Pilot-Plant Development of the Alkali-Cooking Process for Cottonseed Meats. III. Quantitative Effect of Cooking Variables on Solubility of Meal Nitrogen

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IT HAS BEEN KNOWN for a number of years (4, 8) that when cottonseed is cooked under commercial conditions for an extended period of time, at high temperature, in the presence of moisture, denaturation of the protein occurs. The extent of denaturation has been considered to be proportional to the maximum temperatures employed, the duration of the cooking period, and the amount of moisture in contact with the meats although there appears to be little, if any, published information regarding the quantitative relationship involved.

Virtually all commercial cottonseed oil mills heat cottonseed, prior to oil extraction, during cooking or tempering procedures. This is considered necessary to facilitate recovery of the oil from the meats. Heat treatment in some of the present commercial processes also improves the quality of the oil and reduces the toxicity of the meal by inactivation of much of the gossypol present in the raw meats. Moisture content, temperatures, and duration of cooking vary widely in the different mills, even among those using the same methods of oil recovery.

Considerable interest has been shown in recent years by cottonseed-utilization research workers, the industry, and animal nutritionists in seeking means for improving the nutritional properties of cottonseed meal, especially for use in swine and poultry feed.

Protein denaturation has been defined as any nonproteolytic modification of native proteins, giving rise to definite changes in chemical, physical, or biological properties (9). One of the properties of a protein which is changed by denaturation is its solubility in water or aqueous solutions of salts, acids, and alkalies.

Lyman and associates (7) have proposed reduction in solubility of cottonseed meal protein in 0.02N NaOH solution as a measure of heat damage to the protein during processing. This property has shown a parallelism with nutritive value of the meal in several chick-feeding studies (1, 2, 5, 7).

While this property of cottonseed meal is by no means considered to be the final answer to the development of objective chemical or physical measures of its nutritive value, it appears to be of some significance at the present time. It is conveniently determined in the laboratory and therefore may be useful in laboratory control of processing conditions.

In this paper an observation of an approximate quantitative relationship between cooking conditions of cottonseed and denaturation of the protein as measured by the solubility of the meal nitrogen in 0.02N NaOH is presented. In addition to some previously published data, the data obtained under carefully controlled conditions in the pilot plant at this laboratory have been used in the present study (6).

Theoretical Considerations

The fundamental law underlying the rate of reactions is the mass-action law of reaction rate, that is, the reaction rate depends on the effective concentration of reacting materials. If the reaction rate is proportional to the concentration of one reactant, the reaction is called unimolecular. If the reaction rate is proportional to the square of the concentration of a reactant or to the product of the concentration of two reactants, it is said to be of the second order, or to be bimolecular. The more common reactions are either of the first or second order. Many reactions follow the first order equation

$$-\frac{dc}{dt} = kc$$

which expresses mathematically that the rate of decrease of concentration c, with time t, is proportional to the concentration. This equation can be integrated between limits c_0 at t_0 and c at a later time t,

$$k = \frac{2.303}{t} \log c_0/c$$

where c_0 is the concentration of unreacted molecules at the beginning of the reaction period and c the concentration at the elapsed period of time t (3).

In applying the above equation to denaturation of cottonseed protein by heat, c_0 may be considered to be the concentration of undenatured molecules at o time and c the concentration at elapsed time t. Using the data developed by Condon *et al.* (2) under constant moisture conditions, k, the reaction-velocity constant, was calculated to be 0.012 for reduction of nitrogen solubility of butanone-extracted, oil-free cottonseed flakes when autoclaved at 120°C. for various lengths of time, up to 120 min. The value of 0.012 for k is the average of all values calculated from the data in Table I. To illustrate the method of calculation the data at 0 and 30 min. are substituted in the above equation as follows:

$$k = \frac{2.303}{30} \log 0.82/0.56$$

$$\text{whence } k = 0.0127.$$

A comparison of calculated values for nitrogen solubility (percentage of total nitrogen-soluble in 0.02N NaOH), N_s, with those determined by chemical analysis are given in Table I. There appears to be a significant relationship between values for nitrogen solubility calculated by the equation and those val-

TABLE I

Autoclaving time (minutes)	N_s (calculated)	N_s (found)
0	82	82
5	77	76
15	68	73
30	57	56
60	40	38
120	20	29

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ues found by chemical analysis over the range covered by the data (0 to 120 min.). The relationship also suggests that heat denaturation of cottonseed protein, under the experimental conditions studied, as measured by nitrogen solubility in 0.02N NaOH, follows the kinetics of a first-order reaction.

Under practical conditions, in the oil mill, temperatures are not usually held constant, and moisture content of the cottonseed meats usually is changing constantly during the cooking operation. As will be shown later, the moisture content has a pronounced influence on the rate of heat denaturation. For these reasons available data on the relationship between nitrogen solubility of the cooked meats and the moisture content, temperature, and duration of cooking under laboratory conditions, which simulate changing conditions characteristic of commercial cooking-operations, have been studied in an effort to develop an empirical relationship between the factors involved which might be useful to the oil-mill operator.

A study was made of data obtained in recent pilot-plant experiments on cooking cottonseed under carefully controlled conditions (6). These data were obtained on cottonseed meats adjusted to a constant pH of 8.2 by the addition of small quantities of NaOH. Moisture content, temperature, and time of cooking were purposely varied over a rather wide range. The experimental conditions were such that approximately 10 min. were required to bring the cottonseed meats up to the temperature of the experiment, and the moisture content was reduced at a uniform rate over the cooking period to 11%, followed by flash cooling. Upon cooling, the moisture content was reduced to 8 to 10% in each experiment.

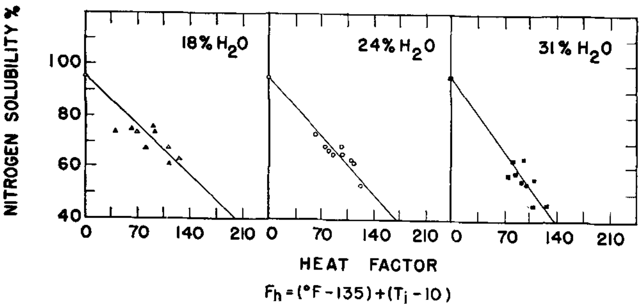

FIG. 1. Relationship between "heat factor" and nitrogen solubility of cottonseed meal.

Since the usual equation for reaction rate was not applicable to these changing conditions of the variables, an empirical relationship was applied to the data. The graphs in Figure 1 were obtained by plotting nitrogen solubility against a "heat factor" at three different moisture-levels. The heat factor was calculated in each instance from the experimental data, using the following equation:

$$\text{Heat factor} = (°\text{F.} - 135°) + (T_i - 10)$$

where °F. is the cooking temperature expressed in Fahrenheit degrees and T_i is the cooking time in minutes. In the first term of the equation 135° is subtracted from the cooking temperature on the assumption that the protein solubility is not affected at or below 135°F. In the second term of the equation 10 min. were deducted from the cooking time because this was the approximate time required to bring the meats up to the cooking temperature in each instance.

It is apparent upon inspection of Figure 1 that the slope of the lines correlating nitrogen solubility with heat factor is markedly different at different moisture-levels during cooking. In other words, the rate of denaturation of the protein by heat is proportional to the amount of moisture present. This observation suggests that a "cooking factor" which combines all three variables: temperature, moisture content, and duration of the cooking period may show a more consistent relationship with extent of denaturation. Accordingly the following mathematical analysis of the data was undertaken. Let

(1) $F_h = \Delta\ °F + T_i$

where F_h = heat factor,

Δ °F. is the temperature increment above 135° F., and T_i is the duration of the heating period in minutes (for these experiments T_i was taken as the time the material was held at the temperature of the experiment after correcting for 10 min. required to bring the material to temperature).

In Figure 1 the slope of the lines at the different moisture-levels may be expressed mathematically in the following equation for a straight line as the factor "m":

(2) $N_s = mF_h + C$,

where N_s = nitrogen solubility,

F_h = heat factor,

m = slope of line, and

C = a constant.

It is obvious from the graphs that "m" will vary with the moisture content. Therefore the values of "m" and "C" were determined for each of the moisture levels studied (*i.e.*, 18, 24, and 31%) by substituting the experimental data in simultaneous equations. The following values were obtained:

% moisture	m	C
18	—0.28	96
24	—0.34	96
31	—0.41	96

When the above values for "m" are plotted against "% moisture," it may be seen that one is a linear function of the other (Figure 2), and therefore their relationship may be expressed by the following linear equation:

(3) $M = fm + A$

where M = % moisture,

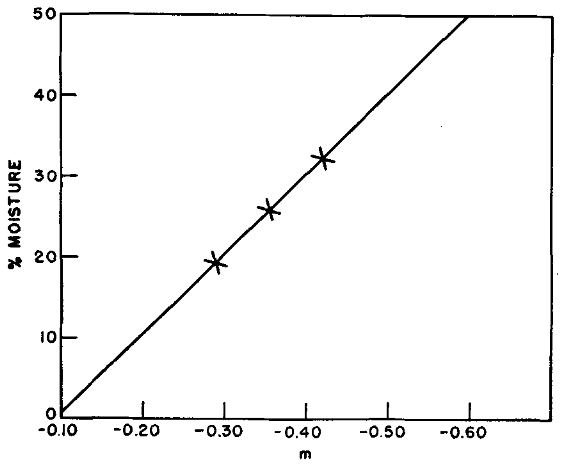

FIG. 2. Variation of rate of denaturation of cottonseed protein with moisture content during cooking.

$f =$ slope of line, and
$A =$ a constant.

Substituting the experimental data in simultaneous equations, as above, and solving for "A" and "f" we find:

$$A = -10$$
$$\text{and } f = -100.$$

Rearranging (3),

$$fm = M - A$$

and substituting derived values for "f" and "A" we have

$$m = -\left[\frac{M + 10}{100}\right]. \tag{4}$$

Combining (1) and (2):

$$N_s = m(\Delta°F. + T_i) + C,$$

$$\text{or (5)} \quad N_s = 96 - (\Delta\ °F. + T_i)\left(\frac{M + 10}{100}\right)$$

The "cooking factor" thus becomes

$$F_c = (\Delta\ °F. + T_i)\left(\frac{m + 10}{100}\right).$$

It may be defined as the numerical reduction in nitrogen solubility of cottonseed meal resulting from cooking of the meats prior to processing.

TABLE II

Comparison of Values for Meal Nitrogen-Solubility (in 0.02N NaOH) Calculated from Pilot-Plant Cooking Data and Values Found by Chemical Analysis

Cooking factor[a] F_c	Initial moisture content, %	Cooking data		% Soluble Nitrogen (N_s)	
		Time (min.)	Temp. (°F.)	Calc.	Found
0	—	Uncooked meats		96	96
12	18	8.5	180	84	75
19	18	14	200	77	74
21	18	40	180	75	75
21	24	25	183	75	74
23	18	13	213	73	69
26	24	41	180	70	68
26	18	55	184	70	74
27	18	40	200	69	71
27	24	23	200	69	67
29	24	15	214	67	66
30	31	37	180	66	57
31	24	57	180	65	65
31	18	55	200	65	68
31	18	40	214	65	62
32	31	40	182	64	64
32	24	40	200	64	68
33	31	25	200	63	57
35	18	55	214	61	63
37	24	40	214	59	63
37	31	55	180	59	64
38	24	55	202	58	61
39	31	40	200	57	55
41	31	31	214	55	54
42	24	55	214	54	52
45	31	40	214	51	46
45	31	55	200	51	56
52	31	58	214	44	46

[a] $F_c = (\Delta\ °F. + T_i)\left(\frac{M + 10}{100}\right)$.

A Comparison of Calculated Values for Nitrogen Solubility With Values Determined by Analysis. Table II gives a comparison of values of N_s calculated from equation (5) and the values found by chemical analysis. These data are plotted graphically in Figure 3. The deviation from ideality is indicated by the line drawn at 45° to the abscissa.

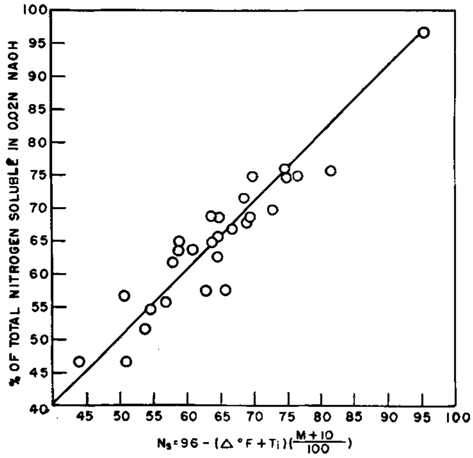

FIG. 3. Comparison of calculated and determined values for nitrogen solubility.

Summary and Conclusions

A study was made of previously published data resulting from experimental cooking of cottonseed from the viewpoint of quantitative relationship between cooking conditions and denaturation of the protein as measured by the solubility of the meal nitrogen in 0.02N NaOH. It was observed that the rate of denaturation of hexane- and butanone-extracted cottonseed meal, autoclaved at constant temperature and moisture content for various lengths of time, closely followed the equation for a first-order reaction. The average reaction velocity constant is 0.012. Since, in commercial oil mill operations, temperature and moisture content of the meats vary during cooking, an analysis of laboratory data obtained under such conditions was made, which resulted in an observation of an empirical relationship between rate of reduction of nitrogen solubility and the three principal factors which affect the rate of denaturation under practical conditions. The factors studied were moisture content, temperature, and duration of the cooking period. In these experiments the meats were adjusted to a constant pH of 8.2. Mathematical treatment of this observation led to an empirical formula relating cooking conditions and denaturation which correlated well with the cooking data. This formula indicates that the temperature increment above 135°F. in degrees F. has about the same effect, numerically, as the cooking time in minutes under the conditions studied. When the sum of these two factors is multiplied by the moisture factor

$$\left(\frac{\%\ \text{moisture} + 10}{100}\right),$$

an approximate, consistent value is obtained which is commensurate with the reduction in nitrogen solubility of the meal cooked under those conditions. The observation suggests the desirability of continuing the study with additional, carefully obtained data under laboratory and plant conditions. It is entirely possible that experiments designed to show the independent effect of moisture content on denaturation at constant temperature may help to explain the theoretical basis for the the equation developed. Such information should be a valuable addition to the knowledge of protein chemistry.

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The Behavior of Distilled Monoglycerides in the Presence of Water[1]

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THE EFFECT of purified monoglycerides on interfacial tension with water has been studied for triglycerides (1, 2) and for petrolatum and amyl acetate (2). Also the effect of monoglycerides on water absorption into cottonseed oil and petrolatum has been reported (1). Comparison with mono- and diglyceride mixtures indicated that the effects were not always proportional to monoglyceride content.

In the absence of fat or petrolatum some unusual qualitative differences became evident. This paper describes gels and other structures resulting from the combination of several distilled monoglycerides with water, and with water plus other materials.

Gels

When distilled monoglycerides (C_{14} or higher, see footnote, Table I) are melted and added to water at the same temperature, a firm "gel" is formed (Figure 1). The gel, usually containing about 15% to 25% by weight of water, is not dispersible in water and is difficultly soluble in oil. If the excess water is decanted, the gel can be worked mechanically into an optically clear solid with a consistency resembling very heavy stopcock grease.

Upon cooling a gel below the melting point of the monoglyceride, crystals begin to form. If crystallization proceeds appreciably, the gel structure deteriorates, and a white creamy paste is produced.

Thus the lower temperature limit of stability is related to melting point; 1-monostearin (m.p. 81°C.) gels are stable above about 80°C., and 1-monoolein (m.p. 35°C.) gels are stable above about 20°C. Mixed monoglycerides, such as those from natural fats and oils, yield mixtures of gel and paste which are not readily dispersed in water.

The upper temperature limit of stability is very close to 100°C. In most cases, if a piece of gel is heated on a spatula, the water begins to boil out before the solid structure deteriorates. Highly unsaturated monoglycerides (*e.g.*, from cottonseed oil or soybean oil) form gels which change at about 98°C. into water and oil layers.

Upon standing exposed to air, a gel loses water. The equilibrium status is not yet fully defined, but the water content at low relative humidity is probably well below 5%. Thus a gel can be kept only in a closed container or under extreme humidity conditions. One monoolein gel was kept in a stoppered bottle at room temperature for more than a year without appreciable change in appearance.

Water is partially soluble in a pure molten monoglyceride. As water is added, the apparent melting point drops somewhat, then the viscosity of the molten material begins to increase. The viscosity becomes high enough at about 10% and 15% by weight of water (monoolein at 25°C.) to result in a soft nonpourable transparent "solid." As the ratio of water is further increased, the solid becomes firmer until no more water can be incorporated.

A typical gel preparation contains 22% to 23% of water. But by severe mechanical manipulation at room temperature, it has been possible to prepare a monoolein gel containing up to about 45% of water without leaking. The gel may be of hydrogen bonded structure since preliminary measurements indicate a conductivity similar to that of the distilled water used ($K = 2.5 \times 10^{-6}$ mhos. per cm.) and appreciably higher than that of a typical water-in-oil emulsion ($K = <5 \times 10^{-8}$ mhos. per cm.).

Gel Preparation

Two techniques were used to prepare gels in order to demonstrate the effect of temperature and of the ratio of water on their physical properties. In the first case the monoglycerides listed in Table I (1 g. in each experiment) were added to water (100 g.) and stirred as the temperature was raised. Two transitions occurred in most cases, one in all cases. At about 10°–15°C. below the melting point the monoglyceride smoothed into a creamy dispersion. Then the monoglyceride coagulated into a non-dispersible lump which was translucent to transparent. This last conversion was called the "gel point."

Upon cooling this mixture of gel and water below the gel point, but above the first transition, and holding for a prolonged period of time with agitation, the

TABLE I

Gel Points of Monoglycerides

Monoglycerides [a]	m.p. °C.	"Gel point" °C.
Distilled monolaurin	54–57	No gel to 99°C.
Distilled monomyristin	62–65	85
Distilled monopalmitin	67–68	68
Distilled monostearin	70–72	70
1-Monomyristin	70.5	No gel to 99°C.
1-Monopalmitin	74–74.5	82
1-Monostearin	81–81.5	81
Distilled monoolein	35	<20

[a] Distilled products usually contain about 90–92% 1-monoglyceride, 5–8% of 2-monoglyceride, and 1–3% of glycerine, diglycerides, and free fatty acids (3).

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[1] Communication No. 240 from the laboratories of Distillation Products Industries.