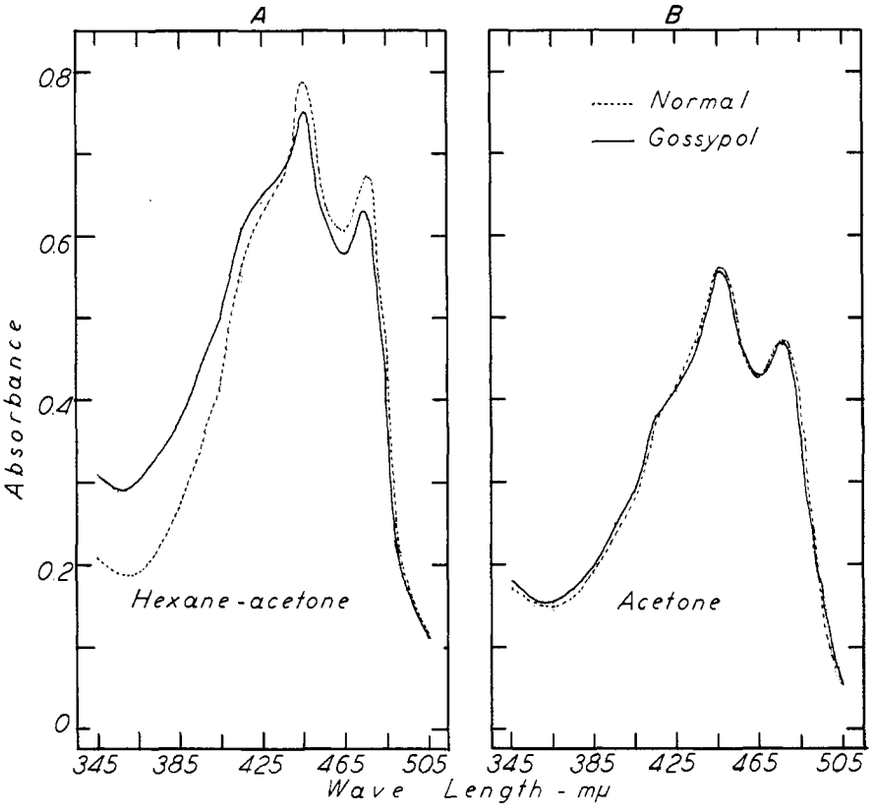


Figure 1. Absorption spectra of hexane-acetone and acetone extracts of normal and gossypol eggs

Extracts of normal eggs and eggs from hens fed 1% hexane-extracted cottonseed meats as a source of gossypol

The absorption spectra of alcohol-ether extracts of normal and gossypol eggs differed only slightly: The gossypol egg extracts absorbed more strongly than normal ones in the range of 325 to 425 mμ. The difference was more readily observed after dilution of the extracts in order to equalize the carotenoid concentrations. For each solvent, extracts were adjusted to a given absorbance at the lutein maximum of 445 mμ (*3*), and absorbance differences were then determined at other wave lengths.

Another solvent mixture which revealed a slight difference between extracts of normal and gossypol eggs was 3 parts by volume of hexane and 1 part of acetone. The hexane had a boiling range of 65° to 74° C. It was purified by treatment with concentrated sulfuric acid, washing with aqueous sodium bicarbonate, drying with calcium chloride, and distilling. Typical curves are presented in Figure 1, A. These curves should be compared with those of Figure 1, B, which show that the absorption spectra of acetone extracts of gossypol eggs are identical with those of normal eggs. The gossypol yolk residues remaining after ten extractions with acetone were still pale yellow, while comparable normal yolk residues were white. The tenth extracts from both eggs were almost always colorless. No free gossypol (*8*) could be detected in the extracted residues of yolks from hens fed 0.0075% free gossypol in the form of hexane-extracted meats. These residues did, however, contain 0.005% total gossypol (*9*). The absorbances in this determination were very low, and the error was high; hence, the method was unsuitable for determining the total gossypol content of eggs from hens which were fed low levels of dietary gossypol.

When the acetone-extracted residues were extracted further with the 3 to 1 hexane-acetone mixture, a yellow pigment was removed from the residue of the gossypol egg, while the extract of the normal egg residue was colorless. The absorption spectra of the hexane-acetone extracts are presented in Figure 2.

The absorption curves of the gossypol yolk extracts have maxima at 375 and 400 mμ, and a shoulder at 420 mμ. There is almost no absorption at wave lengths that show maximum absorption by egg carotenoids (445 and 475 mμ)

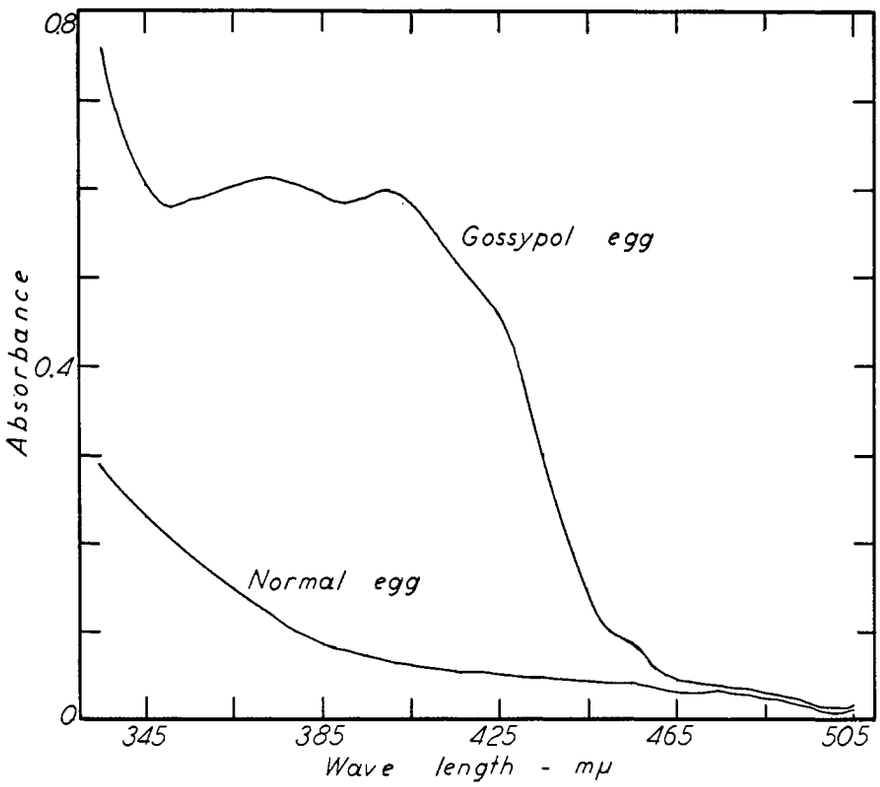


Figure 2. Absorption spectra of hexane-acetone (3 to 1) extracts of normal and gossypol eggs following exhaustive extraction with acetone

(*3*). The peak at 375 mμ is different from that of gossypol, which has a peak at 365 mμ in 3 to 1 hexane-acetone.

Although the distinctive component of the gossypol yolk has not yet been identified chemically, it can be separated from a number of materials with which it is associated when it is first extracted from the egg, and in this procedure the characteristic absorption curve remains essentially unchanged. Its solubility characteristics indicate that the component is associated with the cephalin fraction.

The final extracts made from single yolks had high enough absorbances in a volume of 15 ml. to suggest that fairly low levels of available dietary gossypol might be detectable in this manner. To study this possibility, groups of two hens were fed diets containing 1, 2, 4, 10, and 20% of a screw-pressed cottonseed meal (sample 202). The 1% diet was calculated to contain 0.00048% free gossypol and 0.0071% total gossypol. Samples of eggs from these hens were extracted by the following method.

The yolk material was separated from the albumen and from the vitelline membrane, and mixed thoroughly. Then 10 ml. of yolk were measured into a marked 50-ml. centrifuge tube, 20 ml. of reagent-grade acetone were added, and the mixture was stirred vigorously. After 20 minutes at room temperature, the tube was centrifuged for 5 minutes at moderate speed, and the supernatant was decanted. This process was repeated four times with 20-ml. portions of acetone, followed by five extractions with 15-ml. portions of acetone. Then 15 ml. of a 3 to 1 hexane-acetone mixture were added and stirred. After 1 hour at room temperature (occasional stirring), the tube was centrifuged and the supernatent was filtered by gravity into a marked tube and brought to 15 ml.

The absorbances of 1-cm. thicknesses were determined in a Beckman spectrophotometer, Model B, at wave lengths between 335 and 505 mμ. Significant differences in absorption appeared when the extracts were allowed to stand at room temperature overnight in contact with the residues, and refrigeration caused precipitation from the extracts; hence the entire operation had to be completed within a working day.

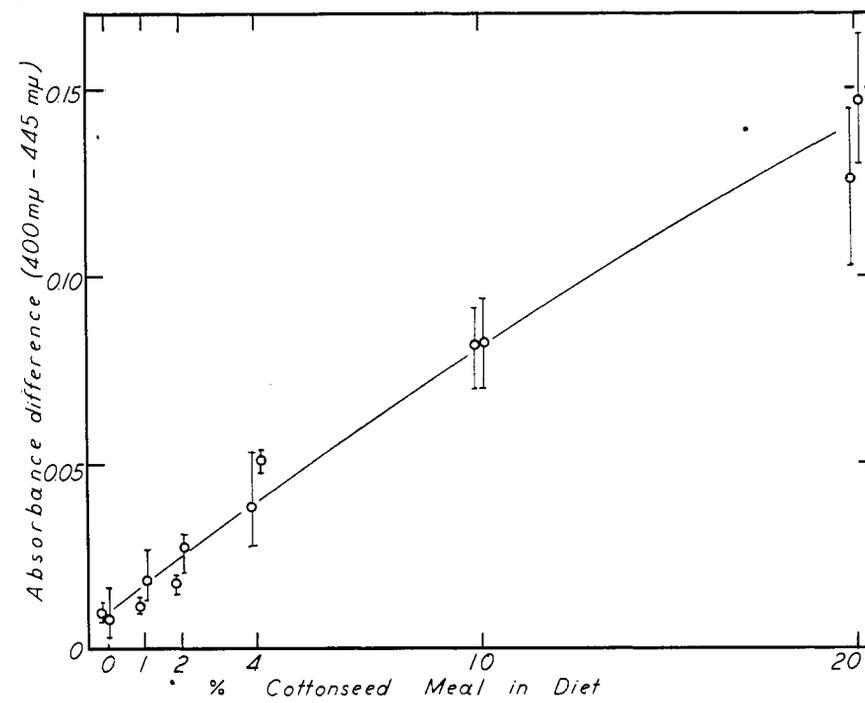
When this method was applied to eggs laid 4 days after the beginning of the feeding period, eggs from hens fed 20% of the meal could easily be detected by the peculiarities of their absorption curves. These effects became more pronounced after 6 days, and by the eleventh day were at a maximum. During the next 35 days, some eggs from each hen were extracted, while others were observed after being placed in an ammonia atmosphere. The results of the extractions are presented in Figure 3.

The wave length most characteristic of the gossypol yolk component is 400 mμ and absorbances at this wave length are related directly to levels of dietary gossypol. The extracts of the eggs of certain hens fed gossypol exhibit an unusually high absorption throughout the range of wave lengths studied. Because the yellow component absorbs very little at 445 mμ (Figure 2), the difference in absorbance at 400 and 445 mμ was used as the best measure of the effect of dietary gossypol on the yolk component.

Extracts were made of two to five eggs

Figure 3. Effect of feeding screw-pressed cottonseed meal on yolk content of component insoluble in acetone but soluble in hexane-acetone

Average for 2 to 5 eggs from each hen plotted as circle. Ranges of eggs from each hen plotted as bar



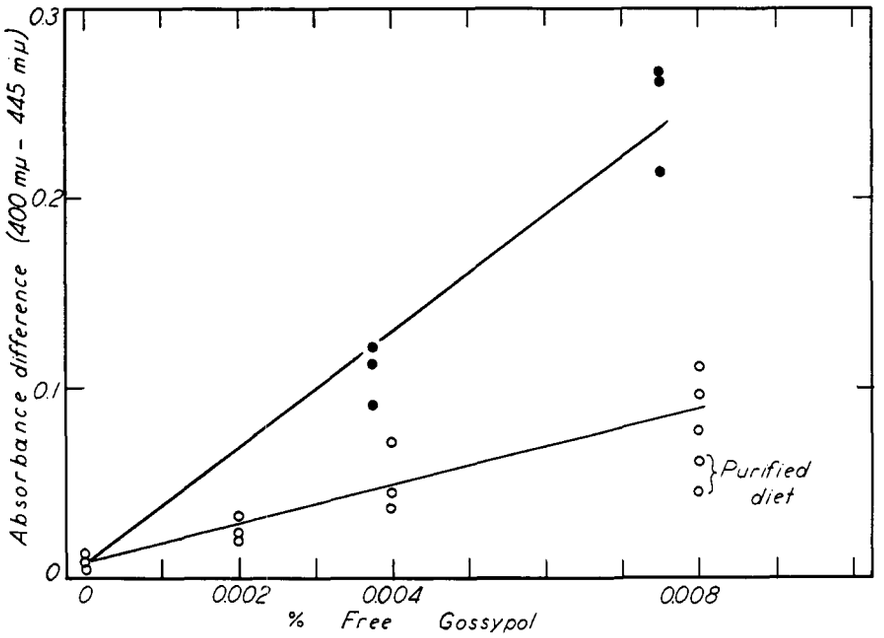


Figure 4. Effect of feeding isolated gossypol (lower curve) and uncooked hexane-extracted cottonseed meats (upper curve) as sources of gossypol on concentration of yellow yolk component

Lower curve. Each point represents one egg from one hen
Upper curve. Each point represents average of 2 eggs from each hen. Stock diets used except where purified is specified

from each hen. The averages and ranges found show that cottonseed meal levels of 2% and higher were easily detected, and that probably the 1% level was also detectable. The meal used contained 0.048% free gossypol (*6*); hence a free gossypol level of 0.001% in the diet is easily detectable.

To compare this method with the ammonia-atmosphere test, eggs from each group were broken into evaporating dishes which were placed in a covered vessel containing a small, open beaker of concentrated ammonium hydroxide. After 1.5 hours at room temperature, the colors of the yolks were scored visually in comparison with normal eggs. In these tests, eggs from hens fed the 10 and 20% levels of cottonseed meal (sample 202) could always be detected, and some of the eggs from hens fed the 4% level could be distinguished by a slight discoloration. Eggs from hens receiving meal levels below 4% were never observed to be discolored.

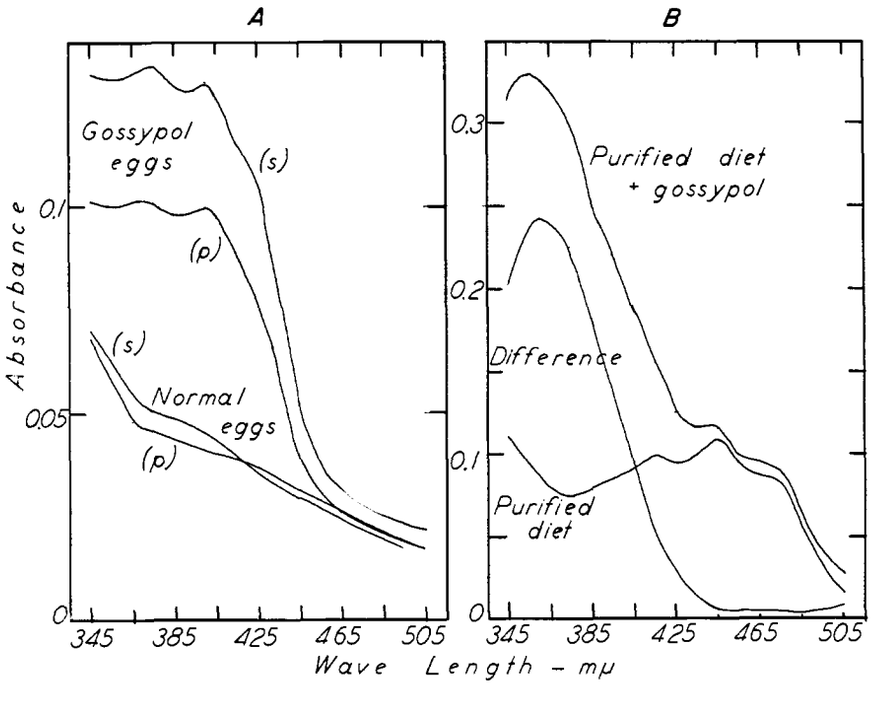
Both hexane-extracted cottonseed meats and screw-pressed meal were effective precursors of the yellow yolk component. If free gossypol is the direct precursor of the component, a diet containing 1% of hexane-extracted meats should be about as effective as one containing 20% of screw-pressed meal, since the free-gossypol contents of the diets are approximately equal. If, on the other hand, some nongossypol component were responsible, the effects of these diets would be markedly different. All the evidence indicated that gossypol was the material responsible. This was clearly shown by an experiment in which gossypol isolated from cottonseed (kindly furnished by the Southern Regional Research Laboratory) was added both to a purified diet low in carotenoids and to the stock diet.

To decrease the carotenoid content of their eggs, hens were fed a purified diet for 14 days: 16% crude casein, 5% gelatin, 10% cellulose (Cellu flour), 5% crude soybean oil, and salts, vitamins, and corn starch to a total of 100%. The yolks of eggs laid by these hens were pale, but not devoid of carotenoids. Previous feeding of these 2-year-old hens had resulted in the accumulation of deposits of pigmented fat, which had apparently supplied some carotenoids to the yolks.

In view of the finding of Heywang *et al.* (*6*) that pure gossypol added to diets was destroyed or inactivated over a period of days, the diets containing isolated gossypol were mixed each morning, and any diet remaining from the previous day was discarded. To assure uniform distribution of the gossypol, it was dissolved in acetone and sprinkled on top of the diet with a pipet. After mixing, the diet was placed in a current of air until the acetone evaporated. The results of feeding the several dietary levels of isolated gossypol are presented in Figure 4. As the gossypol content of the diet was increased, a corresponding increase in the concentration of the yellow component occurred. Qualitatively, the absorption spectra were the same for the extracts of eggs from hens fed the gossypol-containing diet (Figure 5, *A*) and from hens fed cottonseed meal (Figure 2); purified diets produced the same results as stock diets. Isolated gossypol was much less

Figure 5. Absorption curves

A. Typical curves of yellow yolk component in eggs from hens fed stock (s) or purified (p) diets, with or without 0.008% gossypol added to diets
B. Curves of 15 ml. of hexane-acetone (3 to 1) extracts of 1 gram of purified diets with or without 0.008% gossypol. Samples taken 1 day after mixing. Difference curve shows characteristics of gossypol, with no peak at 40 mμ



effective than gossypol in the form of hexane-extracted meats as a precursor of the yellow component, probably because of gossypol inactivation in the diet, as reported by Heywang *et al.* (*6*). Because of interfering pigments, it was impossible to identify gossypol after it was added to the stock diet, but it could be distinguished in the purified diet, as shown in Figure 5, *B*.

Discussion

The results presented here establish a direct relationship between gossypol in the diets of hens and the presence in their fresh egg yolks of a yellow component that can be estimated by quantitative spectrophotometry. This relationship provides the basis for a bioassay of cottonseed meals for available gossypol, and should be particularly valuable in the development of new methods of processing cottonseed.

The role of this component in the discoloration of gossypol eggs during storage is not yet known; studies of discolored eggs show that the distinctive parts of the absorption curve can still be found after 18 months of storage; thus the discoloration is probably not due simply to a breakdown of the yolk component during storage.

Acknowledgment

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MEASUREMENT OF FOOD CHARACTERISTICS

Application of Potentiometric Rotary Viscometer to Measuring Consistency of Food Purees and Pastes

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CONSISTENCY AS APPLIED TO FOOD MATERIALS refers to the subjective impression of "thickness," "body," or resistance to flow. The degree of this property possessed by many food materials—tomato pastes, purees, and catsups, for example—often determines consumer preference and, therefore, the intrinsic market value of the product.

Consistency of food pastes and purees results from the combined contributions of many complex factors. Tomato paste, for example, is basically a two-phase system containing various proportions of a more or less viscous serum and suspended pulp of varying degrees of colloidal suspension and hydration. The enzymatic destruction of pectic substances in the cell walls and middle lamellae of tomato fruit, which may occur during processing (*2, 3*), results in a marked decrease in hydration of the cellular material, and a corresponding increase in the proportion of liquid serum. For this reason retention of the integrity of pectic substances during processing is one of the most important factors in the production of high-consistency tomato products.

The logical measure of consistency in food pastes and purees would be viscosity. However, in systems such as tomato paste the combined contributions of fiber, liquid serum, and hydrated colloids result in a viscous property which is non-Newtonian and exhibits varying degrees of thixotropy. Consistency is, therefore, difficult to measure in these materials by ordinary viscometric methods.

Rotating cylinders and disks tend to beat paths in such materials, and thixotropy causes liquid films to form at the surface of contact of rotor and medium. Results obtained under these conditions have little meaning. Falling-ball types of measurement are not practical because of the extreme thickness of some pastes and purees, as well as their lack of homogeneity.

Two instruments which have been used with moderate success in determining the consistency of tomato catsups and pastes are the Bostwick Consistometer (*7*), which is frequently used for measuring catsup consistencies, and the Penetrometer, which has been employed largely in this laboratory (*4*) to measure tomato paste and catsup consistencies. The former instrument gives satisfactory results, but is limited to the relatively narrow range of consistencies found in catsups. The Penetrometer has a larger range but is subject to certain disadvantages.

This paper describes the application of a potentiometric viscometer with special rotors (Jacobs potentiometric gel-time viscometer; other types of rotor equipment may be used with equal success) to the measurement of consistencies in food products such as