

PARAMETERS FOR THE DETERMINATION OF  
HEAT TREATMENT OF SOYFLOUR

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## INTRODUCTION

As early as 2838 before the Christian era, King Chan Noang of China mentioned the soybean in a medical treatise; that was many years before the first pyramids were built, six "jubilees" before the erection of the Tower of Babel, and 1200 years before the building of Solomon's Temple. The soybean was first brought to Europe in 1712 from China, by the German botanist Englebert Kaempfer.

Recently, increased interest has developed in the nutritive value of soybeans. Various soybean products have been developed for the human diet to partially replace or extend animal proteins. Soya protein, in the form of flour, is being used in ever increasing amounts in a wide variety of food products.

Since 1917, when Osborne and Mendel reported the improvement of the nutritional value of soybean by heat treatment, many studies on the changes of soybean due to heat treatment have been carried out.

A simple, rapid, and sensitive method for "quality control" in industrial processes of soyflour production is badly needed. The purpose of this study was to investigate the changes in soyflour due to heat treatment and methods for revealing the degree of heat treatment of soyflour.

## REVIEW OF LITERATURE

### Soyflour Composition

Soyflour has been defined (Hayward and Diser, 1961) as the screened, graded product obtained after expelling or extracting most of the oil from selected, sound, clean, dehulled soybeans. Generally speaking, all soya products ground finely enough to pass a 100-mesh or smaller screen are referred to as "flour." Soyflours are manufactured in various granulations as desired for specific uses. The moisture content of soyflours varies from 5% to 10%, depending on atmospheric conditions, but usually averages from 5-8%. Ash of soyflours contains 0.25 or 0.26% calcium and 0.58-0.65% phosphorus. Sodium, potassium, magnesium, and trace minerals such as iron, copper, cobalt, zinc, etc., are also present in varying, but appreciable amounts (Hayward and Diser, 1961).

Soyflours also contain approximately 5.5% sucrose and 20-29% polysaccharides, and trace amounts of starch (Hayward and Diser, 1961).

Raw soybeans contain a high level of thiamine (vitamin B<sub>1</sub>). Even though the amount is reduced in soyflour (due to heat treatment), the level of thiamine in soyflour is usually a few times greater than in other cereals (Pomeranz, 1959). Other water-soluble vitamins such as riboflavin, pyridoxine, pantothenic acid, folic acid, and niacin are contained in soyflours in varying amounts. Inositol and choline are present as components of

the lecithin fraction. Other fat-soluble vitamins or vitamin-like substances, such as carotene and tocopherols (vitamin E) are also present. The tocopherols in soyflour possess antioxidant properties and are credited with aiding in the prevention or retardation of the development of rancidity in the foods or other products to which soyflour is added (Hayward and Diser, 1961).

The mixed phosphatide fraction (soy lecithin) in soyflour varies within a range from 1.6-2.5%.

Urease, amylase, protease, allantoinase, ascorbic acid oxidase, carboxylase, catalase,  $\beta$ -glycosidase, glyoxylase, lipase, lipoxidase, phytase, and uricase are all considered to be present in raw soybeans (Circle, 1950).

There are several physiologically active constituents in raw soybeans (Circle, 1950) such as an antioxygenic factor, an allergic factor, a plant growth regulator, a goitrogenic factor, blood coagulant factors, anti-amylase factor, antigrowth and anti-tryptic factor (trypsin inhibitor), saponin, hemagglutinin (soyoin), and estrogenic substances (Booth et al., 1960).

Bitter and beany components of soybeans have been studied and reported as derivatives of lower aliphatic carboxylic acids (Teeter, 1955).

Rackis et al. (1961) reported on the relative distribution and percentage recovery of essential and related amino acids in residue, acid-precipitated, and whey protein fractions obtained from dehulled hexane-extracted soybean meal (or soyflour). The residue fraction contains 16 to 30% of the total amino acids

originally present in soybean meal; 48 to 73% were present in the acid-precipitated protein fraction, and the whey protein fraction contained 6 to 10%. Tryptophan, lysine, cystine, and methionine are susceptible to destruction during acid hydrolysis.

Protein scores of soyflour compared to a provisional amino acid pattern based on the system worked out by the Food and Agricultural Organization of United Nations (FAO) were obtained by Orr and Watt (1957).

#### Heat Effects on Food Protein

The nutritional value of the protein in a heated foodstuff, as compared with that of the unheated material, represents the resultant of two main opposing trends. The first of these is towards increased nutritional value. It is initiated early and at comparatively low temperatures, and is due to one or more of the following factors: 1) increased digestibility resulting from simple denaturation of protein, 2) inactivation of enzyme inhibitors, and 3) destruction of toxic substances in the foodstuff. The second trend, which usually becomes increasingly obvious as heating is prolonged or at higher temperatures, is towards diminished nutritional value. Here, the main factors concerned are: 1) the production of enzyme-resistant parts of the protein molecule either by purely intramolecular rearrangement or by interaction of particular reactive groups with non-protein substances such as reducing sugars, 2) the formation of metabolically unavailable derivatives of free amino acids by reaction with

other food constituents, 3) actual destruction of amino acids, and 4) the loss of substances other than proteins such as vitamins, which are present in the food and are necessary for proper metabolism.

#### Physical and Chemical Changes of Soyflour Constituents due to Heat Treatment

In 1917, Osborne and Mendel showed that heat could improve the nutritive value of soya meal for rats, and made the important observation that the presence or absence of water was an important factor. Whereas dry heating the meal at 110°C for four hours was without effect, steaming moist meal for three hours produced substantial improvement. Hayward et al. (1936) illustrated the extent to which the nutritive value of soybean protein might be improved depending on the conditions of processing.

Heating of proteins in neutral solutions causes a number of changes; i.e., decreases in solubility, loss of specific activity if the protein was a hormone or enzyme, and loss of crystallizability, etc. Collectively, these changes are described by the term "denaturation." In many instances, the distinct process of coagulation of the protein follows its denaturation. A number of native proteins do not contain the free sulfhydryl groups of cysteine as determined by the nitroprusside test; after denaturation, sulfhydryl groups are revealed (White et al., 1959). The rate of denaturation is influenced by temperature, duration of heating, pH, and the concentration of electrolytes. Accompanying denaturation there may be large increases in viscosity and

surface tension, and changes in optical rotation and in other characteristics. Organic solvents might also produce denaturation; these solvents include hexane, the solvent used for extracting oil from soybeans. Both anionic and cationic detergents cause denaturation of protein. Shaking of protein solutions produces surface films of denatured protein.

Evans and St. John (1945) studied the effect of heat on the solubility of soybean protein fractions obtained by extracting soyflour successively with water, 5% potassium chloride, 70% ethanol, and 0.2% potassium hydroxide. Heating at 121°C for up to 30 minutes resulted in a progressive increase in the amount of nitrogen which could be extracted with 0.2% KOH (glutelin fraction) accompanied by a concomitant decrease in nitrogen solubility in water and in 5% KCl.

Liener (1958) defined the destruction of amino acids as the difference between the amounts of an amino acid which could be measured in the acid (or alkaline in the case of tryptophan) hydrolysates of the raw and the heated proteins. Inactivation was defined as the difference between the amounts of an amino acid which could be measured in deproteinized hydrolysates after subjecting the raw and the heated proteins to in vitro enzymatic digestion under standardized conditions.

Rice and Beuk (1953) reported that mild heat treatment of soya products increases availability of methionine, while overheating results in reduced nutritive value, decreased availability of several amino acids, particularly of lysine and arginine.

Eldred and Rodney (1945) considered the reduced availability of lysine in heated protein to be due largely to the formation of enzyme-resistant linkages between the epsilon-amino groups and other unspecified, reactive groups. This hypothesis was tested by Carpenter and co-workers (1958). They allowed dinitrofluorobenzene to react with the free amino-groups of the protein, submitted the product to acid hydrolysis, and measured (colorimetrically) the dinitrophenyl lysine liberated. The epsilon  $\text{NH}_2\text{-X}$  lysine derivative might perhaps be absorbed from the intestine but was for all practical purposes of no nutritional value. Feeding tests, using chicks as the experimental animals, and various meat and fish products as test materials, have shown good agreement between nutritional value and the amount of lysine with free epsilon-amino groups.

Riesen et al. (1947) showed for soya, and Patton et al. (1948) for casein that comparatively mild heat treatment in the presence of glucose produced very significant losses of some amino acids (lysine, arginine, tryptophan) and smaller losses of others (histidine, leucine, isoleucine).

Patton et al. (1948) showed that the heat treatment they used (refluxing for 24 hours) destroyed no appreciable quantity of essential amino acids when water was substituted for the glucose solution. Data contributed by Evans and Butts (1949) showed that the actual destruction of amino acids when soya protein was heated was very small, 9% for cystine and less than 5% for others based on determinations after acid hydrolysis. On the same basis,

heating (autoclaving) in the presence of sucrose resulted in the destruction of about 40% of the arginine and lysine, about 20% of the cystine, and 10% of the histidine. Enzymatic hydrolysis of protein heated alone revealed that some loss of all essential amino acids had occurred in the sense that they had been rendered unavailable for liberation by digestive enzymes although they were evidently regenerated by acid hydrolysis. In the case of lysine, this loss amounted to 30%; the loss of histidine was 16% and of cystine 14%; the losses of other amino acids were small. Following heating in the presence of sucrose, apart from actual destruction, large amounts of the essential amino acids, except isoleucine and valine, had become unavailable, and the losses were much greater than in the absence of sucrose. This was particularly marked in the cases of arginine, cystine, histidine, lysine, and methionine. The decrease in available methionine was especially noteworthy since actual "destruction" was virtually nil, and methionine probably was the amino acid whose concentration was critical with respect to the nutritive value of soya protein. Evans and Butts (1949) suggested the following general explanation of the nutritional damage produced in proteins by heat: 1) certain amino acids, notably those with a free amino or carboxyl group (lysine, histidine, glutamic acid, aspartic acid) react with other constituents of the protein molecule to form enzyme resistant (and therefore unavailable) derivatives hydrolysable by acid, 2) many of the essential amino acids, including those with free amino groups (lysine, arginine,

histidine) and some others, react with carbohydrates to form enzyme resistant compounds, hydrolysable however, by acid, and 3) amino acids with free amino groups and some others (including methionine) are destroyed when heated in the presence of carbohydrates in the sense that they cannot be regenerated from the products of reactions by acid hydrolysis. The latter reaction is, at least in extreme cases, closely associated with the non-enzymatic "browning reaction" at relatively low temperatures; i.e., the Maillard reaction (1912). Steward (1960) summarized our present knowledge in this field as follows: 1) work on the drying of protein hydrolysates containing glucose indicates that the browning effect makes certain of the amino acids unavailable, but without formation of toxic products (Friedman, 1950); 2) substances other than carbohydrates might be implicated; and 3) changes of type occurring in the browning reaction are of negligible nutritional importance in many ordinary heating (cooking) procedures.

The presence of urease in the soybean had been reported by Graaf et al. in 1916, and Nakagawa in 1921 (Circle, 1950). Smith et al. (1956) reported that the soybean hulls were very low in urease activity, and germ, on a weight basis, had nearly twice as much activity as the cotyledons. The urease activity contributed by hulls, germs, and cotyledons to the activity of the whole soybean meal was approximately 0.2%, 3.8%, and 96%, respectively. These data indicated that the removal of hulls, as done in soy-flour processes, would favor an increase in urease value for the

combined cotyledon-germ fractions. Inactivation of urease might be induced due to protein denaturation.

Early investigations of Orestano (1933) on soybean amylase point to its identity as an amylase of the  $\beta$ -type. Teller (1936) indicated that soybeans contained both  $\alpha$ - and  $\beta$ -amylase. According to Newton and Naylor (1939), soybeans contain only  $\beta$ -amylase with a possible slight trace of  $\alpha$ -amylase. The fact that a highly concentrated extract of soybean amylase was capable of reducing the viscosity of a starch paste was not considered to be a conclusive proof for the presence of  $\alpha$ -amylase. The marked change in viscosity of the substrate during digestion with soybean amylase, was attributed to the fact that the enzyme is one of the most powerful sugar-forming plant diastases. A similar conclusion was reached by Laufer *et al.* (1944). Studies with the amylograph by Ofelt *et al.* (1955) demonstrated that 5% of raw soyflour defatted in the laboratory at low temperatures, decreased the viscosity of hard winter wheat starch to an extent nearly equivalent to that produced by 15 SKB units of malt-alpha amylase; this effect was not caused by malt beta-amylase. The existence in raw soybeans of a starch liquefying enzyme as demonstrated by the amylograph was confirmed by Learmonth and Wood (1960) and by Pomeranz and Lindner (1960).

Soybean amylases seem to differ in their properties from amylases of other cereals. Unlike barley and wheat, soybeans do not contain bound beta-amylase (Laufer *et al.*, 1944). Germination does not affect soybean amylase to any extent. The optimum pH of

the soybean amylase has been found to be in the neighborhood of 5.9. A concentrate of soybean beta-amylase was stable when stored at room temperature for up to two years (Newton et al., 1943). Heating ground soybeans for ten days at 100°C did not completely inactivate the amylase.

A starch paste digested by soybean-amylase at 76-78°C for 30 minutes retained over 70% of its saccharogenic activity. This is in contrast to beta-amylases from wheat or barley which are practically destroyed by heating at 70°C for ten minutes. The factor from soybeans responsible for liquefaction of starch was inactivated by raising the pH to 11.0 or lowering the pH to 2.0. The factor in soyflour was heat-labile and was easily destroyed by heating in atmospheric steam for five minutes (Ofelt et al., 1955). Learmonth and Wood (1960) pointed out that the activity of the starch liquefying factor in raw soya diminished rapidly with raising temperature and with falling pH, in contrast to that of wheat malt. The consensus seems to be that soybean seeds are a good source of beta-amylase; the data obtained by Ofelt et al. (1955) point to a fairly rich level of a starch-liquefying agent.

Bailey et al. (1935) suggested that the levels of amylolytic enzymes in soyflour were high enough to make them functional diastatic supplements. Bohn and Favor (1945) reported that including 10 parts soya flour per 100 parts wheat flour had an effect on gassing power similar to the use of 1% commercial malt 20° Lintner. The data presented by Simon and Melnick (1950) showed that the beta-amylase activity of bakery type or of optimally

heated soyflour was small. Only one sample out of 20 tested, in a survey of commercial soyflours by Ofelt et al. (1955), had a liquefying action. The authors, therefore, concluded that heat treatment normally applied to soyflours in commercial processing was sufficient to destroy the factor responsible for liquefying starch pastes. Learmonth and Wood (1960) similarly concluded that the alpha-amylase activity of raw soya was not significant in commercial breadmaking.

Ham and Sandstedt (1944) and Bowman (1944) discovered the trypsin inhibitor, a substance that retarded the digestion of protein by trypsin in vitro. This seemed to explain satisfactorily the loss of sulfur amino acids in digestion and also to explain the low nutritional value of the raw soybean. Kunitz (1946) isolated and crystallized the trypsin inhibitor from soybeans (SBTI). More recently, Rackis et al. (1959) isolated a second SBTI which had an antitryptic activity about 60% greater than the Kunitz inhibitor. Liener (1951) tested both crude and crystalline inhibitors on rats and found that the crude preparation was toxic; the crystalline inhibitor injected at an equally high level of activity failed to show toxicity. Chernick et al. (1948) and Lyman et al. (1957) reported that feeding either crude SBTI or raw soybean meal enlarged the pancreas and greatly stimulated its activity. They measured the activity of trypsin, pepsin, lipases, and amylases in the gut after feeding diets containing raw and heated meal. For a very brief period after food intake, the activity of the trypsin in the gut was reduced,

but this low tryptic activity was soon followed by increased secretion of enzymes so that after six hours, the secretion of trypsin was three times greater than normal. The lipases and amylases were three to four times more than normal, but the pepsin was unaffected. They concluded that it would seem unlikely that in the rat, growth inhibition could result from insufficient intestinal proteolysis. Lyman (1957) reported that less nitrogen was ingested by rats fed raw meal and that the raw meal nitrogen was not as well utilized as nitrogen from heated meal. Lyman concluded that the inhibiting action might be exerted through a loss of essential amino acids from endogenous sources rather than depression of normal intestinal protein hydrolysis.

The works of Borchers and Ackerson (1950) and Smith (1961) showed that the effect of the inhibitor on growth appeared to disappear at high levels of protein intake although in these high protein diets the protein efficiency ratio was low. Brambila et al. (1961) also reported a slight growth depression when chicks were fed a crystalline soybean trypsin inhibitor, but the depression was not nearly as great as that obtained with 15% raw soybean meal. Jensen and Saxena (1963) concluded that although trypsin inhibitors in raw soybeans might have some physiological effects on birds, they apparently did not account for the major part of growth depression. Fisher et al. (1957) obtained equal egg production with hens fed either raw or heated soybean meal provided the diet was properly supplemented with methionine and vitamin B<sub>12</sub>.

Bielorai and Bondi (1963) found an inverse relationship between the extent of proteolysis occurring in pancreatic digests of certain plant protein feeds and the amount of trichloroacetic acid (TCA) precipitable nitrogen present in these digests. They used the method that the nitrogen compounds resistant to digestion were characterized by their precipitability with TCA to study trypsin inhibiting factors, and found that the solutions obtained by acid-extraction of soybean protein had no inhibiting action on proteolysis when added to casein. They also found that heavy precipitates were obtained by the addition of trichloroacetic acid to pancreatic digests of raw soybean meal. The amount of these precipitates decreased considerably when treatment of raw soybean meal lead to inactivation, or removal of antitryptic factors preceded the digestion, as was done in toasting or extraction with dilute acid.

Liener (1953) purified a hemagglutinin from raw soybeans and showed that this substance (once called soyin; different from the term "soyin" used by Willstätter (1926) to designate the proteolytic enzymes of soybeans) depressed rat growth. Smith (1961) could not confirm rat growth inhibition by hemagglutinin.

Potter and Kummerow (1954) contended that part of the improvement in the nutritive value of soybeans effected by heat was due to the destructive hydrolysis of the toxic saponin, soyasapogenol.

## Methods for the Measurement of the Heat Treatment of Soyflour

Various methods and techniques have been developed for in vitro testing of the efficiency or extent of heat treatment received by soyflour during processing. Obviously, the aim is to develop a quick index to the nutritive value of soyflour on the basis of the correlation between heat treatment and biological value. Some methods can be used only for revealing the degree of overheated but not for underheated samples. Some methods can be used for a wide range covering underheated, optimum, and overheated soyflour; but those methods usually are time consuming and complicated. A simple method to determine the effect of heat treatment on the nutritional value of soyflour is still badly needed.

Animal Growth Test. Chicks and rats were used by several workers to study the nutritive changes of heat-treated soybean meal. Hegsted et al. (1947) and Mitchell (1954) reported independently that man resembles closely the growing rat in his metabolic utilization of food proteins. They indicated that the results of rat growth tests were, on the whole, applicable to the evaluation of human diets. Several terms are used to indicate nutritive value of protein. In 1909, Osborne, Mendel, and Ferry introduced the concept of Protein Efficiency Ratio (P.E.R.) which was the grams gain of animal per gram protein consumed. In 1953, Bender and Miller described a method of estimating Net Protein Value and the term, Net Protein Utilization (N.P.U.) was defined as:

$$\text{N.P.U.} = \frac{\text{Body N of test group} - \text{body N of non-protein group}}{\text{N consumed by test group}} + \frac{\text{N consumed by non-protein group}}{\text{N consumed by test group}}$$

Microbiological Method. The need for a simple, rapid, and inexpensive method for protein evaluation had served to focus attention on the possibilities of using microbiological assays. A variety of methods had been proposed.

Protozoa. Several methods made use of the ciliated protozoan *Tetrahymena*. Campbell (1960) reported that use of the protozoan offered the advantage that the tested samples did not require hydrolysis prior to assay. Good correlations with animal assays were reported, however, for only a limited number of protein foods. Ford (1960) pointed out that the procedures used in protozoan assays were unfamiliar to most microbiologists and chemists, and complex compared with usual microbiological assays. In a large scale study of methods, Bunyan and Price (1960) found difficulty in obtaining agreement between replicates, using protozoans as test organisms.

Bacteria. Halevy and Grossowicz (1953), using a strain of *Streptococcus faecalis*, measured the nutritive value of egg albumin, gelatin, gluten, and zein in relation to casein following in vitro digestion of the proteins with a crude preparation of pancreatic proteinases. A simple method of protein evaluation was also described by Teeri et al. (1956) with *S. faecalis* No. 9790 in a medium in which the amino acids were supplied only by enzyme hydrolysates of proteins. *Leuconostoc mesenteroides* P-60

was used by Horn et al. (1954) to determine the effect of heat treatment on the nutritive value of cottonseed proteins after enzyme and acid hydrolysis. In these tests it was assumed that the growth of the test organism was dependent upon the pattern of amino acids available in the protein hydrolysate. Although results for a limited number of proteins agreed with literature values determined by animal assay, it was not shown that they could be used as general procedures to evaluate the proteins in natural foods. Rogers et al. (1959) compared results by these methods with P.E.R. estimations on a series of foods. With S. faecalis 9790, autolysis occurred in media containing hydrolysates of proteins deficient in lysine, and erratic results were encountered. Bunyan and Price (1960) compared results obtained with the micro-assay method with N.P.U. determinations and found no correlation between the methods on a large series of meat meals. With L. mesenteroides P-60, growth was influenced only by the most limiting amino acid relative to the growth requirements of the test organism. Since the same amino acid was not always limiting in sample and standard, erratic results were sometimes obtained.

Recently, Ford (1960) reported detailed studies on a comparison of assays using Streptococcus zymogenes with biological assays of protein quality. He found that this organism did not have an absolute requirement for lysine and suggested that S. faecalis rather than S. zymogenes be used for evaluating proteins in which lysine is the limiting amino acid. Although

there was good correlation between assays by the latter organism and N.P.U. data, nevertheless, it was unlikely that the cystine-methionine interrelationship for this organism was quantitatively similar to those in higher animals. This was suggested by the fact that casein was rated 100 while whole egg was rated 90 (Campbell, 1961). The test was rather lengthy, and procedure needed additional standardization of testing conditions in order to obtain more reproducible results as well as elimination of erratic determinations from interfering components of the soyflour.

Available Methionine Test. Ascarelli *et al.* (1962) proposed that the amount of methionine in the filtrate of an enzymic digest was an indication of the availability of this amino acid, and by employing Horn's method (1946) he found that heating of soybean meal for 15 min. at 120°C doubled the amount of available methionine. Heating for an additional period of 15 min. again increased the available methionine by the same amount, while further heating neither improved nor decreased the availability of the methionine.

Antitryptic Factor - Activity. Ham and Sandstedt (1944) extracted the factor by a solution buffered at pH 4.2. Ascarelli (1962) modified the test by diluting the extract and measuring the activity of inhibition exerted on the digestion of casein by crystalline trypsin. The extent of digestion was measured by the increase in absorption at 280 m $\mu$  of the filtrate obtained after precipitation of protein by trichloroacetic acid. A similar

method had been used by Bielcorai (1963) for studying the activity of antitryptic factors.

The results showed that heating of soyflour resulted in inactivation of antitryptic factors.

Urease Activity. Since Caskey and Knapp (1944) reported that urease activity decreased as heating of soybean meal increased, urease was considered as a useful measurement of heat treatment of soyflour. Boyn et al. (1961) showed there was no evidence of a simple relationship between urease activity values and the result of gross protein value, nor was there any suggestion that the soybean meals giving medium urease values were of highest nutritive value as had been suggested in earlier work (Croston et al., 1955). Ascarelli (1962) also showed that most of the urease activity was destroyed during the usual industrial oil extraction even without toasting.

Amylase Activity. Pomeranz and Linder (1960) showed that  $\alpha$ -amylase activity, measured with the Brabender Amylograph, could not be used to follow heat treatment of soyflour.

Solubility. Evans and St. John (1945) studied the changes in solubility of protein fractions of solvent-extracted soybean meal which result from wet heat treatment. By the method of Lund and Sandstrom (1943), the proteins of soybean meal samples were fractionated into albumins (water-soluble fraction), globulins (fraction soluble in 5% potassium chloride), prolamine (fraction soluble in 70% ethanol), glutelins (fraction soluble in 0.2% potassium hydroxide), and residual protein (fraction not

dispersible in any solvent used). Their work showed that observed changes in solubility resulted from progressive increases in severity of heating. It was evident that moderate heating tended to reduce the quantity of "albumin" and that much of this fraction appeared to be displaced into "residual protein" and "glutelin" fractions. Heating of greater severity appeared to interfere seriously with the dispersibility of the proteins in alkali as evidenced by progressive reduction in quantity of "glutelin" and increase in "residual protein." Thus, the effect of heat treatment of raw soybean meal on its content of alkali soluble protein seemed to parallel roughly its action on protein quality of soybeans; i.e., moderate heating increased, and severe heating decreased protein quality. The apparent persistence of a water-soluble, heat-stable "albumin" component of soy probably was related to the observation that from 4 to 8% of the total nitrogen of fat-free soybean meals was non-protein. The weight gain on the supplemented basal diet of chicks was subtracted from the gains on the other diets, and the additional gain in weight per unit of supplementary test protein was expressed as a percentage of the extra gain in weight per unit of supplementary casein protein to give the Gross Protein Value. With soybean and sunflower seed meals, none of the solvent systems tested yielded a satisfactory correlation with Gross Protein Value. Boyne et al. (1961) reported similar results.

Pomeranz and Lindner (1960) proposed to measure changes in protein solubility during heating soybean meal by determination of

the refraction index of dilute sodium hydroxide extracts of meals. These authors pointed out that "the test seems attractive as a screening test because of simplicity and speed." Later, Dangouman and Debruyne (1962) reported similar results.

Dye Absorption. The ability of soybean meal to absorb dyes containing a phthalein group had been utilized by Frölich (1954) to predict the quality of the food protein. Ascarelli et al. (1962) carried out the test with cresol red according to the modification of Olomucki and Bornstein (1960) and reported that there was very good agreement between the growth obtained in the biological test and the amount of dye absorbed by soybean meal. Binding of cresol red increases with heat treatment; the increased capacity of binding is probably due to an increased availability of amphoteric groups resulting from increased protein denaturation. Udy (1956) used Orange G (1-phenylazo-2-naphthol-6, 8-disulfonic acid sodium salt) in the determination of the protein content of wheat. Orange G, under acidic conditions, binds specifically free amino groups, the imidazole group of histidine, or the guanidyl group of arginine, provided they are in a free or dissociated state (Fraenkel-Conrat and Cooper, 1944). Moran et al. (1963) reported that differences in the binding capacity of soybean meal were caused primarily by differences in the availability of the epsilon-amino group of lysine, and they also reported that the dye binding capacity of soybean meals heated for varying periods of time was closely correlated with growth of chicks fed the meals.

Graham et al. (1949) developed a test which involved fluorophotometric comparisons between phosphate-buffer extracts of soybean meal samples and a standard solution of quinine sulfate. The quantity of soluble fluorescent materials in meals apparently was influenced by the severity of heat treatment. Balloun et al. (1953) reported that this method was an adequate means of detecting overheating in soyflour, but of little value with screw-pressed products.

Loska and Melnick (1950) reported that salts of strong acids such as calcium chloride, magnesium chloride, ferric chloride, and sodium bisulphate were effective agents for the precipitation of soy proteins. Hydrochloric acid was also an effective curdling reagent. Maximal yields of soy curd, using magnesium chloride as the precipitating reagent, occurred at pH 5.8, whereas isoelectric precipitation using hydrochloric acid was at pH 4.5. Their results showed the curd volume was an accurate index of the percentage of soluble protein in the soyflours which were subjected to various heat treatments.

## MATERIALS AND METHODS

### Soyflour Samples

Seventeen samples of soyflour were obtained from four manufacturers. The samples varied in protein content from 46-53%, and in protein solubility (as % of total protein) from 8-90%. The samples were believed to be typical of products marketed for use

in food industries. Only two of the tested samples (both from one manufacturer) were active in hydrolyzing pregelatinized wheat starch to sugars fermentable by baker's yeast, and in reducing the viscosity of pregelatinized waxy maize starch. In addition to commercial samples, one of the enzymatically active soyflours containing 90% soluble protein was heated in the laboratory for 5-240 minutes either in an oven at 120°C or in an autoclave at 15 p.s.i.

#### Measurements

Urease Activity. Urease activity was determined according to A.O.C.S. Tentative Methods (1946).

Fluorescence Value. The method used was a modification of the procedure of Cole and Milner (1953) as proposed by Linko and Sogn (1960). One g. of soyflour was weighed into an Erlenmeyer flask containing 25 ml. of 0.2 M hydrochloric acid. The contents were shaken by hand four times at 15-minute intervals and allowed to stand overnight at 25°C. After filtering through Whatman No. 5 paper the clear solutions were diluted 20 times, and used for fluorescence determination. Measurements were made with a Coleman Photoelectric Fluorimeter with B<sub>1</sub>-S and PC-1 filters; the instrument was standardized to read 60 with 0.1 p.p.m. sodium fluorescein solution.

Color Measurement with Reflection Meter. Measurements were made on samples of soyflours placed into depressions of a spot plate and flattened with a spatula.

A photoelectric reflection meter Model 610 (Photovolt Corporation, N.Y.) was used. The instrument was standardized according to the manufacturer's instruction manual. Samples were tested by placing the searching unit of the instrument on soyflour in the spot plate cavity. Three measurements were taken for each sample, employing in each case the green, blue, and amber tristimulus filters.

Color Measurement with Agron. Reflectance measurements were made according to the procedure described by Gillis (1963). Two measurements were taken for each sample. In the first, the standard plate was employed for the range of 0-95; in the second, the scale range 52.5-85 was used for more accurate and sensitive measurements.

Determination of Protein Solubility in Water. Two g. of the soyflour sample were suspended in 15 ml. of distilled water in a stoppered Erlenmeyer flask and shaken for one hour in a laboratory shaker. The contents of the flask were centrifuged for 20 minutes at 1,500 r.p.m. Five ml. of the supernatant solution were taken for the determination of nitrogen content according to the A.A.C.C. Method (1962). The values obtained were then calculated, and the protein solubilities of the soyflour samples were expressed as % of soluble nitrogen content/total nitrogen content.

Cresol Red Absorption Test. Dye absorption of cresol red by soyflour was determined according to the method by Olomucki and Bornstein (1960).

Gassing Power of Soyflour. The starch used as substrate in

gassing power studies was pregelatinized wheat starch from Stein, Hall and Company, Inc., New York, N.Y.

The beta-amylase was purchased from Wallerstein Laboratories, Staten Island, N.Y.; the wheat malt alpha-amylase had an activity of 414 SKB units per g. (Sandstedt et al., 1939).

The nitrogen base medium for fermentation tests with yeast (in experiments with pregelatinized wheat starch) was prepared in the laboratory according to the formula in the Difco Manual (1953).

Yeast suspensions were made by suspending 7.5 g. of Fleischman's wet cake baker's yeast in 95 ml. of a 2% NaCl solution.

The wheat or alpha-amylase was added as a filtered suspension in 0.2% CaCl<sub>2</sub> solution.

One of the flour samples used was experimentally milled on a Bühler mill from a composite grist of several hard winter wheat varieties grown at a number of locations through the Great Plains in 1962. The composition of the flour on a 14% moisture basis was 0.37% ash and 12.9% protein. Additionally, four samples of wheat were milled on a "Miag" Multomat mill; their characteristics are shown in Table 1.

For gassing power determination with pregelatinized wheat starch, 10-g. mixtures of starch and soyflour were weighed in one side of the aluminum cup of the Sandstedt-Blish pressuremeter (Sandstedt et al., 1939). At one-minute intervals 5 ml. of nitrogen base medium, 5 ml. of yeast suspension, and 15 ml. of water were added. The consistency of the past was comparable to

Table 1. Characteristics of the flour samples milled on a Miag Multomat mill.

Wheat	: :Extraction: : %	: : Ash : %	: Protein : content :(N x 5.7)	:Parinograph :absorption : %
Hard Red Winter	73.2	0.43	10.7	66.4
Soft Red Winter	70.9	0.36	9.1	66.0
Northern Spring	72.7	0.44	13.1	64.6
Durum	67.0	0.56	11.9	63.6

that of a wheat dough in breadmaking. For gassing power determinations with wheat flour, 10-g. mixtures of the wheat flour and the soyflour were mixed with 5 ml. of yeast suspension and 2.5 ml. of water. The contents of the aluminum cup were mixed well prior to tightening the lids, and the pressuremeters placed in a water bath at 30°C. After five minutes the pressure was released, and five pressure readings were taken at one-hour intervals.

Amylograph (Measurement of Amylase Activity). The starch used as the substrate to measure viscosity changes was Amaizo 721-A pregelatinized waxy starch from American Maize Products Company, New York, N.Y.

Measurement of Starch Liquefying Properties of Soyflour Amylases. Thirty-three and five-tenths g. of pregelatinized waxy maize starch were added slowly to 320 ml. of 0.002 M phosphate buffer solution pH 5.3 and mixed for two minutes by a laboratory stirrer at 1450 r.p.m. in a beaker.

The apparently homogenous dispersion was transferred with the aid of 120 ml. of additional buffer solution to an Amylograph bowl. The Brabender Amylograph was employed to measure changes

in starch viscosity as a result of amylase action. The instrument bowl was operated at 75 r.p.m.; the enzymatic action was measured at 37° C. After the starch was transferred to the Amylograph bowl, the instrument was operated for ten minutes for temperature equilibration. Then the soyflour was added, and the viscosity was recorded for 20 minutes.

Microbiological Assay. The method of Ford (1960) was used. The growth responses of S. *zymogenes* were determined by measuring the optical densities of the cultures in the tubes at 580 m $\mu$  with a Bausch and Lomb Photocolorimeter.

The growth response of bacteria on soyflour digests was computed after subtraction of turbidity of non-inoculated tubes. The average growth response per tube was compared with the average response of casein digests (as 100%) passed through the analytical procedure.

Curd Sedimentation Values. The curd forming capacity of heat-treated soyflour was measured according to the modified Zeleny's Sedimentation Test for wheat flour (Pinckney et al., 1957). The reading was taken after standing for 90 minutes.

## RESULTS AND DISCUSSION

The effect of heat treatment on the urease activity of soyflour is shown in Table 2. Since the increase in pH is directly proportional to urease activity of the soyflour, the increase in pH is used to indicate the urease activity. The results shown in Table 2 indicate  $\Delta$  pH values of 1.2 to 0.1 for the autoclaved

Table 2. The effects of length and kind of heat treatment on the urease activity of soyflour.

Heating period (min.)	Urease activities of soyflour <sup>1</sup>	
	Autoclaved at 15 p.s.i.	Heated in oven at 120°C.
0	1.2	1.2
5	1.1	1.2
15	1.1	1.1
30	1.1	1.1
60	1.0	1.0
120	0.35	1.0
240	0.20	0.8
480	0.10	0.25

<sup>1</sup> Expressed as  $\Delta$  pH.

samples heated from 0 minute to 480 minutes and  $\Delta$  pH values of 1.2 to 0.25 for the oven-heated samples. There are no significant differences among samples heated between 0 minute to 30 minutes for either autoclaved or oven-heated samples. After 60-minutes heat treatment there is a rapid decrease in urease activity. The decrease in urease activities for autoclave-heated samples is faster than that of oven-heated samples as the result of the moisture present during the autoclaving. This poses the problem as to whether or not, the decrease in urease activities in moist-heated samples is consistent with the decrease of the biological value of the samples.

According to Bird et al. (1947), an increase in pH of 0.3 or less indicates that the soyflour has a slight urease activity but has been sufficiently heat treated for good nutrition. A pH change above 0.3 is generally shown by soyflours either unheated or slightly heated.

The value of urease activity in assessing heat treatment of soyflours is limited. Urease activity varies with soybean varieties and processing during oil extraction. Additionally, length and conditions of storage of either the beans or the processed flour, which are totally unrelated to heat processing but which may significantly affect enzymatic activity, are liable to alter urease levels in soy products. It is generally accepted that high urease activity is indicative of under treatment, but that the test is of little value in the determination of excessively heated samples.

The effects of length and kind of heat treatment on the fluorescence values of soyflour are shown in Table 3. The fluorescence values of autoclaved soyflour increased rapidly after 60-minutes heat treatment; and that of oven-heated increased slowly after 15-minutes treatment. For both cases, there were significant increases of fluorescence values after 60-minutes heat treatment. But there were no differences among those of slightly heated samples. Therefore, the fluorescence values of heat-treated soyflour cannot be used to indicate slightly heated, mildly heated, or properly heated soyflours. The test may be used to indicate severely heated (over-heated) soyflour. This is in agreement with the report of Balloun *et al.* (1953) who found that fluorescence values were adequate in indicating overheated soyflour, but had no merit as a criterion for determining whether a sample was under-heated or has received adequate heating for optimum nutritional value. The presence of moisture during

Table 3. Effect of length and kind of heat treatment on the fluorescence values of soyflour.

Heating period (min.)	Fluorescence value of soyflour	
	Autoclaved at 15 p.s.i.	Heated in oven at 120°C.
0	23	23
5	24	23
15	24	27
30	24	
60	24	33
120	35	37
240	49	39
480	90	51

heating (autoclaving) had a marked effect on the formation of fluorescent substances in treated soyflour. It is well established that the oil extraction procedure employed and the particle size, age, and previous history of the tested sample may affect the results of the fluorescence determination. It is, therefore, essential that the test be carried out under standardized conditions and that only soyflours obtained from similarly oil-extracted meals be compared.

To obtain an objective and visual appearance of the reproducible index of color, the heat-treated soyflours were compared with a reflection colorimeter. Tristimulus measurements employing the three filters supplied with the instruments were made, as shown in Table 4.

The blue filter appears to be the most appropriate as it shows highest sensitivity for detection of small differences among the slightly heat-treated samples.

Table 4. Comparisons of different filters in the measurement of the color changes of heat-treated soyflours with Reflection Model 610.

Length of autoclaving soyflour at 15 p.s.i. (min.)	Reflection value		
	Amber <sup>1</sup>	Blue	Green
0	89.0	73.0	86.0
5	88.0	70.0	85.0
10	87.5	69.5	84.0
15	85.0	65.5	82.0
20	84.0	65.0	81.5
30	85.0	65.5	81.5
60	70.0	44.5	66.0
120	50.0	25.0	47.0
240	34.0	11.5	27.0
480	26.0	8.0	21.0

<sup>1</sup> Colors of filters used.

Table 5 shows reflection measurements of samples varying in kind and length of heat treatment. The autoclaved samples showed a faster decrease of reflection values than oven-heated samples. Table 6 shows replicate determinations of four of each of the tested samples of soyflour. The small differences between replicate determinations seem to originate primarily from variations in smoothness of tested soyflour surfaces.

Table 7 shows color reflection values of heat-treated soyflour employing the Agtron instrument. This instrument is more expensive than the simpler reflection meter described previously. The Agtron has been designed for testing powdered substances, and the preparation of samples for testing has been fairly well standardized. As shown in Table 7, standardization with reflection plates 00-95 for the reading range of 0 to 100 gave a better

Table 5. Effect of kind and length of heat treatment on the color reflection of soyflour.

Length of heating (min.)	Reflection value			
	With blue filter		With green filter	
	Oven-heated: at 120°C.	Autoclaved: at 15 p.s.i.	Oven-heated: at 120°C.	Autoclaved: at 15 p.s.i.
0	70	70	85	85
5	70	68	83	82
10	66	66	81	82
30	62	64	78	77
60	59	56	75	73
120	54	44	72	64
240	53	31	72	52
480	51	19	71	36

Table 6. Replicate reflectance measurements.

Length of heating (min.)	Reflection values with blue filter					Standard error
	1	2	3	4	Average	
0	69.0	68.0	69.0	68.5	68.6	0.15
15	67.5	67.0	67.0	67.5	67.3	0.09
30	66.0	65.0	65.0	65.0	65.25	0.17
90	60.0	58.5	58.5	60.0	59.25	0.20
240	18.0	18.0	18.0	18.0	18.0	0.00

overall comparison of the heated samples than using reflection plates 52.5 and 85. For testing small differences in slightly heated samples, the use of the latter two plates seems more advantageous.

The results given in Table 7 were obtained from testing two sets of soyflours heated under what seemed to be the same experimental conditions. The results vary from one set of data to the other due to the difficulty of reproducing short heating times of small samples in the autoclave.

Table 7. Agron reflection of soyflour autoclaved at 15 p.s.i.

Length of : heat treat-: ment (min.):	Instrument reading			
	Experiment I		Experiment II	
	A <sup>1</sup>	B <sup>2</sup>	A <sup>1</sup>	B <sup>2</sup>
0	85	91.8	85	93
15	84	90.8	85	92.5
30	83	90.1	79	90
45	83	90.1	80	72
60	82.5	85	83	78.2
90	79	73	68	84
120	59	6	43.5	25.5
240	38.5	0	24	0
480	30.5	0	16	0

<sup>1</sup> Instrumental reading (of range 0 to 100) standardized with standard reflection plate 00 to 95.

<sup>2</sup> Instrumental reading (of range 0 to 100) standardized with standard plate 52.5 to 85.

Table 8 shows cresol red dye absorption values of the soyflour heat-treated samples. Dye absorption value increased with increasing length of heat treatment. Moran *et al.* (1963) interpreted the increased capacity to bind cresol red as due to an increased availability of amphoteric groups resulting from increased protein denaturation. The values obtained in Table 8 were from 0.825 to 3.45 for autoclaved, and 1.25 to 2.75 for dry heated samples. These values, in general, were slightly smaller than the values obtained by Olomucki *et al.* (1960) and Pomeranz and Lindner (1960). No consistent and significant differences in binding of Orange G, according to the method of Moran *et al.*, (1963) were recorded.

Changes in protein solubilities as a result of heat treatment of soyflours are shown in Table 9. Increasing length of heat

Table 8. Cresol red absorption of heat-treated soyflours.

Length of heating (min.)	Dye absorption <sup>1</sup>	
	Autoclaved at 15 p.s.i.	Heated in oven at 120°C.
0	0.825	0.825
5	0.825	1.25
15	0.825	1.25
30	1.25	1.25
60	1.70	1.45
120	2.16	3.05
240	3.45	2.40
480	3.45	2.75

<sup>1</sup> Expressed as mg./g. of soyflour sample.

Table 9. Water soluble protein of heat-treated soyflour.

Length of heat treatment (min.)	Protein solubilities <sup>1</sup>	
	Autoclaved at 15 p.s.i.	Oven-heated at 120°C.
0	90.0	90.0
5	77.5	73.8
15	91.1	82.5
30	93.0	71.3
60	43.6	46.3
120	20.0	30.0
240	6.3	27.5
480	13.8	17.5

<sup>1</sup> Expressed as % of total protein.

treatment decreases protein solubility both in autoclaved and in dry heated samples. Autoclaved soyflour shows more rapid decreases in protein solubilities than dry heated samples.

This method seems to show differences in extent of heat treatment of soyflour. The overall differences are similar to

those observed during urease determinations, but protein solubility seems to be a more sensitive index covering a wider range of heat treatment than urease. The urease test is, however, a simpler and faster test than the protein solubility determination.

The effects of adding various amounts of diastatically active soyflour to pregelatinized wheat starch on gassing power are summarized in Table 10, and on starch viscosity in Fig. 1.

Table 10. Effect of adding soyflour to pregelatinized wheat starch on gassing power.

Soyflour (%)	Pressure (cm. mercury)	
	After 2 hours	After 5 hours
0	1.7	2.3
0.5	14.4	53.7
1.0	17.7	66.5
2.0	19.0	76.4
3.5	20.5	86.5
5.0	22.9	98.9
7.5	23.8	102.4
10.0	23.9	103.9
15.0	24.9	108.5
20.0	25.1	108.9

A comparison with the gassing power of sucrose added to pregelatinized wheat starch is shown in Table 11.

Table 11. Effect of adding sucrose to pregelatinized wheat starch on gassing power.

Sucrose (%)	Pressure (cm. mercury)	
	After 2 hours	After 5 hours
1.6	20.4	25.1
4.0	30.7	40.1
6.0	34.7	55.6
12.0	30.7	85.9
24.0	34.3	105.9

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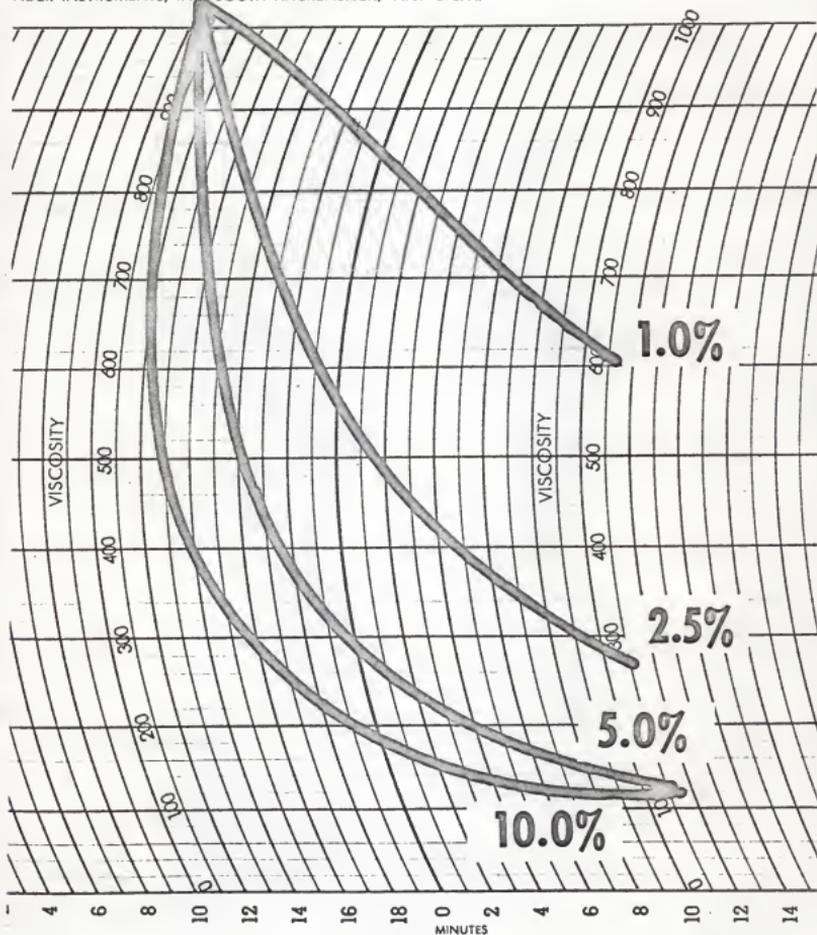


Fig. 1. The effect of adding various levels (% of starch) of enzymatically active soyflour on liquefaction of pregelatinized waxy maize starch.

As mentioned earlier, only two out of the 17 samples of commercial soyflours tested had a significant effect on gassing power when added to the pregelatinized starch. Whereas the addition of the heated soyflour samples resulted in increased pressuremeter readings, this increase remained stationary after the first hour, indicating a probable contribution of fermentable sugars from soyflour rather than an enzymatic breakdown of wheat starch.

The extremely high gassing power response from adding soyflour to pregelatinized wheat starch was a result of using an enzymatically available starch. The results summarized in Table 12 were obtained when employing wheat flour as substrate for the action of soybean amylases. The addition of 5% soyflour increased the levels of fermentable sugars in all the tested samples, except the durum flour. The increase was smaller than from pregelatinized starch, as a result of limited levels of available, mechanically damaged starch in wheat flours. This limitation does not seem to hold for the flour from the vitreous durum wheat which is more severely damaged during milling than the other tested flours.

Table 12. Effect of adding 5% soyflour to wheat flours on gassing power.

Flour	: Pressure (cm. mercury) <sup>1</sup>	
	:Without soyflour:	With 5% soyflour
RBS 62	20.1	31.6
Hard red spring	21.2	37.0
Hard red winter	31.3	38.3
Soft red winter	15.7	20.5
Durum	40.3	38.3

<sup>1</sup> Reading after 5 hours.

The effect of heat treatment on enzymatic activity of soy-flours is summarized in Table 13. When these results are compared with those in Fig. 2, showing the effect of autoclaved soyflour on starch viscosity, it is observed that the sample autoclaved for 30 minutes had a markedly reduced starch liquefaction capacity, but that the  $\beta$ -amylolytic action was practically unaffected. Heating for 60 or 120 minutes destroyed both amylolytic actions. A similar picture is obtained by comparing the results summarized in Table 14 with those shown in Fig. 3. In both cases, soyflours heated commercially to give a protein solubility range between 20-90% were used. Whereas the strongly heated soyflour (sol. protein 20%) had no amylolytic activity, the sample less severely heated had practically no reduced  $\beta$ -amylase activity, but a markedly lowered starch liquefying action. It seems, therefore, that it is possible to inactivate differentially the two amylases in soyflour by heat treatment, the alpha-amylase being more heat labile than the beta-amylase. This is in agreement with the findings of Ofelt *et al.* (1955) and of Newton *et al.* (1943) who reported a high heat lability of soybean alpha-amylase, and relative heat stability of the beta-amylase, respectively. With regard to the levels of alpha-amylase found, adding 10% enzymatically-active soyflour was equivalent in its liquefying action to adding about 4 SKB units from wheat malt (Fig. 4). Adding up to 20 mg. beta-amylase had no measurable effect. It should be noted, however, that the soyflour used in this study was commercially processed and that such processing might have resulted in partial heat

Table 13. Effect of heat treatment of soyflour on gassing power of pregelatinized wheat starch containing 10% supplement.

Length of treatment (min.)	Pressure (cm. mercury)			
	Dry heated soyflour (at 121°C)		Autoclaved soyflour (at 15 p.s.i.)	
	After 2 hr.	After 5 hr.	After 2 hr.	After 5 hr.
0	25.1	106.7	23.2	112.3
5	25.0	109.9	21.3	109.0
10	25.3	110.0	21.8	111.0
30	22.3	94.3	22.0	105.5
60	19.9	56.2	8.2	15.0
120	16.9	34.6	8.6	11.9
240	11.9	20.0	8.9	14.6
480	8.9	11.1	5.8	5.9

Table 14. Effect of adding 10% commercially heated soyflour samples varying in protein solubility on gassing power of pregelatinized wheat starch.

Protein solubility <sup>1</sup>	Pressure (cm. mercury)	
	After 2 1/2 hr.	After 5 hr.
90	35.5	105.1
40	36.1	104.5
20	15.1	23.3

<sup>1</sup> Expressed as % of total protein content.

inactivation of the labile alpha-amylase. Additionally, it is feasible that the liquefying action of soyflour added to native starch heated to about 90°C within 40 minutes apparently is higher as a result of the contribution of the relatively heat stable and highly potent soybean beta-amylase. The contribution of beta-amylase to the very rapid viscosity drop observed by using pregelatinized starch and a low temperature of 37°C, would be small.

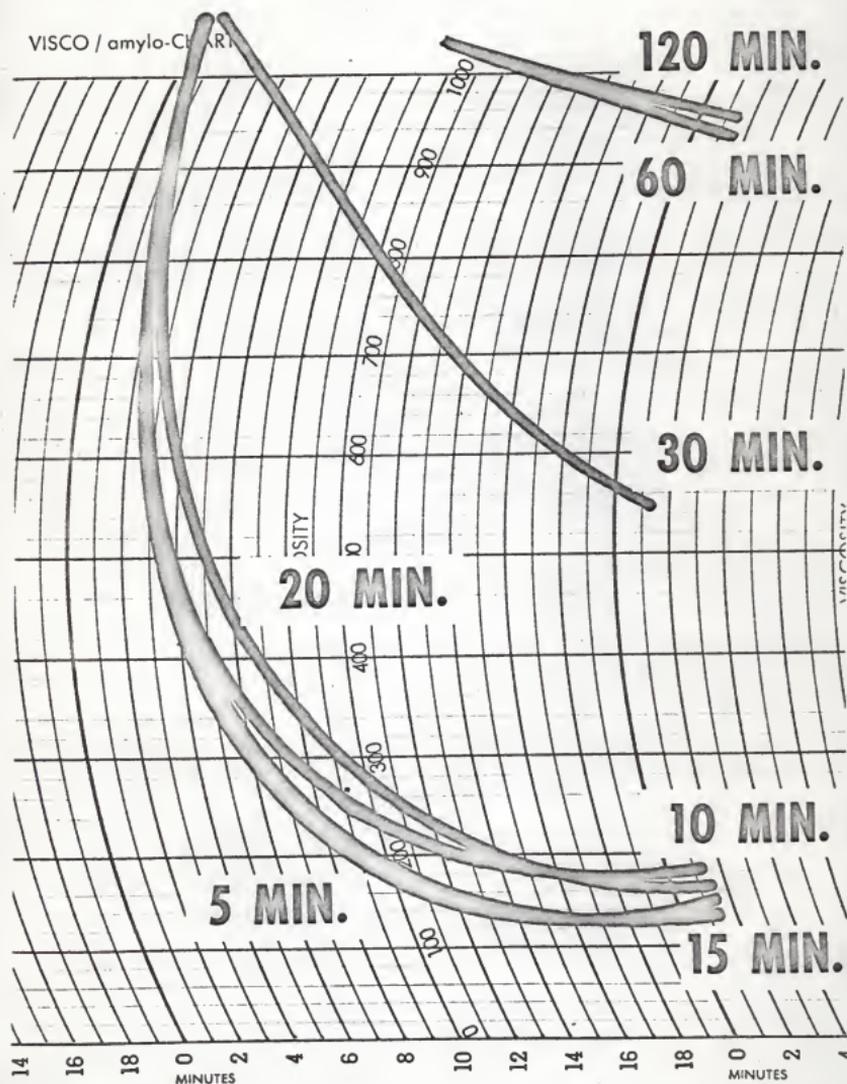


Fig. 2. Effect of the length of heat treatment (minutes) in an autoclave on starch liquefying action of soyflour (added at a 10% level).

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VISCO / amylo-CHART

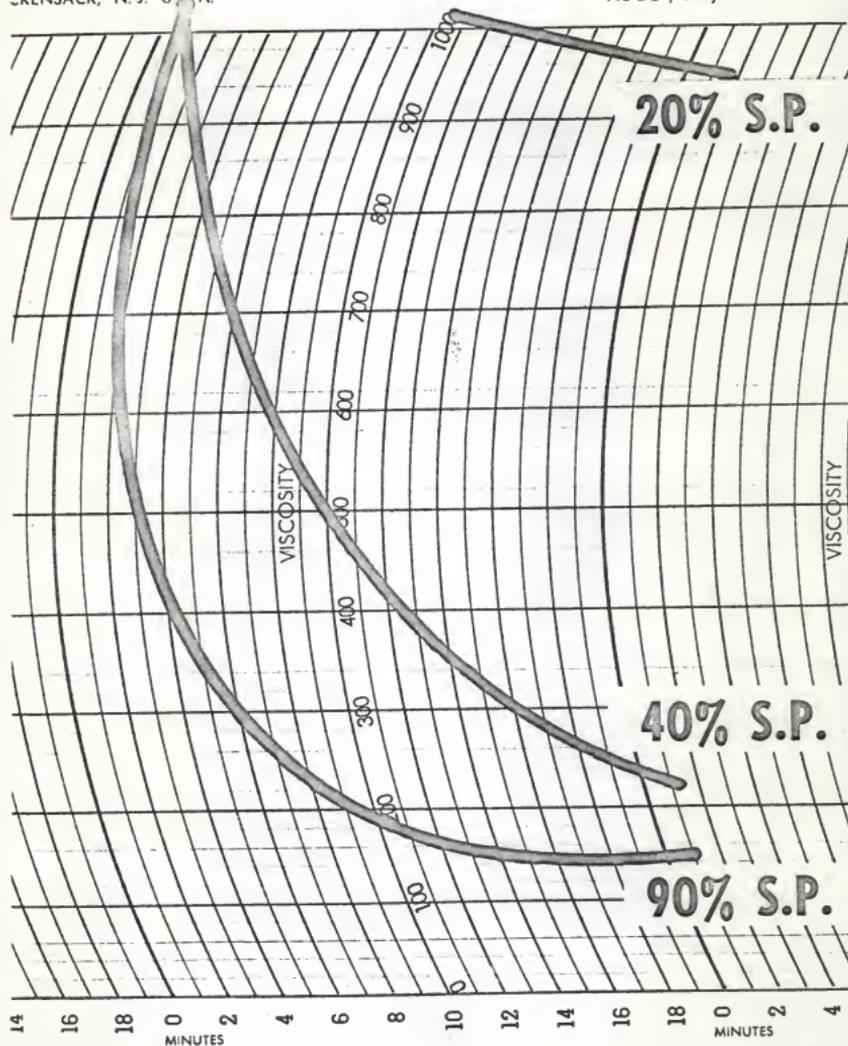


Fig. 3. Liquefying action of commercially processed soyflours (added at a 10% level) varying in protein solubility.

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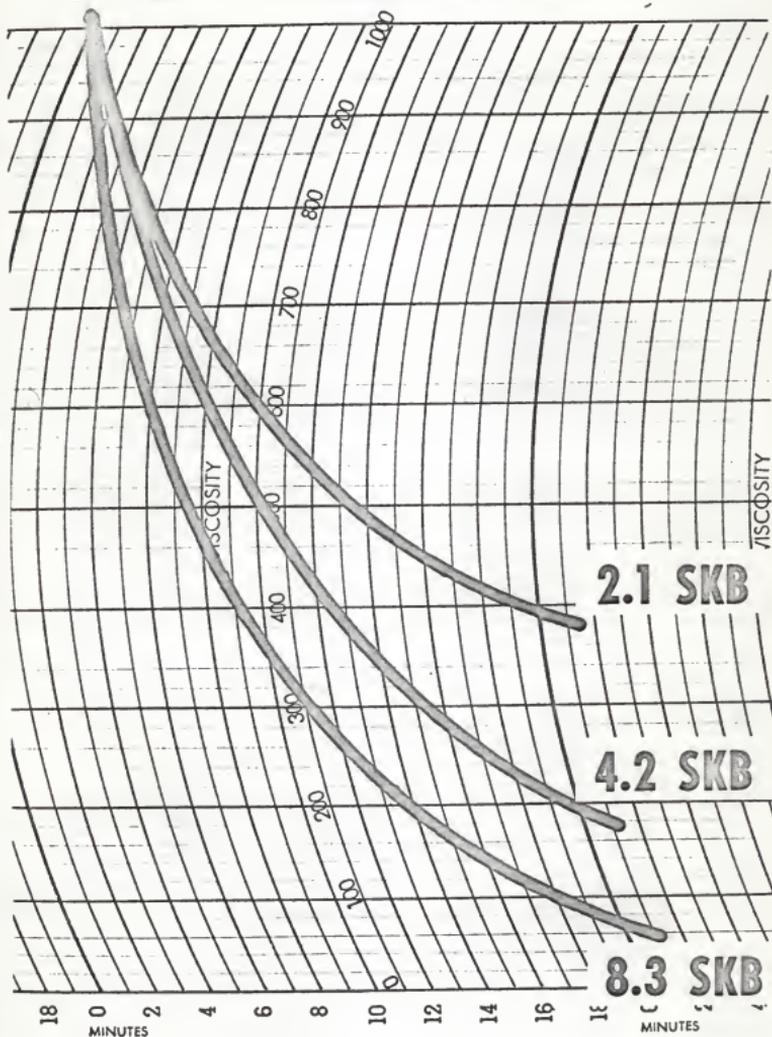


Fig. 4. The effect of adding various levels (SKB units per 33.5 g. of starch) of wheat-malt alpha-amylase on viscosity of pregelatinized waxy maize starch.

Preliminary experiments with acid hydrolyzed soyflours employing L. mesenteroides, S. faecalis 8043, or S. zymogenes 8055 as test organisms gave no differentiation between samples heat treated to a different extent. This seems to be due to the fact that the severe treatment (autoclaving 1.0 g. of product with 25.0 ml. of 2.0 N hydrochloric acid for five hours at 15 p.s.i.) tends to obscure any relatively minor differences resulting from toasting of soyflours. Employing enzymatically digested soyflours, only S. zymogenes showed a variation in growth response to the tested flours.

The growth response of S. zymogenes on papain digests of laboratory heated soyflours is shown in Table 15. Relative growth response value (R.G.V.) is defined as the % of optical density of tubes containing soyflour digests relative to that of casein digests. R.G.V. of autoclaved samples decreased more rapidly than those of dry heated samples. There was some improvement of nutritive value as a result of slight heat treatment by autoclaving as shown by an increased R.G.V. from 79.1% (0 min.) to 80.7% (15 min.). R.G.V. decreased abruptly as a result of over-heating.

Table 16 shows the R.G.V. of commercial heat-treated soyflour. The extent of heat treatment is illustrated by protein solubilities. The slightly heated commercial soyflour (70% S.P.) had the highest R.G.V. among the four soyflours. Toasting decreased the availability to the test microorganism substantially. The nutritive values of the isolated water dispersible

Table 15. Growth responses of S. zymogenes on papain digests of laboratory-heated soyflours.

Length of heat treatment (min.)	:Relative growth response value (R.G.V.) <sup>1</sup>	
	: Autoclaved at	: Oven-heated at
	: 15 p.s.i.	: 120°C.
	: (%)	
0	79.1	79.1
5	72.5	75.0
15	80.7	65.7
30	78.1	76.0
60	62.2	72.5
120	12.7	52.2
240	17.9	52.6

<sup>1</sup> Assuming casein as 100%.

Table 16. Growth responses of S. zymogenes on papain digests<sup>1</sup> of commercial soy products.

Product	:	:	:	Relative
				growth
			Water	response
	Total	:dispersible	:	value
	protein	protein <sup>2</sup>	:	(R.G.V.) <sup>3</sup>
			(%)	
Soyflour-unheated	50	90		79.1
Soyflour-sl. toasted	50	70		96.6
Soyflour-toasted	50	35-45		74.8
Soyflour-db. toasted	50	8-20		68.1
Isolated soya protein	90	-		38.4
Sodium proteinate	90	100		38.4
Deflavored 70% protein	70	-		72.1

<sup>1</sup> Containing equal amounts of total protein.

<sup>2</sup> As % of total protein.

<sup>3</sup> Assuming casein as 100%.

protein and non-dispersible protein were substantially lower than the R.G.V. of soyflours. Desugared protein (Protein 70) was comparable to soyflour.

The results summarized in Tables 15 and 16 were obtained by using liquid media prepared in the laboratory according to the procedure outlined by Ford (1960). As the preparation of such media is lengthy and requires maintenance of stocks of a large number of chemicals, attempts were made to employ a dry medium containing all the necessary nutrients. Such media dissolved in appropriate amounts of water are commonly employed in microbiological assays of vitamins or amino acids. Comparison of results obtained by testing a series of soyflour samples employing the laboratory compounded and dehydrated (experimental batch prepared by Difco Laboratories, Detroit) media are shown in Table 17. From these results it would seem that both media give comparable values, but that the range and sensitivity of the liquid medium were larger.

Table 17. Growth responses of *S. EVMOGENES* on papain digests of laboratory-heated soyflour's employing different media.

Sample No.	Relative growth response value (R.G.V.) <sup>1</sup>	
	Dry medium	Liquid medium
1	56.3	79.1
2	29.4	5.9
3	72.0	117.0
4	62.5	86.9
5	78.1	169.0

<sup>1</sup> Assuming casein as 100%.

In both media, but especially in the dehydrated dry medium, it is essential to run for each sample and at all levels of substrate, an uninoculated control, and compute the results of turbidity by difference.

The results of curd sedimentation tests are shown in Plate I and Table 18. No sedimentation occurred in the unheated and slightly heated soyflours. Heating beyond 45 minutes in an autoclave at 15 p.s.i. decreased the volume of sedimentation appreciably. The differences seem to result from changes in water-imbibition capacity of soyflours which have undergone various degrees of heat treatment. This simple method seems to be a good test for the detection of overheated soyflours; it is of little value, however, in differentiation between mildly heated samples. Attempts to increase the range of sedimentation values and sensitivity of the test in the region of mild heat treatment by employing a number of reagents; i.e., quaternary ammonium compounds, anionic detergents, and basic solution were unsuccessful. It is interesting to note the variation in foam formed above the samples; heat treatment reduced this capacity of foam formation as well as surface tension of the solution as determined by surface tension measurements employing capillary tubes.

EXPLANATION OF PLATE I

Curd sedimentation of heat-treated soyflour.  
The picture was taken after a standing time  
of 90 minutes.

## PLATE I



Table 18. Curd sedimentation of heat-treated soyflour.

Cylinder No.	Length of heating (min.)	Sedimentation volume <sup>1</sup> (ml.)
1	0	74
2	15	74
3	30	74
4	45	73
5	60	51
6	120	24
7	240	18
8	480	16

<sup>1</sup> Values of sedimentation were taken after a standing time of 90 minutes.

#### SUMMARY AND CONCLUSIONS

Urease activity and protein solubility decreased with the length of heat treatment of soyflours. Autoclaving at 15 p.s.i. lowered the enzymatic activity and protein solubility in water at a faster rate than dry heating in the oven at 121°C. Both tests are well suited to follow mild heat treatment; protein solubility varies over a wider range of heat treatment; and the urease activity method is preferred due to its simplicity. The fluorescence test can be used primarily to detect over-heated samples. Reflectance tests, employing a reflectance colorimeter or the Agron instrument, have been found as simple, rapid, and non-destructive tests for heat treatment covering a wide range of heat treatment. An increase in the absorption of cresol red paralleled the length of heat treatment. No significant change in Orange G absorption of heated soyflours was recorded.

Two samples of commercially processed soyflour had a high amylolytic activity with regard to production of fermentable sugars by baker's yeast, and to reduction of viscosity of pregelatinized starch as measured in the amylograph. Enzymatically active soyflours heated commercially or in the laboratory had lowered amylolytic activities, but mild heat-treatment inactivated the saccharifying enzyme to a smaller extent than it inactivated the starch liquefying enzyme. Adding 10% soyflour reduced the viscosity of pregelatinized starch to an extent comparable to adding 4 SKB units from wheat-malt alpha-amylase.

Growth of S. zymogenes, as measured by the turbidity produced by the test organism grown on papain digests of soyflour samples, was increased by mild heat treatment and decreased in overheated samples. The volume of sedimentation of soyflour mixed with an acid solution, the surface tension of the solution, and the foam above the solution decreased as a result of heat treatment of the tested soyflours.

#### SUGGESTIONS FOR FUTURE WORK

The purpose of this work was to investigate the possibilities of devising simple and rapid indices of heat treatment of soyflour, correlated to changes in biological value of the flours. The results obtained have suggested several additional areas for future research.

It seems feasible that useful information might be obtained by following physical or physico-chemical changes; i.e., surface

tension in soyflours undergoing various heat treatments.

Fractionation of soyflours by the use of a series of solvents varying in polarity might elucidate the components responsible for water imbibition, visual browning and fluorescence, and changes in nutritional value. Use of modern techniques; i.e., starch gel electrophoresis, ultracentrifugation, and molecular sieves might be helpful in following the changes occurring in heated soyflour proteins.

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by

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and Feed Milling Industries

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Urease activity and protein solubility decreased with the length of heat treatment of soyflours. Autoclaving at 15 p.s.i. lowered the enzymatic activity and protein solubility in water at a faster rate than dry heating in the oven at 120°C. Both tests are well suited to follow mild heat treatment; protein solubility varies over a wider range of heat treatment; the urease activity method is preferred due to its simplicity. The fluorescence test can be used primarily to detect overheated samples. Reflectance tests employing a reflectance colorimeter or the Agtron instrument have been found as simple, rapid, and non-destructive tests for heat treatment of soyflour, covering a wide range of heat treatment. An increase in absorption of cresol red paralleled the severity of heat treatment. Enzymatically active soyflours heated commercially or in the laboratory had lowered amylolytic activities, but mild heat treatment inactivated the saccharifying enzyme to a smaller extent than it inactivated the starch liquefying enzyme. Adding 10% soyflour reduced the viscosity of pregelatinized starch to an extent comparable to adding 4 SKB units from wheat-malt alpha-amylase. Growth of Streptococcus zymogenes, as measured by turbidity produced by the test organism grown on papain digests of soyflour samples, was increased by mild heat treatment and decreased in overheated samples. The volume of curd sedimentation of soyflour mixed with an acid solution, the surface tension of the solution, and the foam above the solution decreased as a result of heat treatment of the tested soyflour.