THIS BOOK IS OF POOR LEGIBILITY DUE TO LIGHT PRINTING THROUGH OUT IT'S ENTIRETY.

THIS IS AS RECEIVED FROM THE CUSTOMER.

FACTORS AFFECTING STARCH DAMAGE AND ITS MEASUREMENT IN EXTRUSION COOKED CEREALS

by

KEITH CHARLES BEHNKE

B.S., Kansas State University, 1968

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCES

Department of Grain Science and Industry

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1973

Approved by:

Major Professor

TABLE OF CONTENTS

INTRODUCTION

A great deal of interest has been shown in the last several years by the feed industry in new methods of processing to obtain products of higher digestibility and better efficiency. An example of this is the interest shown in steam flaking, dry roasting, micronizing and high moisture fermented grain. One particular form of processing that has been used for years in the pet food and fish food industries is that of extrusion cooking.

Recently, the extrusion process has been gaining attention in the field of livestock feed production. The reason for this interest is the fact that, as animals are pushed to their genetic production limit, feeders find it difficult to supply an adequate quantity of highly utilizable energy in the daily ration to maintain the highest possible rate of production. With the extrusion process, it is possible to alter the secondary starch structure to the point that energy is released at a compatable rate with production. As a result, it is theorized that, as feed consumption increases, the expected decline in energy utilization efficiency will not be as great. As a consequence of this, the efficiency of feed utilization should be increased.

On a national scale, there has been relatively little work reported on the use of extruded products in livestock feeds. There are probably many reasons for this but probably the most important is the lack of basic fundamental information as to the application of extrusion cooking to ground or reconstituted cereal grains.

The purpose of the research presented in this thesis is to review the literature concerning application of the extrusion process to the livestock industry and specifically evaluate methods which can be used to determine the degree of starch damage obtained when the process is applied to ground cereal grains.

LITERATURE REVIEW

I. Processing Grains for Nutritional Benefit

Cereal grains and their by-products have been used as a primary source of available energy in livestock feeding. This is true not only for the swine and poultry industries but for the cattle and sheep industries as well.

Many feeders in the past assumed that slightly cracked grain or even whole grain was adequately utilized by the animal (1). Grinding as well as pelleting has been shown to be of benefit for all classes of livestock. It was shown in four feeding trials that a pelleted ration gave approximately a 10% better gain than the same ration in meal form. Arizona workers found that feed consumption decreased as the percentage of fines in the ration increased, thus enforcing the case for pelleting (2).

Another form of processing that has generated a great deal of interest is that of steam-flaking of grains. Investigations at Colorado showed that steam-flaking corn increased its utilization (Table I) (3).

Work reported by Ensor (4) showed a definite increase in efficiency when steamed corn was used in place of raw ground corn (Table II). The steaming in these studies was at atmospheric pressure and with a time interval of from 15-25 minutes. Work reported in 1967, showed that by elevating the pressure under which the grain was cooked, a better product could be obtained in much less time (1). It was found, with actual feedlot data collected on 158,000 fed cattle over a period of four years, that the feed per pound of gain for high pressure treated flaked grain was 7.44 pounds as compared with 8.55 pounds for grain steamed at atmospheric pressure and then flaked.

Table I. Results of steam-flaking corn for cattle feeding trials.*

	Flaked	Cracked
Average daily gain, lb.	2.16	2.11
Feed per pound gain, lb.	7.57	8.41

*Matsushima, J.K. Feedstuffs. 41 (15), 39 (1969).

Table II. Effect of steaming corn on steer performance.*

	Corn	
	Raw	Steamed
Initial wt.	2.75	2.76
Average daily gain, lb.	2.05	2.55
Feed per pound gain, lb.	3.99	3.30

*Ensor, E.S. J. Dairy Sci. 42, 190, 1959.

Earlier work presented by researchers in Arizona indicated that steam processed or "cooked" grain does not alter palatibility or feed intake and that dry matter digestibility, as determined by the lignin ratio technique, was substantially increased (5). It was also reported that feed required per pound of gain for the cooked grain was significantly reduced from that of ground grain.

Most reported work agrees with the supposition that steam processing or cooking improves the utilization of cereal grains but there are several exceptions noted (6,7,8). Most of these deal with work done on barley. Steaming whole grain alone does not necessarily increase its utilization. The process is more efficient when accompanied by a physical change such as flaking (Table III). This was confirmed in work involving in vitro gas production by a washed cell suspension of rumen microorganisms when acting on steam-flaked and steamed-nonflaked material (9).

Most grain processing systems require heat and physical change to accomplish the modification necessary to increase the utilization of the constitutents. One process that has gained some attention recently that does not require an exogeneous source of heat is that of reconstituted grain. Reconstitution is simply the raising of moisture content of grain to 25-30% and storage under anaerobic conditions for a period of 15 days or more (10). In trials comparing reconstituted sorghum grain with steamed-flaked sorghum grain, a decrease in feed intake for the same average daily gain was noted in favor of the reconstituted grain (11). In the same study, it was shown that protein digestibility was better for the reconstituted grain than either dry rolled or steam-flaked grain. As might be expected, the reconstituted grains, in general, show no gelatinization of the starch (by chemical analytical procedures), yet, in vitro gas production from

Table III. Effect of rolling and pressure on starch digestion of cooked sorghum grain and barley.*

	In Vitro starch digestion by rumen microorganisms.						
Grain	Sorghum grain				Barley		
Pressure cooked, psi	20	40	60	80	20	40	60
	%	%	%	%	%	%	%
Unflaked	15.6	16.3	25.1	32.4	19.2	20.7	38.2
Poor flake	15.8	--	30.0	42.2	--	34.2	42.7
Excellent flake	28.8	29.6	47.2	--	32.3	48.5	48.0
Flat flake	31.7	33.0	45.2	53.2	37.6	52.4	53.8

*Osman, Thuerer and Hale, Arizona Cattle Feeders Day Report. May 5, 1966.

reconstituted grain was nearly as high as those found from steamed-flaked grain in which nearly total gelatinization was observed (12). This would indicate that the starch fraction of the grain became more susceptible to microbial enzymatic attack and that protein solubility was possibly increased, resulting in higher digestibility.

In recent years, a process that has existed for quite some time has beed adapted to the feed manufacturing industry. This process is commonly known as extrusion-cooking and uses a combination of moisture, extreme pressure, high temperature, and mechanical friction to accomplish in seconds the same physical and chemical changes in grains that normally would take several minutes, hours or even days to accomplish by the procedures reviewed above.

The unique characteristic of the extrusion cooking process is its ability to achieve total starch damage, limited starch damage or any degree of damage between. When cereal grains are cooked, the starch is damaged and becomes more susceptible to enzymatic and microbial breakdown (13,14).

The objective of a recent study in Kansas was to evaluate the feasibility of extrusion cooked sorghum grain as a means of enhancing more efficient ration utilization and performance by swine (15). In one trial reported in this study sixty pigs were fed ad libitum a ration with 0,20,40, 60, or 80% expanded sorghum grain. Daily gains were not significantly different although, pigs fed the 40 or 60% levels of cooked grain tended to gain more rapidly. In a second trial reported in the same study, no differences were noted in daily gain but daily feed consumption was less, resulting in better feed conversion.

No significant increase in the incidence of gastric ulcers in swine fed the cooked grain was noted in this study. This finding was contrary to

other studies reporting significant increases in gastric ulcers (16,17,18).

Studies using extrusion cooked grain as part of the ration for ruminant animals resulted in a 50% decrease in concentration of rumen ammonia when compared to an unprocessed ration of the same general composition (19). The same work revealed that concentration of rumen bacterial protein was 50% greater in the ration with expanded sorghum than in the unprocessed ration. This suggested that bacteria may have been converting significantly more ammonia nitrogen to bacterial protein.

In a similar study, the effect of an expanded sorghum grain and finely ground hay ration was compared to that of a long stem alfalfa and cracked sorghum grain ration (20). When fed independently or together, both the expanded grain and ground hay reduced the rumen pH and the acetic to propionic acid ratio. A decrease in milk fat content and increase in milk protein was reported when the expanded grain and ground hay were fed in combination.

Since extrusion cooking process is capable of causing total starch damage, new concepts in product development have come about. One such product is Starea (21). Starea is a mixture of finely ground cereal grain and urea which is processed by extrusion-cooking to a high degree of starch damage. Research revealed a lack of toxicity when dietary protein was supplemented with non protein nitrogen in the form of Starea rather than urea.

The use of urea in ruminants has been restricted by poor conversion of urea nitrogen to microbial protein, by potential toxicity, by low palatibility and by processing and storing problems related to its hygroscopicity (22). Starea reduces or eliminates these problems.

A limiting factor concerning the use of urea is its potential toxicity. The rumen microflora have an efficient urease enzyme system capable of rapid

urea degradation. The resulting ammonia is released into the rumen fluid for utilization in synthesis of microbial protein (20). If synthesis does not keep pace with ammonia production, excess ammonia results and is absorbed into the venous blood surrounding the rumen. The liver converts most of this ammonia to urea and it is eliminated in the urine. If blood ammonia is in excess, ammonia toxicity and death are possible results.

A study using expanded grain as a highly available energy source resulted in significantly lower rumen ammonia concentrations and more efficient microbial protein synthesis (20).

In an earlier study by the same workers it was found that feeding extruded grain and finely ground alfalfa resulted in higher milk yield and more protein and lactose when compared to cracked grain and chopped hay (23). Efficiency in converting feed protein to milk protein was improved.

The above factors indicate the reason for the success of Starea. The starch portion of Starea is presented to the rumen microflora in a highly available form compatable with urea degradation and protein synthesis (24,25). The hydrolysis products of starch breakdown not only provide the required energy but a source of carbon skeletons needed for amino acid synthesis.

In toxicity studies using identical twins, Starea was compared to grain plus urea at various levels (21). The extruded grain urea product was significantly less toxic than the same mixture not processed.

When the extrusion cooked Starea was compared with soybean meal or urea as protein supplements for lactating cows, it was nearly equal to soybean meal and superior to urea (26). Grain intake was lowest for the diets containing unprocessed urea ($P < .05$).

In rumen metabolism studies, Starea was compared with cracked sorghum

grain plus urea, fine grind sorghum grain plus urea and expanded sorghum grain plus urea (27). All diets were formulated on an isonitrogenous basis. Rumen fluid was analyzed for ammonia content by a micro-diffusion method. By day two of the trial the rumen ammonia content of animals fed Starea was significantly less ($P < .01$) than the other rations. By day seven of the trial, the animals fed Starea, expanded grain and finely ground grain rations were significantly different ($P < .01$) from those fed the control ration.

In in vitro studies, the rumen inoculum from urea-adapted animals was incubated with Starea (34%,39% or 44% crude protein equivalent), expanded corn plus urea and ground corn plus urea (28). Bacterial residues were analyzed for amino acids and protein and the fluid was analyzed for in vitro ammonia nitrogen. Results indicated that Starea supplements improved non protein nitrogen urea utilization more than expanded corn plus urea but not significantly so ($P > .05$). All substrates containing extrusion processed grain had significantly higher protein synthesis and lower ammonia nitrogen levels than the ground corn plus urea substrates.

II. Grain Composition

Before one discusses changes that have taken place within the physical structure of cereal grains, the native structure and components should be defined.

The most common grains used in livestock feeding in the United States are corn and sorghum grain. Wheat is of importance but is often too expensive to be used in animal rations.

Corn is the most widely used grain in livestock feeding. The proximate analysis of corn for oil, starch, fiber, ash and protein is a useful aid in evaluating corn for livestock feeding purposes. Table III shows a range of

published values as well as an average value which is useful in formulating rations (29). The starch portion of corn grain contributes to its high energy value. On the average, 71% of the kernel of ordinary dent corn is comprised of starch (30). This starch, on the average, is composed of 27% amylose and 73% amylopectin. Some breeds of corn (waxy maize) have been produced with starch comprised of 100% amylopectin while others contain as much as 50% amylose in the starch (31).

Fiber is also a source of energy, particularly in the ruminant. The pericarp of corn, which contributes the bulk of the fiber fraction, is about 40% cellulose and 40% pentoglycan (32). These two components are found primarily in the cell walls of the pericarp and other fiberous tissue. The lignin portion accounts for the remainder of the fiber fraction.

A small percentage of free sugars are found in the mature corn kernel. Of the approximately 2.5% free sugars available, about one-half to three-fourths is sucrose with the remainder being D-Glucose and D-Fructose.

Approximately 3-4% of the corn kernel is comprised of oils, glycerides and other ether extractable materials (33). The mixture is complex and variable and will not be discussed here except to say that the lipid content is thought to measurably affect extrusion processing characteristics.

Corn protein is a mixture of several distinct protein types: salt-soluble globulins; alcohol-soluble prolamin, zein; and the alkali-soluble protein glutelin (34). The average percentages of the total protein are given as follows: globulin-2%, zein-48%, glutelin-25%, insoluble-2%.

Grain sorghum is the second largest grain crop utilized for livestock feeds. About 95% of the total annual production is used for feeding purposes with the remainder used in wet and dry milling operations (30). The important feature of sorghum grain is that in periods of drought, the plant

Table III. Proximate analysis of corn grain*

	Range	Average
Moisture,% (wet basis)	7-23	16.7
Starch,% (D.B.)	64-78	71.5
Protein, (Nx 6.25),% (D.B.)	8-14	9.91
Fat,% (D.B.)	3.1-5.7	4.78
Ash,% (D.B.)	1.1-3.9	1.42
Fiber (crude),% (D.B.)	1.8-3.5	2.66
Sugar, total,% (D.B.)	1.0-3.0	2.58

*D.F. Miller, "COMPOSITION OF CEREAL GRAINS AND FORAGES," Pub. #585 NAS,NRC Washington, D.C., 1958. (29).

becomes practically dormant allowing it to withstand this stress much better than corn. For this reason sorghum grain has gained much importance in the more arid regions of the United States.

The morphological structure of sorghum grain is almost identical to that of corn in respect to the germ and endosperm. Table IV shows a gross breakdown of the constituents of grain sorghum (29).

The pericarp or fiberous portion of grain sorghum is similar in structure to that of corn except that it is covered with a thick layer of wax and includes many very small starch granules in the misocarp layer (35,36).

Sorghum grain differs from corn in that it has a lower fat content, but slightly higher protein and starch content. It also seems more variable in protein content than does corn. This has caused problems in feed formulation (37).

Sorghum grain also differs from corn in that its protein is much less soluble in normal extraction solvents (38). Five samples of sorghum grain were extracted to fractionate the protein. The average contents were as follows: albumins-3.8%, globulins-4.0%, prolamines-5.8%, glutilins-17.7%, and insoluble protein residue-59.1%. The insoluble residue for high lysine corn in the same study contained 10.6% of the total protein. This value compares favorably with normal dent corn.

The starch of grain sorghum is very similar to that of corn except that average granule diameter is a little larger than that of corn starch: 15.0 μ for milo starch compared to 9.2 μ for corn starch on the same basis (39). The amylose and amylopectin content is nearly the same for both grains.

III. Starch Composition

As stated earlier a primary component of cereal grains that the feeder is most interested in is starch because of its energy yielding structure.

Table IV. Proximate analysis of sorghum grain*

	Range (% Dry Basis)	Average (% Dry Basis)
Water (% wet basis)	8-20	15.5
Starch	60-77	74.1
Protein (N x 6.25)	6.6-16	11.2
Fat	1.4-6.1	3.7
Ash	1.2-7.1	1.5
Fiber (crude)	6.4-13.4	2.6
Pentoglycans	1.8-4.9	2.5
Sugars (as dextrose)	0.5-2.5	1.8
Tannin	0.0003-0.17	0.1
Wax	0.2-0.5	0.3

*D.F. Miller, "COMPOSITION OF CEREAL GRAINS AND FORAGES," Pub. #585, NAS-NRC. Washington, D.C., 1958. (29).

Starch is comprised of repeating glucose units with the intermolecular linkages being identified primarily through methlation studies (40). Methylation of starch followed by acid hydrolysis produces several methyl ethers of glucose with the predominant one being 2,3,6-trimethyl glucose. This indicates the principle linkage is between the one and four carbons. Smaller amounts of 2,3,4,6-tetramethyl glucose and 2,3-dimethyl glucose define the end groups and branching points, respectively.

Starch was shown to exist as two distinct entities by using physiochemical methods (41). The first separation of starch into two components was accomplished using an electrophoretic method. This technique has been replaced by numerous fractionation methods which are much more efficient (42,43). The two fractions have been designated as amylose and amylopectin. Methylation studies of the amylose portion reveal only 2,3,4,6-tetramethyl and 2,3,6-trimethyl glucose while the amylopectin, when methylated, yields a significant amount of 2,3-dimethyl glucose in addition to the other two glucose derivitives (44). These results indicate the amylose fraction is made up of straight chains of repeating glucose units with 1-4 glucosidic links while the 2,3-dimethyl glucose derivitive of amylopectin indicates that, in addition to the straight chains, there are branch points with 1-6 glucosidic links. The amount of branching varies among varieties of starch but is normally 3-5% of the glucosidic links in cereal grains.

The amylose portion of the starch is visualized as being long, relatively straight, chains, with little variation due to source (45). In solution the shape of amylose corresponds to a helical structure with each turn in the helix consisting of six glucose units. This is true only when complexing agents are present (46).

The amylopectin fraction is a highly branched molecule with the degree

of branching depending upon the source of the starch (44).

It has been proposed that differences in staining with iodine between the two fractions is due to their structure (47). The most prevailant theory concerning this phenomenon is that the iodine molecules line up inside the helix structure of amylose giving the characteristic blue-purple color while it is more loosly held in the amylopectin, resulting in the reddish color.

It is a well recognized fact that starch is the principle energy storage unit in the plant. Starch is synthesized within the plant cell and organized into granules during the maturation of the plant. There are many theories as to how the starch granules are actually formed but few are given much credence (48). The most accepted theory is that the starch granule forms by deposition of concentric layers of starch molecules which build up gradually during plant maturation. There are probably two separate systems which produce amylose and amylopectin simultaneously in very close proximity.

There has been a great deal of effort toward determining the types of bonding that hold the granule together. The two primary techniques used to describe the bonding are the mode of swelling of various starches and the extent of solubilization during pasting (49). It has been determined that corn and grain sorghum starches show a limited, two-stage swelling, indicative of two sets of bonding forces which relax at different temperatures. In contrast, potato starch showed a rapid and unrestricted swelling at relatively low temperatures indicating weaker uniform bonding. The solubility patterns generally follow the same trend as the swelling.

A method of exploring starch granule structure using the dissolving action of amylases has been proposed by several workers (50,51). Recent work using enzyme applications has shown there are great physical differences

between the corn starch structure and that of potato starch (52). The following starches were shown to have increasing resistance to amylase decomposition (low to high resistance): waxy maize, tapioca, waxy sorghum, sorghum, corn, wheat, rice, arrowroot, potato. Two general patterns of enzymatic solubilization were observed: A) extensive erosion and random fragmentation of corn and sorghum starches and B) selective, granule by granule, destruction of potato and other starches. Conclusive evidence as to why the differences were noted was not established but the suggestion was made that cereal starches may be more porous in structure, therefore, more susceptible to enzymatic hydrolysis than potato starch which is less permeable. No evidence was found to confirm the theory that the granule is covered with a restrictive outer membrane and that amylolysis of the various starches is not related to consideration of molecular association or crystallization patterns. Of the amylases tested amylase showed the least amount of activity in solubilization of raw starch whereas alpha-amylase had the greatest amount of activity. This was true regardless of the starch source.

One of the more recent methods of characterizing starch granules from various sources has been made possible through the use of X-ray defraction (53). Both modified and unmodified samples of several starches were characterized using unfiltered Cu radiation. It was found that moist-heating of potato starch did not alter the physical appearance but profoundly changed the physical properties such as sorption capabilities. Corn starch demonstrated these same changes but to a lesser degree.

Perhaps one of the most useful techniques for studying starch granule structure is that of electron microscopy. Most work found in the literature has been based on unmodified starch with the emphasis placed on following

the mechanism of granule development after pollination (54). The electron microscopic procedure was used to follow the effect of acid modification of mature corn starch granules. All unmodified starch granules contained folds and buckles which radiated from the periphery and had concentric rings with a periodicity varying from 0.15 to 0.7 μ . The periodicity for any one granule was found to be essentially constant from the periphery to the hilum (center).

IV. Starch Gelatinization

Starch granules exist in the endosperm of cereal grain surrounded by a protein matrix. Normal processing in feed preparation results in little damage to the starch granule. The fractions of the starch granule are said to be held together by relatively weak hydrogen bonds (49). This being the case, a polar solvent should be able to enter the molecule and break these bonds causing hydration of the granule. This forms the basis for a relatively simple definition of starch gelatinization. A recent proposal states that gelatinization is "the irreversible rupture of the native, secondary-bond forces in the crystalline regions of a starch granule" (55). Gelatinization involves only the breaking of the secondary-bonds of the polymer molecule while the other bond forces are left intact.

It is possible to obtain complete granule disintigration through the use of disrupting organic compounds such as disodium ethylenediamine-tetra acetate or dimethyl sulfoxide. Under normal conditions gelatinization occurs in the presence of water, heat and/or pressure. Gelatinization may be aided by the addition of compounds, such as urea, which help break hydrogen bonds (56).

The term gelatinization, under normal useage, has been a rather ill-defined term. Some of the more commonly used indicators of gelatinization

have been the granules swelling tangentially and loss of characteristic polarization crosses, commonly refered to as loss of birefringence. The activities were observed only above a specific range of temperatures dependent upon starch species. Below this temperature swelling was considered reversible (57).

Perhaps the most important change that occurs in starch during gelatinization is irreversible swelling. As gelatinization begins, the initial swelling is thought to take place in the more assessible and amorphous intermicellar areas of the granules. This initial swelling is due to water breaking weak hydrogen bonds and, possibly, forming water bridges between -OH groups (57,58). As temperature increases, water is driven into the more crystalline region in the interior of the granule. This, in turn, causes rupture of more secondary hydrogen bonds and results in hydration of the granule. The temperature of gelatinization is the most commonly refered to term when discussing this property of starches and is the range of temperatures at which the particular starch lossed 50% of its birefringence. The most common method of determining temperature of gelatinization uses the Kofler microscope hot-stage which provides a simple and accurate means for determining loss of birefringence (59).

Starches have been classified into three groups according to their degree of association or resistance to gelatinization (in decreasing order): 1) cereals, 2) root or pith and 3) tuber (57). In this study, cereal starches showed a characteristic two-stage swelling, an indication of the presence of two sets of internal bonding forces which probably correspond to the highly associated crystalline area and the less associated amorphous area. At the opposite end of the spectrum the tuber (potato) exhibits an exceptionally high degree of swelling and ease of gelatinization. This is

indicative of weak internal bonding and a high degree of continuity of structure.

A recent study investigated the various iodine staining patterns following digestion of α- and β- amylases (48). The starches exhibited three types of staining patterns characteristic of their structures. Potato and tapioca starches gave essentially an "S" shaped curve as amylolytic activity progressed. Wheat, corn and sorghum starches retained nearly all their staining capacity throughout the digestion period (30 Minutes). Waxy sorghum and waxy maize had little stainable material due to their low amylose content.

The same study delt with changes of infared spectra of the various starches during gelatinization following temperature rise. It was observed that the shape of the absorption band changed with degree of gelatinization. These changes are indicative of decreased hydrogen bonding in the structure. The most predominant change, described as a sharpening of the hydroxyl absorption band, occured close to the gelatinization temperature. In this study gelatinization was defined as loss of 50% birefringence.

V. Determination of Starch Damage

Research has been done to characterize the extent of starch damage. Nearly all reported work has delt with mechanical damage sustained by starch during flour milling operations. This is important since damaged starch is principally responsible for gas production during the later stage of fermentation and proofing. It also contributes materially to flour-water absorption (60). Excess starch damage may lead to a dark crust color and poor crumb structure in bread (61).

Damaged starch has been found to differ from normal starch in many ways. Important differences are: water absorption, solubility, suscepti-

bility to staining with iodine and certain dyes and digestibility by amylases (62). Conceivably, any of these properties could be used to quantify the damage in starch but solubility and increased enzyme digestibility appear most practical at this time. Each of these properties have been used at some time or another by various researchers.

Early attempts to measure starch damage involved observation of staining characteristics of starch following addition of congo red dye (60). This method is not quantitative but is acceptable if a gross estimate is desired.

It was recognized that starch which appeared to be damaged had an increased susceptibility to enzymatic attack. Early attempts to quantify starch damage by this property involved removal of all enzyme activity by acidification, neutralization and then the addition of extracts of α — and β — amylase (63). This procedure met with limited success and formed the basis for development of several enzymatic methods for determination of starch damage.

One of the first and perhaps most useful enzymatic techniques developed was that of Sandstedt and Mattern (62). This method was based on the differential rate of digestion between damaged and undamaged starch by amylase.

The development of the method used mixtures of soft wheat starch with very little damage and hard wheat starch with severe damage as the substrate. The enzyme used was a standardized, laboratory prepared, malt extract containing primarily α— amylase with some β— amylase present. In the procedure, the substrate was incubated with the enzyme preparation for periods of one and two hours. After the incubation period the enzyme was neutralized and reducing sugars were measured and expressed as maltose.

The slope of the curve between one hour and two hours was extrapolated to zero time and the value obtained expressed as percent starch damage.

It was found that after the first hour of digestion, the curves were essentially parallel and differed only in degree of starch damage.

Another method using enzyme susceptibility was developed by Sullivan, et al., (64). In this procedure the endogenous enzyme activity of the sample was inactivated using trichloracetic acid. The TCA was removed with butanol and ethyl ether washings and the sample dried. Two 5 gm samples of the flour were used with one being treated with 80 mg of β-- amylase. The maltose in both samples was measured using a standard ferricyanide method. The difference in milligrams of maltose per 10 gm of flour between the two determinations represented the Starch Damage Index (SDI). Several flours were used and it was found the starch damage index for each correlated well to loaf volume and crumb structure index when the flours were test baked.

A rapid method for determination of starch damage was suggested by Donelson and Yamazaki (65). The procedure was essentially that of Sandstedt and Mattern (62), except the digestion period was considerably shorter (15 minutes), made possible by the use of an additional 0.10 gm Rhozyme 33[a] and a smaller sample (1 gm).

The rapid hydrolysis of starch by this particular enzyme system was illustrated by the fact that all curves of time versus mg maltose leveled off after only 15 minutes regardless of the percent of damaged starch present. The maltose produced was determined using the ferricyanide method. A comparison was made between the proposed method and the Sandstedt-Mattern

---

[a]Marschall Co., Elkart, Ind.

procedure. A high correlation (r = 0.89) was obtained between the two methods. (65)

It was later discovered that the original Donelson-Yamazaki procedure was useful only with starches of relatively low damage. A study on samples with higher degrees of damage showed that serial reductions in sample size as starch damage increased improved results (66). Results were consistant within the range covered (10-30% damage).

A rather sophisticated procedure based on the differential iodine absorption rates by damaged and undamaged starches was proposed by Medcalf and Gilles (67). This procedure used amprometric titration to measure the rate of absorption. A sensitive galvanometer was used to measure the conductance between two platinum wire electrodes 1 mm apart in solution during timed additions of standardized potassium iodate solution. Percent iodine absorbed (gI/100 gm sample) was plotted against time to give a curve showing the rate of iodine absorption.

Experimental results showed that iodine absorption became essentially linear after 8-10 minutes. Based on this observation, the procedure was modified by taking only two readings, one at 9 minutes and one at 14 minutes. The iodine absorption was plotted against time at these two points and extrapolated to time zero. The value at time zero for a particular sample was multiplied by 100 to give the Iodine Absorption Value (IAV).

The method was compared with that of Donelson and Yamazaki using several flours and starches. It appeared there was satisfactory agreement although no correlation analysis was performed.

A colorimetric method for damaged starch has been reported by Williams and Fegol (68). The method included the extraction of soluble starch with a solution of sulfasalicylic acid and then color development with the addition

of iodine. Absorbance was determined at 550nm against a reagent blank. Evidently no attempt was made to correlate color development (absorbance at 550nm) to percentage of starch damage as all reported values were expressed as absorbance.

The procedure was compared with five previous procedures and found to be highly correlated with all of them ($r > 0.90$).

A modification of the Sandstedt-Mattern method was developed by Sung (69). The method used commercially obtained β – amylase (Wallerstein, N.Y.). as opposed to laboratory enzyme preparation of Sandstedt and Mattern. Reducing sugar determination at the end of one hour was deleted and results of the two hour digestion were reported as milligrams maltose per gram instead of converting the value to percent starch damaged.

An analysis was run to determine the required amount of β -- amylase. No benefit was observed above 50 mg per gram substrate, although 60 mg/gram substrate was used in each case to insure an excess.

A mannometric procedure for determining the effect of processing on grain was proposed by Trei, et al. (9) and later modified by Roth (70). The original procedure consisted of incubating a 2.0 gm sample with 75 ml warm, buffered rumen inoculum for a period of three hours. The volume of fluid displaced from a mannometric device was corrected for gas produced by the control (75 ml inoculum) and expressed as milliters of gas per gram of dry matter incubated. The technique was used to differentiate between steamed, steam-flaked and unprocessed sorghum grain and barley. High correlations were found between gas production and starch digestion.

The modification used by Roth (70) was the replacement of the rumen inoculum with a yeast-enzyme system capable of hydrolyzing the starch and converting it to gas. Gas was measured and reported in the same manner as in the work by Trei et al. (9).

EXPERIMENTAL PROCEDURE

Samples of feed grade yellow dent corn, sorghum and hard red winter (HRW) wheat were obtained commercially by the Kansas State University Feed Mill. The material was cleaned initially to remove extraneous material. It was then ground under various conditions to obtain a range of average particle sizes.

The research information reported is divided into three phases to facilitate reporting of analytical procedures and to insure continuity of information.

PHASE I. Raw Sample Preparation

For the first investigation, samples of the above cereal grains were ground using a 25 hp. Sprout-Waldren[a] (SW) Tear-Drop screen hammermill and a 3 hp. Gruendler hammermill[b] (180° screen area). To obtain a coarse grind, the SW hammermill was equipped with a 0.318 cm hole diameter screen. A medium grind was obtained using the same hammermill equipped with a 0.159 cm hole diameter screen. A finer grind was obtained when samples of each grain were ground through the Gruendler hammermill equipped with a 0.159 cm hole diameter screen.

Particle size was determined by the method of Headley and Pfost (71). A computer program was used to facilitate computation of mean particle diameter, surface area, particle number and to eliminate rounding errors.

Sample preparation for the second series of studies was similar to that of the first except that a wider variation in particle size was desired.

---

[a]Sprout-Waldren Co., Muncy, Pa.
[b]Gruendler Pulverizer Co., St. Louis, Mo.

Samples of each grain were ground through a 25 hp. Jacobson[a] hammermill equipped with a screen of 0.635 cm diameter hole openings to give a coarse grind. A medium grind was obtained when samples of each grain were ground through the same hammermill equipped with a 0.159 cm diameter hole opening screen. To obtain an extremely fine grind, portions of each grain were ground using a Micro-Bud[b] micro-pulverizer with an air classifier set at 500 RPM. Particle size was determined using the method reported previously.

A third study was designed as a preliminary experiment to determine the effect of moisture content on further processing. In this study, all samples were ground through the Gruendler hammermill equipped with a screen with 0.318 cm hole diameter openings. The samples, designated as CM-1, CM-2 and CM-3, were corn and were treated as follows: sample CM-1 was ground and extrusion cooked with no further treatment. Sample CM-2 was ground dry then tempered to 15% moisture for 24 hours before processing. Sample CM-3 was raised to 15% moisture and tempered for 24 hours prior to grinding and processing. Each sample was processed immediately after the tempering period.

In each case moisture was determined at 110°C for two hours in an air oven. Moisture was determined after grinding and just prior to further processing.

PHASE II. Extrusion Cooking Process

A 200 pound sample of each ground grain by particle size was processed through the extruder for experiments 1 and 2. For the third experiment, the sample weight was 100 pounds for each grain.

---

[a]Jacobson Machine Works. Minneapolis, Minn.
[b]Metals Disintegrating Co., Pittsburg, Pa.

The material in each experiment was processed through a Wenger Model[a] X-25 continuous extrusion-cooker and dried on a horizontal drier using ambient air.

Following drying the expanded material was ground through a laboratory mill equipped with a 0.159 cm screen, mixed and an analytical sample taken for analysis. Moisture was again determined on the ground samples.

PHASE III. Starch Damage Determination

A major change that occurs in extruded cereal products is the damage sustained by the starch fraction. Three enzymatic methods were used to evaluate this change in samples from experiments 1 and 2. Sung's (2) method with modifications was used as the standard reporting procedure. This method was the only procedure used to evaluate the results of trial 3 and the results of the particle size studies (experiments 1 and 2).

Sung's method was based on the standard AACC method using ferricyanide to determine reducing power. The enzyme used was β - amylase (Wallerstein Co., N.Y.) which had been standardized at 5000SKB units by the manufacturer.

A 1.0 gm sample of air dried processed material weighed to the nearest 0.1 mg was used for each test. Triplicate samples were used in each case. Approximately one half teaspoon ignited quartz was added and mixed well with the ground sample. Forty one milliliters of 0.01M sodium acetate-acetic acid buffer solution (pH 4.6) were added to the flask from a burette and mixed gently with the sample. The flasks were then placed in a 40°C water bath and allowed to warm to the desired temperature. Five milliliters of 40°C distilled water containing 60 mg β - amylase were added rapidly to each flask and mixed thoroughly. The mixture was incubated for exactly one

---

[a]Wenger Manufacturing Co., Inc. Sabetha, Ks.

hour in a thermostatically controlled waterbath (40°C). At the end of the incubation period, 2 ml of 3.58N sulfuric acid was added and mixed well. This was followed by the addition of 2 ml sodium tunstate. The sample mixture was allowed to stand for 2 minutes to inactivate the enzyme after which it was filtered (Whatman#4 or equivalant), discarding the first 8 to 10 drops.

A 5.0 ml aliquot of the filtrate was added to a large test tube to which 10.0 ml of 0.1N alkaline ferricyanide solution had been previously added. The solutions were mixed thoroughly and the tubes placed in boiling water for exactly 20 minutes. Care was taken to insure that the boiling water level was 3 to 4 cm above the solution level in the tubes.

At the end of the boiling period the tubes were cooled rapidly in a running waterbath. The alkaline ferricyanide-filtrate mixture was transfered to a clean 125 ml Erlenmeyer flask with three washings of acetic acid-salt solution of 25 ml total volume. The addition of the acetic acid-salt solution was done with care to avoid rapid expansion in volume. One milliliter of soluble starch-potassium iodide indicater was added prior to titration with 0.1N sodium thiosulfate. A magnetic stirrer was used to aid in titration. The conversion table of Sandstedt and Mattern (62) was used to convert ml of 0.1N ferricyanide reduced to mg maltose equivalent. Concentration of solutions and standardization procedures were identical to those used by Sandstedt and Mattern (62).

The second procedure used was a modification of the above method in which the reducing sugar determination was made using a colorimetric procedure adapted during this research. The determination is based on the reduction of dinitrosalicylic acid (DNS) by the reducing sugars present after enzymatic digestion (72). The concentration of the reduced DNS was determined spectrophotometrically.

The DNS solution was prepared as follows. Ten grams of 3-5 dinitrosalicylic acid was dissolved in 200 ml 2N sodium hydroxide (16 gm/200 ml) with warming. Three hundred grams of sodium potassium tartarate were added to 500 ml distilled water and stirred until dissolved. The two solutions were mixed and brought to 1 liter volume with distilled water.

This solution was stable for the duration of this investigation (3 months) but as a precaution the DNS solution was stored in a brown bottle with refrigeration. Weekly determinations were made with a fresh maltose solution to insure no change was taking place. No crystallization was noted during the period of use. Enough solution was prepared to use for the entire investigation.

A standard curve was used to determine values for unknowns. The curve was determined using a standard maltose solution (10 mg per ml) in a sodium acetate-acetic acid (pH 4.6) buffer. The standard maltose solution was diluted in 10 ml volumetric flasks to concentrations from 0 to 10 mg per ml in one mg per ml increments. One ml of each maltose concentration was thoroughly mixed with 1 ml of the DNS solution in glass tubes and placed in a boiling water bath for exactly 5 minutes. The tubes were then cooled rapidly in running water and the solution diluted with 28 ml distilled water and mixed thoroughly with shaking. Absorbance was read at 540 nm on a Bausch and Lomb Spectronic 20 colorimeter using standardized photometric tubes. Absorbance was plotted against concentration (mg/ml) to obtain the standard curve (Fig. 3).

The procedure based on colorimetric starch damage determination was as follows. A one gram sample was weighed to the nearest 0.1 mg and placed in a 125 ml Erlenmeyer flask together with one half teaspoon ignited quartz. To this material was added 45 ml 0.01M sodium acetate-acetic acid buffer

(pH 4.6) and the suspension mixed and warmed to 40°C in a thermostatically controlled water bath. To this suspension was added 5.0 ml distilled water containing 60 mg β-amylase (Wallerstein Co., N.Y.). The material was incubated at 40°C for one hour with shaking at 5 to 10 minute intervals. At the end of the incubation period, the flasks were placed in boiling water for three minutes to inactivate the enzyme after which they were cooled in running water. To each flask was added about 0.25 gm Celite (Johns-Manville or equivalent) and the flasks allowed to stand briefly.

The solution was then filtered through Whatman Number 4 filter paper. A 1.0 ml aliquot of the filtrate was added to 1.0 ml of the DNS solutions were thoroughly mixed and the tubes placed in boiling water for exactly five minutes. At the end of the boiling period the tubes were cooled in running water. The time from the end of the incubation period to the end of the color development boiling period should not exceed twenty minutes.

The tubes containing the reduced DNS solution were brought to room temperature and 28.0 ml distilled water added. The tubes were stoppered and swirled to obtain a thorough mix.

A 4 ml aliquot from each tube was placed in separate, matched, photometric tubes and absorbance determined at 540 nm with a Bausch and Lomb Spectronic 20. A reagent blank (all except grain) was used to obtain a zero absorbance on the photometer. A standard extrusion cooked milo was used with each analytical run to indicate possible procedural variations.

The colorimetric procedure was checked for linearity using mixtures of highly cooked corn and ground corn. The mixture varied from 0 to 100% cooked corn with ground corn making up the balance. Increments were at 20% intervals.

To obtain milligram maltose per gram the value from the standard curve

was multiplied by the total milliliters of solution in the reaction flask (in this case: value x 50).

The third method used to determine starch damage was an in vitro gas production technique in which the rate of enzymatic hydrolysis was measured by the milliliters of gas produced by an excess of yeast. The method was a modification of the technique used by Roth (70) with the major change being the mannometric apparatus (Fig. 1). Results were expressed as gas produced per gram dry matter per hour.

In this technique, 0.75 gm of the ground material was weighed to the nearest 0.1 mg into a 50 ml flask containing one half teaspoon ignited quartz and mixed. To this mixture 0.25 gm of commercially prepared dry yeast was added and incorporated.

The enzyme used was Diazyme 160 (Marschall Co., Elkart, Ind.) which had been standardized by the manufacturer. A 0.1% enzyme solution was prepared prior to each run to insure freshness. Due to the insoluble nature of the enzyme carrier, the flask containing the enzyme solution was shaken prior to taking a 10 ml aliquot for addition to the grain-yeast mixture.

The 10 ml aliquot of enzyme solution was added to the reaction flask with gentle swirling to insure adequate mixing. The flasks were immediatly connected to the mannometric apparatus. The reaction was allowed to proceed for exactly 3 minutes after the last flask had been connected in order to allow the air to be evacuated from the system. At the end of the 3 minute period, the transfer tubes were placed in the collection cylinders. A blank determination (yeast plus enzyme) was made with each run.

The fermentation period lasted for 3 hours with gas production as determined by water displacement recorded at hourly intervals. The results were calculated using the following formulas:

net ml gas produced per hour=

$$\frac{\text{total ml gas (sample) - total ml (Blank)}}{\text{total time (hours)}}$$

ml gas produced per hour per gm DM=

$$\frac{\text{net gas per hour}}{\text{gm DM in sample}}$$

The fermentation period was carried out with the reaction flasks partially submerged in a 37°C water bath held constant by thermostatic control. The mannometric fluid was adjusted to below pH 1.0 with sulfuric acid to prevent fermentation gas from being dissolved. Each sample was run in triplicate to reduce analytical errors.

RESULTS AND DISCUSSION

This research was undertaken to investigate certain aspects of the extrusion cooking process as it applies to feed manufacturing. A primary goal was to determine the effect of mean particle diameter of the ground cereal grains on the degree of starch damage sustained during the cooking process. For the process to have practical application, it should be compatible with processes employing other equipment found in the manufacturing facility.

In the first experiment an arbitrary selection of grinding conditions was made with the known limitations of the extrusion processing equipment in mind. Table I shows the results of particle size analysis conducted on raw samples of sorghum, corn and wheat prior to extruding. An important fact that should be noted from Table I is that standard deviation values for the grains do not differ greatly. This indicates that, while the particle size distributions are not identical for each grain, the distributions are similar enough that meaningful comparisons can be drawn from within grains and between grains.

Table II is a summary of conditions under which the ground samples were extruded. The grind classification was made on the basis of the particle size determination. An attempt was made to keep all conditions of processing between grains and within grains the same. As can be seen from the data, the wheat portion of the run caused a reduction in production rate. This was probably due to the characteristic gluten development of wheat which causes its dough to become quite tenacious when wetted and mixed. It was felt that this caused no serious variation in the results of the starch damage determination.

The results of the starch damage determination are shown in Table III. This determination was made using the modified Sung method. The range of

Table I. Results of particle size analysis for raw material for study one*

Sample Number	Grain	Geometric mean particle diameter (microns)	Surface area per gram (cm^2)	Geometric mean Standard deviation
P-1-1	Grain Sorghum	361	147	1.82
P-1-3	Grain Sorghum	433	115	1.82
P-1-2	Grain Sorghum	483	110	1.63
P-2-1	Corn	396	129	1.69
P-2-3	Corn	462	103	1.75
P-2-2	Corn	480	108	1.45
P-3-3	Wheat	377	139	1.82
P-3-1	Wheat	391	129	1.66
P-3-2	Wheat	509	112	1.82

*Determinations were made according to the method of Headley and Pfost (1970) (71).

Table II. Conditions of extrusion processing for study one[a]

Sample number	Grain	Production rate[b] (kg/hr)	Moisture added[c] (kg/hr)	Moisture added[d] (%)	Total moisture (%)	Die head temperature (°C)
P-1-1	Grain Sorghum	454	30.23	6.65	19.85	140.5
P-1-2	Grain Sorghum	454	30.23	6.65	19.85	138
P-1-3	Grain Sorghum	454	30.23	6.65	19.85	138
P-2-1	Corn	454	30.23	6.65	18.40	140.5
P-2-2	Corn	454	30.23	6.65	18.40	140.5
P-2-3	Corn	454	30.23	6.65	18.40	140.5
P-3-1	Wheat	318	30.23	9.5	17.45	143
P-3-2	Wheat	318	30.23	9.5	17.45	143
P-3-3	Wheat	318	30.23	9.5	17.45	143

a) All samples were processed through die with one 0.953 cm hole
b) From calibration curve using fine grind sorghum grain
c) From calibration chart supplied with Sho-Rate flow meter (Brooks Instrument Co. Nat Feild, PA.)
d) Calculated as $\frac{\text{lbs water added per hour}}{\text{lbs product per hour}} \times 100$
e) Moisture determined on raw material plus that added during processing

particle sizes was smaller than anticipated and the results of the starch damage determination reflected these small differences. Each sample was analyzed in triplicate to minimize analytical error.

The effect of particle size of the raw material on the degree of starch damage is graphically shown in Figure I. As can be seen, the scatter diagram shows a variation of points but also shows a definite trend downward and to the right.

This study was undertaken with the assumption that amount of damage to starch during controlled processing was linearly related to the particle size of the raw material. To statistically analyze this assumption, a computer program was written which included a test of significance of departure from linear regression and an analysis of variance for curvilinear regression as well as regression equations for both linear and quadratic in order to determine the best fit line.

Table IV is a summary of the statistical analysis to determine departure from linearity. When the Standard F test was applied to the data, a significant departure from linearity ($P < .05$) was found, suggesting the data was curvilinear. Table V is an analysis of variance table for curvilinear regression. The information in this table is useful in determining the coefficient of correlation for the curvilinear regression curve. The coefficient of correlation for linearity was determined by standard procedures and found to be low ($r = -0.51$). To determine the coefficient of correlation for curvilinearity the following formulas were used (Snedcor) (73).

$$r^2 = \frac{\text{Sums of Squares due to Regression}}{\text{Sums of Squares for Total Deviation}}$$

$$r = \sqrt{r^2}$$

When these equations were applied to the data retrived from Table V the coefficient of correlation due to curvilinear regression was $r = -0.68$.

Table III. Results of starch damage determination[a]

Sample number	Grain	Particle diameter (microns)	mg maltose per gram
P-1-1	Grain sorghum	361	214 207 212
P-1-2	Grain sorghum	483	178 178 175
P-1-3	Grain sorghum	433	221 221 218
P-2-1	Corn	396	225 226 227
P-2-2	Corn	480	225 224 222
P-2-3	Corn	462	236 231 228
P-3-1	Wheat	391	232 233 240
P-3-2	Wheat	509	200 197 196
P-3-3	Wheat	377	223 231 232

a.) Starch damage determined by modified Sung Procedure. (69).

Table IV. Test of significant departure from linear regression

Source of variation	DF	SS	MS	F
Deviation from linear	25	6297.2		
Deviation from curved	24	4530.8	188	
Reduction in SS	1	1761.4	1766.4	9.357*

*Significant at P = .05

Table V. Analysis of variance for curvilinear regression

Source of variation	DF	SS	MS
Regression	2	3817.19	1908.397
Deviation	24	4530.8	188.8
Total	26	8348.0	

THIS BOOK CONTAINS NUMEROUS PAGES WITH DIAGRAMS THAT ARE CROOKED COMPARED TO THE REST OF THE INFORMATION ON THE PAGE.

THIS IS AS RECEIVED FROM CUSTOMER.

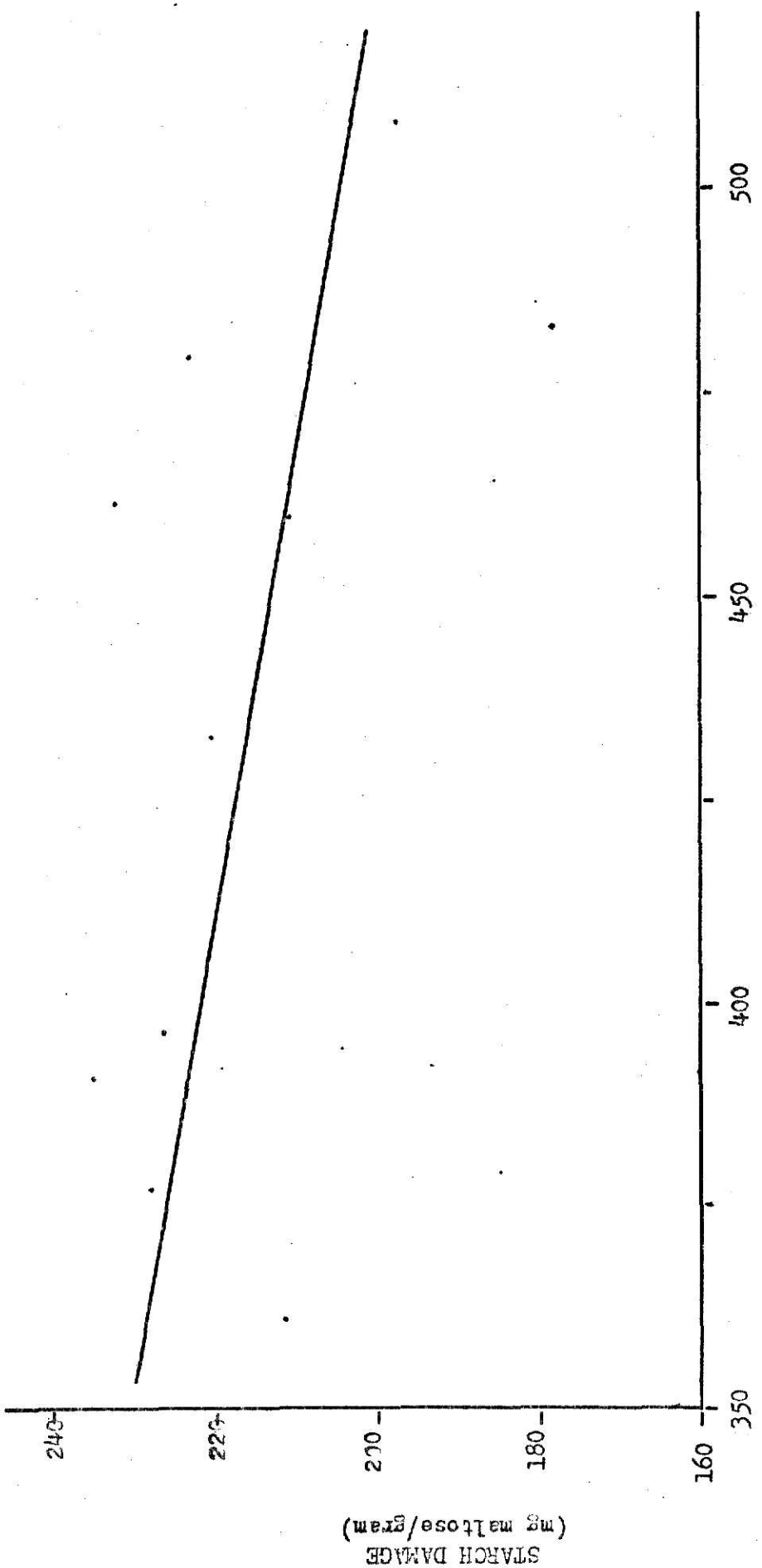

Figure 1. Effect of particle size on the degree of starch damage.

Both coefficients are statistically significant ($P < .01$) but the curvilinear application gives the best fit.

The line equations for the present study were:

1) for linear
$y = -0.173x + 291.4$

2) for curvilinear
$y = -0.005x^2 + 4.06x - 614.1$

The results of this study were encouraging but it was felt an improvement could be made by changing the experimental design. A second study was undertaken with the same basic parameters except that new guidelines were used in selecting the particle sizes of the raw material. A much broader range was desired to more precisely evaluate results.

Results of the particle size analysis on the raw material used are shown in Table VI. A wider dispersion of mean particle diameter was evident. Once again grind classification was based on the results of the particle size analysis.

Table VII gives a summary of extrusion cooking conditions for samples in experiment two. There was more variation in the conditions of study two but was considered to have little, if any effect, on the starch damage determination.

Table VIII shows the results of starch damage determinations as compared to particle size. It should be noted that differences in starch damage and particle size were greater than in experiment one.

Figure 2 gives a graphic display of the averages of triplicate analysis plotted against mean particle diameter. It should be noted there is apparently a better correlation between particle size and starch damage than in experiment one (Table I). When normal statistical procedures were applied to the data the coefficient of correlation for linearity was highly significant ($r = -.91$) ($P - .01$).

Table VI. Results of particle size analysis for raw material used in Study Two(a)

Sample number	Grain	Geometric Mean particle diameter (microns)	Surface area per gram (cm^2)	Geometric Mean standard deviation
P-M-1	Grain Sorghum	182	158	1.68
P-M-2	Grain Sorghum	459	105	1.67
P-M-3	Grain Sorghum	605	85	1.64
P-C-1	Corn	156	172	1.65
P-C-2	Corn	400	127	1.67
P-C-3	Corn	517	131	1.73
P-W-1	Wheat	145	164	1.62
P-W-2	Wheat	348	142	1.65
P-W-3	Wheat	580	94	1.90

(a) Determinations were made according to the method of Headley and Pfost (1970) (71).

Table VII. Conditions of extrusion processing for study two[a]

Sample	Grain	Production rate[b] (kg/hr)	Moisture added[c] (kg/hr)	Moisture added[d] (%)	Total Moisture[e] (%)	Die head temperature (°C)
P-M-1	Grain Sorghum	375	37.7	10.0	23.2	146
P-M-2	Grain Sorghum	375	37.7	10.0	23.2	146
P-M-3	Grain Sorghum	375	37.7	10.0	23.2	146
P-C-1	Corn	318	33.9	10.7	23.2	149
P-C-2	Corn	318	33.9	10.7	23.2	146
P-C-3	Corn	318	33.9	10.7	23.2	149
P-W-1	Wheat	250	37.7	15.1	25.0	141
P-W-2	Wheat	250	37.7	15.1	25.0	138
P-W-3	Wheat	250	37.7	15.1	25.0	135

a) All samples were processed through a die with a single 0.95 cm hole
b) From calibration curve using fine grind sorghum grain
c) From calibration chart supplied with Sho-Rate flowmeter (Brooks Instrument Co., Natfield, PA.).
d) Calculated as $\frac{\text{lbs water per hour}}{\text{lbs product per hour}} \times 100$
e) Moisture determined on raw material plus that added during processing

Table VIII. Degree of starch damage for samples in study two

Sample number	Grain	mg maltose/gm[a,b]
P-M-1	Grain sorghum	253 252 246
P-M-2	Grain sorghum	172 175 171
P-M-3	Grain sorghum	166 166 167
P-C-1	Corn	228 228 224
P-C-2	Corn	183 182 179
P-C-3	Corn	171 170 170
P-W-1	Wheat	257 258 257
P-W-2	Wheat	199 198 199
P-W-3	Wheat	186 189 187

a) Starch damage determined by β - amylase hydrolysis method (Sung) (69).

b) Values are averages of triplicate analysis.

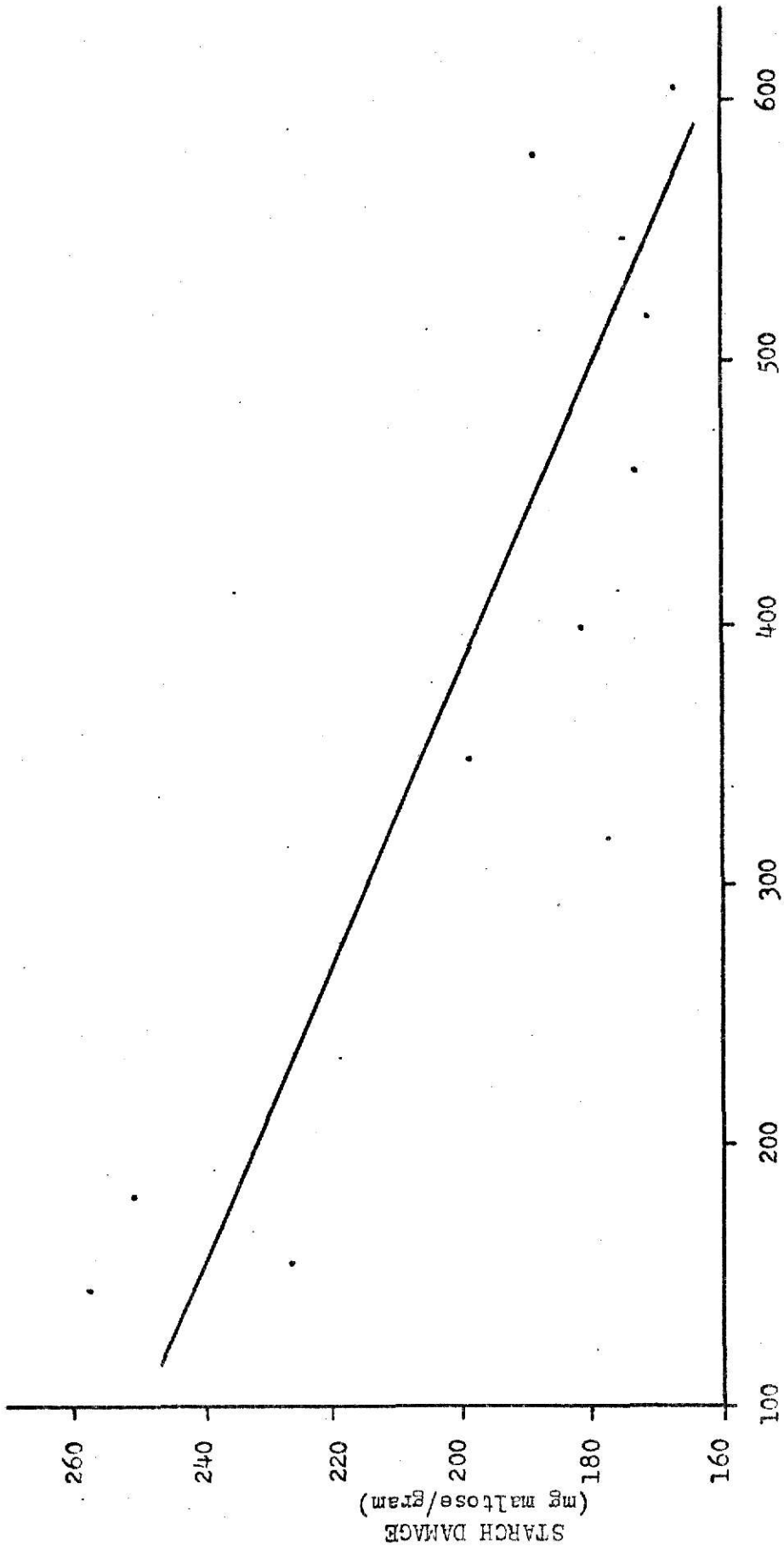

Figure 2. Effect of particle size on the degree of starch damage

Table IX. Test of significant departure from linear regression (study two).

Source of variation	DF	SS	MS	F
Deviation from linear	25	5118.0		
Deviation from curvilinear	24	2880.6	120.0	
Reduction in SS	1	2237.3	2237.3	18.64*

*Significant at P = 0.01

Table X. Analysis of variance for curvilinear regression (run two).

Source of variation	DF	SS	MS
Regression	2	26549.3	13274.6
Deviation	24	2880.7	120.02
Total	26	29439.1	

The data were also analyzed for departure from linearity. Table IX gives the results of the test for significant departure from linearity. Data were significantly different from linear ($P < .01$), again suggesting a curvilinear equation fits the data more accurately. Table X gives results of an analysis of variance table which contains the information needed to compute the coefficient of correlation value for curvilinear regression. When the data were applied to the determined curvilinear equation, a correlation coefficient of $r = -.95$ was found as opposed to $r = -.91$ for linear. The line equations for each catagory are as follows:

1) for linear $y = 0.176x + 267.6$
2) for curvilinear $y = 0.004x^2 - 0.4997x + 313.3$

The graphic representation of the linear equations are presented in Fig. 2.

In both studies one and two the calculated curvilinear lines fit the data somewhat better than the calculated linear lines. This would suggest the distribution was affected by some factor other than mean particle diameter. An investigation using surface area data did not improve the departure error. Very close agreement was found between the linear line equations for both experiments suggesting that departure error found may be due to normal population variance.

EFFECT OF MOISTURE ON EXTRUSION COOKING

This portion of the study was preliminary in nature with an expanded study planned at a later date.

As discussed earlier sample CM-1 was ground and extruded at its natural moisture content. Sample CM-2 was ground and then tempered for 24 hours to a total moisture content of 15.1%. Sample CM-3 was raised to 15.2% moisture prior to grinding.

The results of the particle size determination on the ground material (Table XI) indicates that moisture addition did tend to increase mean particle diameter while surface area and particle count were decreased.

The conditions of extrusion cooking were held constant with the exception of moisture content which was varied prior to processing. The data concerning the cooking process is shown in Table XII.

Table XIII shows the results of the starch damage determinations. There appeared to be no differences related to the method of applying additional moisture. This was contrary to what was expected because of the increase in particle size. Some benefit appeared to be gained from the additional moisture regardless of when it was added.

Table XI. Results of particle size determination for moisture study[a]

Sample #	Mean particle diameter (microns)	Surface area (cm^2)	Particle number (per gram)
CM-1	485	120.9	78,968
CM-2	485	120.9	78,968
CM-3	571.34	96.6	27,902

a) Determined by the procedure of Headley and Pfost (71).

Table XII. Conditions of extrusion cooking for moisture study

Sample #	Moisture at Cooking (%)	Production rate (kg/hr)	Water Addition (%)	Total Moisture (%)	Die temp. (°C)
CM-1	12.1	454.5	6.65	18.75	149
CM-2	15.1	454.5	6.65	21.75	149
CM-3	15.2	454.5	6.65	21.75	149

Table XIII. Results of starch damage determination for moisture study

Sample #	Beginning moisture (%)	Starch damage ($\frac{mg\ maltose}{gm.}$)
CM-1	12.1	243
	12.1	244
CM-2	15.1	252
	15.1	252
CM-3	15.2	253
	15.2	252

A COMPARATIVE STUDY OF TECHNIQUES FOR STARCH DAMAGE DETERMINATION

During the preliminary work leading to this investigation a void in information concerning the determination of starch damage in extruded cereal grains appeared to be present. Specifically the techniques that could be adapted for quality control work appeared to be limited. Nearly all work on starch damage determinations has been done using a slurry (6 to 15% concentration) of purified starch from various sources that have been heated under highly controlled laboratory conditions. This procedure is quite different from conditions found in a feed milling facility. In view of the above considerations, it was decided to conduct a comparative study using three techniques selected for their adaptibility to quality control work. The techniques used were all based on the rate of enzymatic degradation followed by either reducing sugar determination or gas production by yeast in vitro.

A review of current procedures for starch damage was conducted and most were eliminated as they did not meet the requirements of a quality control procedure. The initial publication of Sandstedt and Mattern (62) formed the basis for subsequent enzymatic techniques and most of the procedures of this type are extremely time consuming and require the use of a large number of stock solutions as well as two solutions that require standardization.

The method of Sung (69) was developed from that of Sandstedt and Mattern (62) and was used in this study as the reference technique. This is also the method used by this laboratory as a standard reporting procedure when dealing with extruded products. The advantage of this procedure is that the enzymatic digestion period is reduced from two hours to one hour and a commercially prepared and standardized enzyme is used. The method requires lengthy titrations and standardization of solutions.

The colorimetric procedure was developed with the desire to eliminate the standardization of titration solutions and the titration procedure. The DNS solution, when combined with reducing sugars produced during digestion, forms a brown colored solution which absorbs strongly at 540 nm (72). Using the specification discussed earlier, the concentrations of reducing sugars produced follow Beer's law for absorbance.

To avoid calculations associated with colorimetric procedures, a standard curve was determined from which the concentration of the reducing sugars per milliliter could be read directly.

Figure 3 is a reproduction of the standard curve used in this procedure. The points on the curve are average values of absorbance versus concentration for three separate runs of the procedure. Maltose was used as the standard because it is the only reducing sugar produced by β - amylase. The same procedure could be adapted for use with other enzyme systems dependent only upon the reducing sugar produced.

In order to prove linearity for the results of the colormetric procedure, a mixture of raw and extrusion cooked grain was analyzed. Table XIV lists the mixtures of the material and the results of the determinations. All determinations were made in triplicate. Figure 4 shows the linearity obtained. A good correlation appears between the cooked grain content and the degree of starch damage as determined by this procedure. Based on the results of this experiment, this procedure was assumed to be valid over the range of values determined.

The mannometric apparatus used for the invitro gas production work was different from that described by Roth (70) and Trei, et al. (9). Figure 5 is a photograph of the apparatus in operation. The device was simple to construct. The main requirement was that care was necessary to insure each

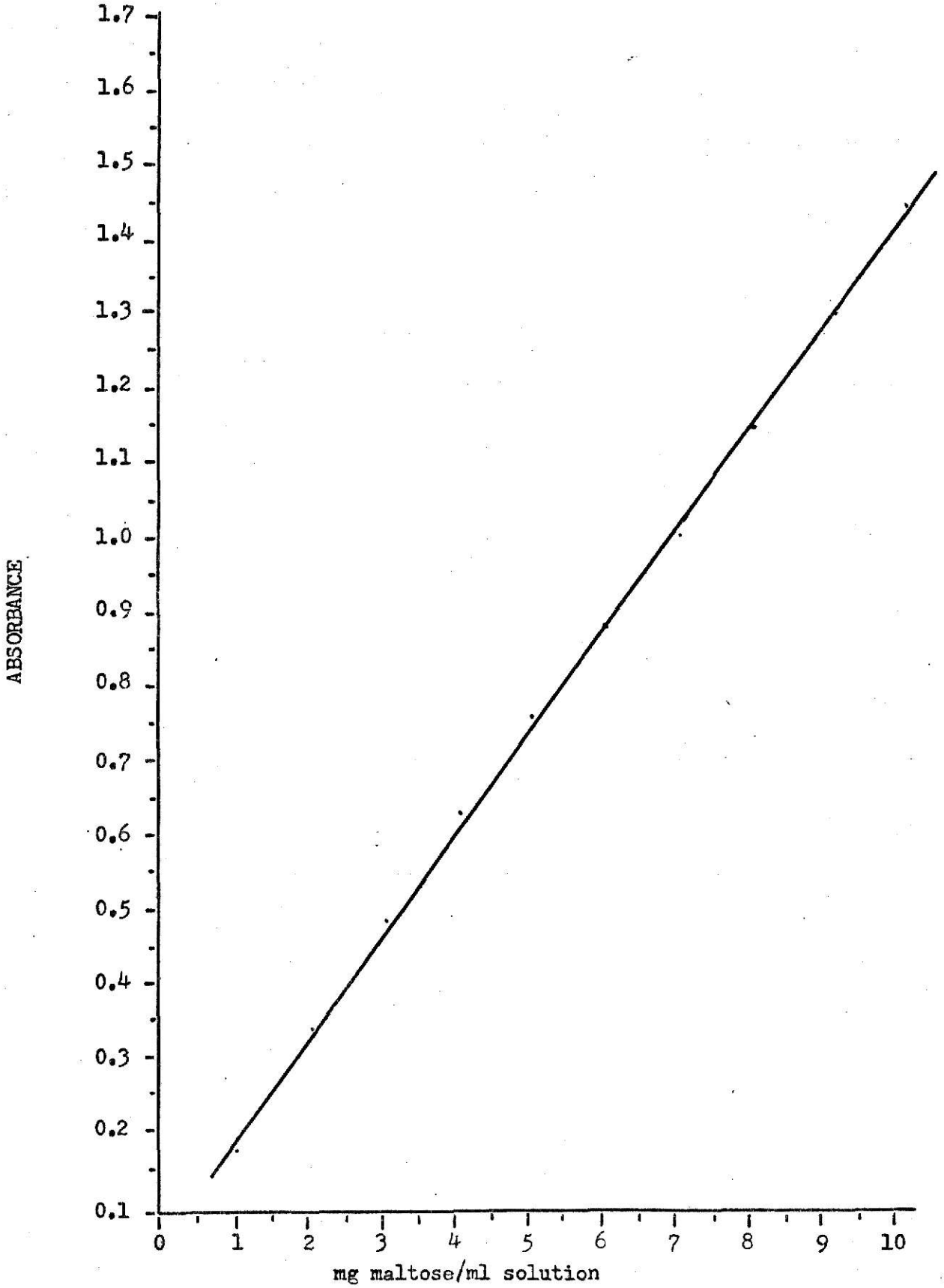

Figure 3. Standard curve for colorimetric procedure.

Table XIV. Effect of varying amounts of cooked grain on linearity of colorimetric procedure

Sample #	Raw corn[a] (mg) ( )	Cooked corn[b] (mg) ( )	Percent of sample as cooked grain	Starch damage[c] ($\frac{\text{mg maltose}}{\text{gm sample}}$)
1	1000	-	0.0	60.0
2	800	200	20.0	114.0
3	600	400	40.0	158.0
4	400	600	60.0	196.0
5	200	800	80.0	243.0
6	-	1,000	100.0	294.0

a) Finely ground corn

b) Finely ground corn extrusion-cooked

c) Average of triplicate determination

device was constructed the same as the rest. This was necessary to insure that results from one device would be the same if identical material were run in a different apparatus. A deficiency or problem of the apparatus is that the results are unique to that equipment and probably could not be duplicated in another laboratory. This does not delete from the value of the procedure as a quality control technique as the results are relative to the standard selected.

This procedure suffers from the fact that a large amount of time was required to complete the assay (3 hours). Run time can be cut to two hours but it was felt that some accuracy would be lost. The main advantage of this procedure is that the operator is free during most of the digestion period to accomplish other things in the laboratory as the procedure does not require constant attention. If immediate results are a requirement, the disadvantages probably outweigh the advantages. One advantage of a technique of this type is its correlation with in vivo carbohydrate digestion. Also, the same apparatus can be used as an artificial rumen for research purposes, as was done following the completion of the work reported here.

The samples used in this comparative study are the same samples used in the previously described work. The samples vary widely in their degrees of starch damage and should represent a good cross section of a total population. The analytical procedure was performed as described earlier with the results of each analysis shown in Table XV. The values represented are the averages of triplicate determinations. For brievity, the column headings are as follows: "Sung's" to describe the method using reducing sugar determination by titration, "colorimetric" for the colorimetric procedure, and "gas" for the mannometric method.

The statistical analysis for this study was done using a computer

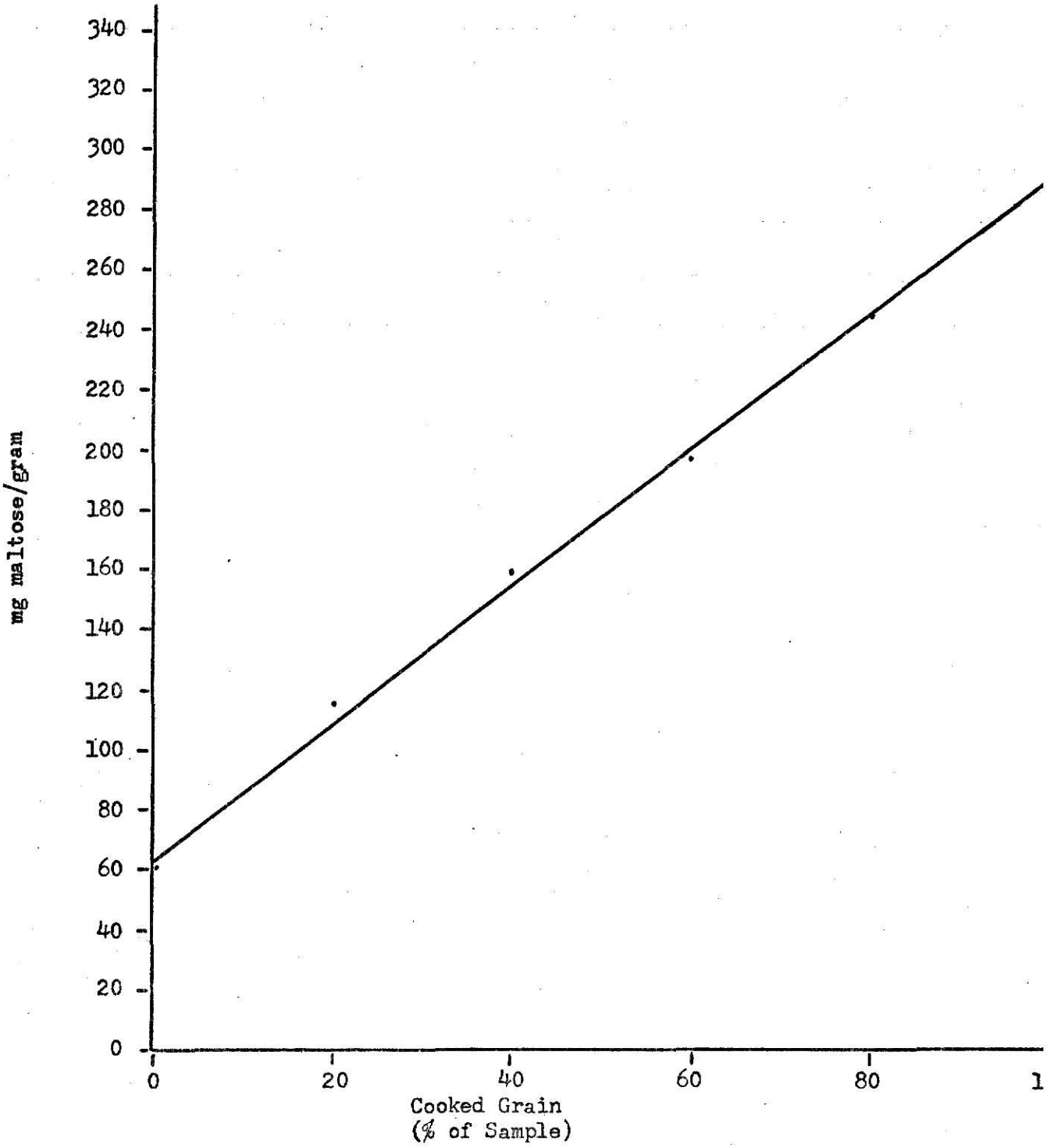

Figure 4. Linearity determination for colorimetric method.

THIS BOOK CONTAINS SEVERAL DOCUMENTS THAT ARE OF POOR QUALITY DUE TO BEING A PHOTOCOPY OF A PHOTO.

THIS IS AS RECEIVED FROM CUSTOMER.

Figure 5. Photograph of manometric apparatus.

program. This program calculates the coefficient of correlation directly, and gives the line equations and analysis of variance table. The analysis was performed on the results of each procedure for each grain, then on the combination of analytical results in all combinations. This was necessary to determine if one procedure is more fitted to a particular grain and, also, to indicate areas where a procedure may give erroneous results.

Perhaps the most useful information gained from this work was the coefficients of correlation. This figure indicates the degree to which the results from one analytical procedure correspond to the results of a different procedure on the same sample. It is not necessary to have the results in the same units (ie. mg maltose/gm) in order to calculate this value. Table XVI shows the results of this statistical treatment for each combination of analytical procedures performed on the individual grains. The only value of questionable significance is that resulting from the comparison of Sung's method with the colorimetric procedure. There is evidently some compound in grain sorghum, other than reducing sugars produced by hydrolysis, that tends to oxidize the DNS or that has a characteristic absorbance at 540 nm. A thorough search of the literature did not reveal the answer but this author tends to believe the latter hypothesis.

Table XVII shows the results of the statistical analysis when the individual data from the grains were combined to form the population.

The results of this work indicate that each analytical procedure would be adequate for quality control work. There was more varibility found in testing all procedures than had been indicated in the literature. This can be explained by the fact that samples used here demonstrate much higher degrees of damage than those indicated in the literature. The material used in previously reported work was normally flour which had been milled in a

Table XV. Results of starch damage determinations by each procedure

Material	Sample No.	Sung's (mg maltose/gm)	Colorimetric (mg maltose/gm)	Gas (ml gas/hr/gm)
Milo	P-1-1	211	240	35.37
	P-1-2	177	223	30.79
	P-1-3	220	282	39.50
Corn	P-2-1	226	274	37.69
	P-2-2	223	271	38.46
	P-2-3	232	284	38.01
Wheat	P-3-1	235	318	38.78
	P-3-2	198	258	36.85
	P-3-3	228	305	39.71
Corn	P-C-1	227	294	39.12
	P-C-2	182	227	34.26
	P-C-3	171	216	31.84
Wheat	P-W-1	258	337	39.67
	P-W-2	199	258	35.23
	P-W-3	187	241	31.23
Milo	P-M-1	251	290	41.85
	P-M-2	173	253	32.78
	P-M-3	167	238	30.60

normal fashion. This would hardly compare to the ground whole grain used in the extrusion process for this work.

In order to convert the results of one procedure to the equivalent units of another procedure, the line equations are included in Table XVIII. These equations are the results of statistical analysis of the entire sample population. The line equations for the other sample combinations were calculated but no significant differences were found, therefore, these equations were not included in this work.

The analysis of variance for deviation from a linear relationship to a curvilinear relationship was also calculated for all sample combinations but in no case were the deviations from linear great enough to be significant at any normal level.

As an example of the dispersion obtained when the procedures are compared, Figure 6 is included. It is interesting to note the appearance of wide dispersion, yet, the coefficient of correlation is nearly 0.9. The graphs for the other sample data combinations are not included as the line equations and coefficient of correlation values adequately describe their relative dispersion.

Table XVI. Coefficients of correlation for determinations on individual grain

Grain	Number of samples	Sung's X Gas	Sung's X Colorimetric	Colorimetric X gas
Corn	6	0.9664[a]	0.9747[a]	0.9033[a]
Wheat	6	0.8501[b]	0.9942[a]	0.8762[a]
Grain Sorghum	6	0.9648[a]	0.8014[b]	0.9230[a]

a) Significant at P - 0.01

b) Significant at P - 0.05

Table XVII. Coefficient of correlation for determinations on combinations of data from all grains [(a)]

Combinations of grains	Number of samples	Sung's X gas	Sung's X Colorimetric	Colorimetric X gas
Corn and Grain sorghum	12	0.9592	0.8700	0.8584
Corn and Wheat	12	0.8993	0.9577	0.8642
Grain sorghum and wheat	12	0.9236	0.8755	0.8344
Corn, wheat and grain sorghum	18	0.9283	0.8893	0.8395

a) All values are significant at P - 0.01

Table XVIII. Line equations for comparison of results of procedures used

Axis x	Axis y	Line equation
Sung's vs colorimetric		$y = 1.0664x + 44.115$
Sung's vs gas		$y = .1172x + 11.699$
Colorimetric vs gas		$y = 0.0856x + 13.2056$

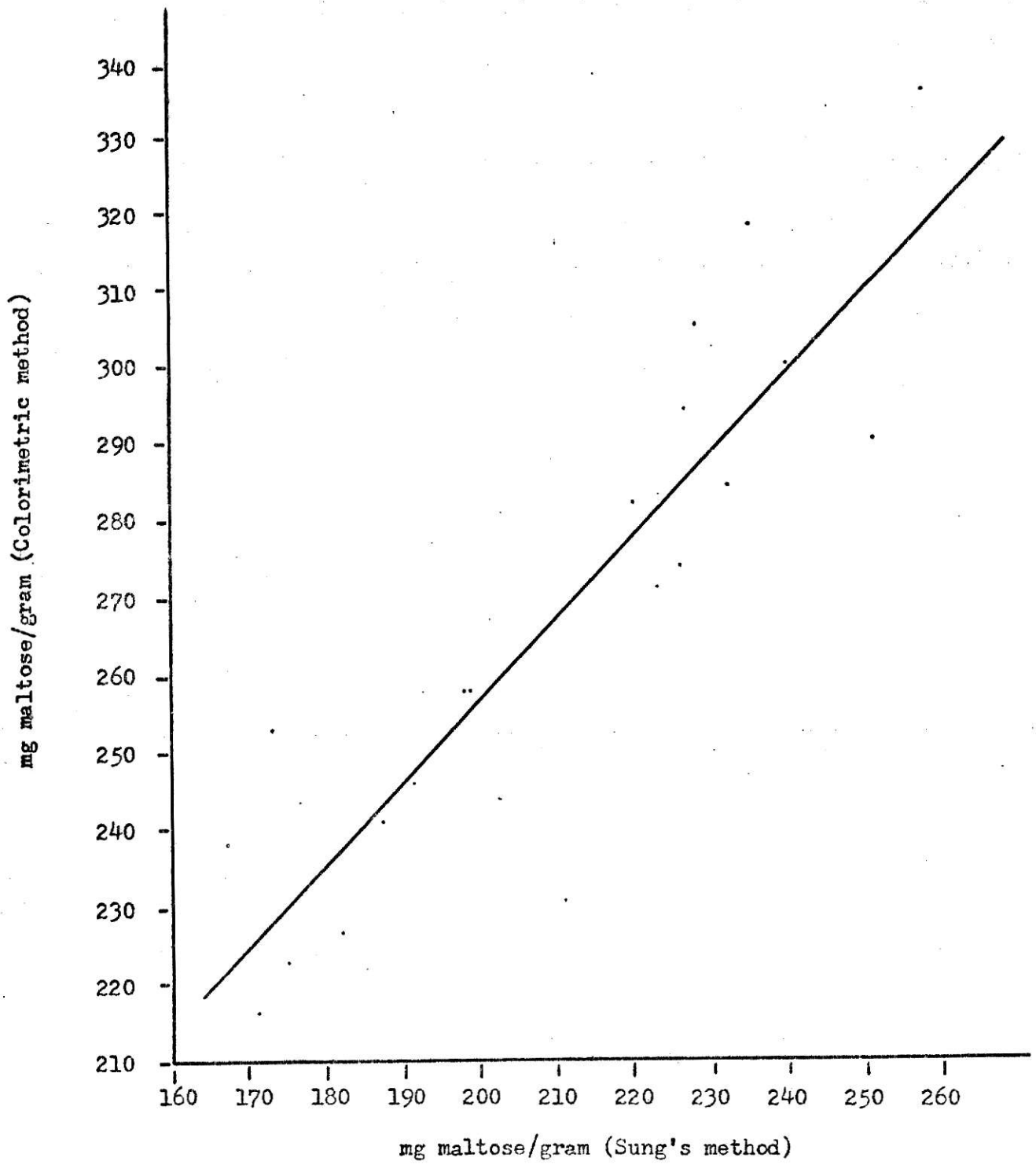

Figure 6. Sung's method vs. Colorimetric method for combined data.

SUMMARY

Samples of various particle size classifications of feed grade cereal grains were processed under normal production conditions of extrusion cooking. It was found that there was a definite linear relationship between the mean particle diameter of the raw material and the degree of starch damage sustained during processing. More importantly, a significant decrease in variation was found when the resulting data was analyzed for curvilinearity. This would suggest that perhaps the degree of damage may be related to surface area as well as particle diameter.

It was noted that moisture added prior to processing had a tendency to increase the degree of starch damage. The grain sample which was tempered for a period of time prior to grinding produced a larger particle size but the degree of starch damage was not significantly different than that observed when the moisture was added during processing. This would suggest that total moisture content had an affect on the extrusion process but no additional starch cooking was effected by moisture additions in the grinding stage.

Three methods of starch damage evaluation were compared in this investigation. A colorimetric procedure was developed during this work to decrease the time of analysis. It was found that all three methods were acceptible as quality control procedures, with the longer titration method being the most accurate. Coefficients of correlation were determined as comparison values for the methods. The colorimetric method showed some interference from some compound in grain sorghum with the values showing more variability than expected. The colorimetric procedure has the advantage of saving a great deal of time and eliminating all standard solutions. Line equations are provided in order to describe dispersion and to be able to convert the value obtained by one procedure to units of another procedure.

LITERATURE CITED

1. Erwin, E.S. Processing Grains for Cattle. Feedstuffs, Vol. 39., No. 7. p. 29. (1967).

2. Hubbert, F., Taylor, B., Hale, W.H., Carey, W.C. and Stanley, E.B. Pelleted rations for feeder cattle. Arizona Agr. Expt. Sta. Cattle Feeders' Day Report. (1962).

3. Matshshima, J.K. Colorado tests new extrusion process for flaking grain in cattle ration. Feedstuffs. 41, No. 15. p. 39. (1969).

4. Ensor, E.S. Special diets for the production of low fat milk and more efficient gains in body weight. J. Dairy Sci., 42:189 (1959).

5. Ensor, E.S. Pressure cooking grains for cattle rations. Feedstuffs. Vol. 38, No. 7. (1966).

6. Hale, W.H., Cuiten, L., Saba, W.J., Taylor, B. and Theurer, B.J. Effect of steam processing and flaking milo and barley on performance and digestion by steers. J. Animal Sci., 25:392 (1966).

7. Garrett, W.M. Comparitive feeding value of steam-rolled or ground barley and milo for feedlot cattle. J. Animal Sci., 24:726 (1965).

8. Hale, W.H., Saba, W.J., Cuiten, L., Mehen, S., Justed, T., Figroid, W., Taylor, B., and Theurer, B. Digestion of steam processed milo and barley by cattle. Arizona Cattle Feeders' Day Report (1965).

9. Trei, J., Hale, W.H., and Theurer, B. Affect of grain processing on *in vitro* gas production. J. Animal Sci., 30:825 (1970).

10. Hale, W.H., Grain processing as it effects beef cattle. Presented at a Feed Processing Seminar. Kansas State University. October 1970. (Mimiograp

11. McGinty, D.D. and Breuer, L.H. Digestibility of dry and reconstituted sorghum by cattle. (abst.) J. Animal Sci., 26:223 (1967).

12. McNeil, J.W. Texas Agr. Experiment Sta. Consolidated Report. No. PR-2775-2280 (1970).

13. Husted, W.T., Mehen, S.M., Hale, W.H., Little, M. and Theurer, B. Digestibility of milo processed by different methods. J. Animal Sci., 27:533 (1968).

14. Osman, H.G., Theurer, B., Hale, W.H., and Mehen, S.M. Influence of grain processing on *in vitro* enzymatic starch digestion of barley and milo. J. Animal Sci. 25:593 (1966).

15. Cowman, G.L. The utilization of cooker-extruder processed sorghum grain by growing finishing swine. An unpublished PhD dissertation. Kansas State University, Manhattan, Kansas (1970).

16. Riker, J.T., Perry, T.W., Pickett, R.A. and Curtin, T.M. Influence of various grains on the incidence of esophagogastric ulcers in swine. J. Animal Sci., 26:731 (1967).

17. Pickett, R.A. and Perry, T.W. Effect of methods of preparation and feeding of corn on the performance of growing-finishing swine. Purdue Swine Day Progress Report 625 (1966).

18. Newer, A.J., Perry, T.W., Pickett, R.A., Curtin, T.M., Featherston, W.R., and Befson, W.H. Value of various additives to ulcer producing gelatinized corn diets fed to swine. J. Animal Sci. 24:113 (1963).

19. Meyer, R.M., Bartley, E.E.. Deyoe, C.W., and Colenbranuer, V.F. Feed processing I. Ration effects on rumen microbial protein synthesis and amino acid composition. J. Dairy Sci., 50:1327 (1967).

20. Colenbrander, V.F., Bartley, E.E., Morrill, J.L., Deyoe, C.W., Meyer, R.M. and Pfost, H.B. Feed Processing IV. Effect of feeding expanded grain and ground hay on concentration of rumen ammonia. J. Dairy Sci., 51:1974 (1968).

21. Bartley, E.E., Deyoe, C.W., and Pfost, H.B. A new urea product for ruminants. Feedstuffs 40 (17) p. 9. (1968).

22. Chalupa, W. Problems in feeding urea to ruminants. J. Animal Sci. 27:207 (1968).

23. Colenbrander, V.F., Bartley, E.E., Morrill, J.L., Deyoe, C.W. and Pfost, H.B. Feed Processing II. Effect of feeding expanded grain and finely ground hay on milk composition, yield and rumen metabolism. J Dairy Sci. 50:1966 (1967).

24. Husted, W.T., Mehen, S.M., Hale, W.H., Little, M. and Theurer, B. Digestibility of milo processed by different methods. J. Animal Sci. 27:231 (1968).

25. Osman, H.G., Theurer, B., Hale, W.H. and Mehen, S.M. Influence of grain processing on invitro enzymatic starch digestion of barley and milo. J. Animal Sci., 25:593 (1966).

26. Helmer, L.G., Bartley, E.E., and Deyoe, C.W. Feed Processing VI. Comparison of Starea, urea, and soybean meal as protein sources for lactating dairy cows. J. Dairy Sci., 53:883 (1970).

27. Stiles, D.A., Bartley, E.E., and Deyoe, C.W. Feed Processing VII. Effect of an expansion processed mixture of grain and urea (Starea) on rumen metabolism in cattle and on urea toxicity. J. Dairy Sci. 53:1436 (1970).

28. Helmer, L.G., Bartley, E.E., Deyoe, C.W., Meyer, R.M. and Pfost, H.B. Feed Processing V. Effects of an expansion-processed mixture of grain and urea (Starea) on nitrogen utilization in vitro. J. Dairy Sci. 53:330 (1969).

29. Miller, F.D. "Composition of Cereal Grains and Forages" Pub. No. 585. NAS-NRC Washington, D.C. (1958).

30. Whistler, R.L., ed. Starch: Chemistry and Technology. Vol. II. Academic Press. N.Y. (1967).

31. Vineyard, J.M., Bear, R.P., MacMasters, M.M. and Deatherage, W.L. Development of "amylomaize" -Corn hybrids with high amylose starch: II Results of breeding efforts. Agron. J., 50:595 (1958).

32. Wolf, M.J. Preparation and some properties of hemicelluloses from corn hulls. Cer. Chem., 30:451 (1953).

33. Beadle, J.B., Just, D.E., Morgan, R.E., and Reiners, R.A. Composition of corn oil. J. Am. Oil Chemist's Soc., 42:90 (1965).

34. Bresseani, I.R. and Mertz, E.T. Studies on Corn Proteins. IV. Protein and amino acid content of different corn varieties. Cereal Chem. 35:227 (1958).

35. Sanders, E.H. Developmental morphology of the kernel in grain sorghum. Cer. Chem. 32.12 (1955).

36. Collier, J.W. Caryopsis development of several grain sorghum varieties and hybreds. Crop Sci., 3:419 (1963).

37. Miller, G.D., Deyoe, C.W. Walter, T.L. and Smith, F.W. Variation in protein level in Kansas sorghum grain. Agron. J. 56:302 (1964).

38. Skoch, L.V. Amino Acid Composition of the Fractionated Proteins of Sorghum Grain and High-Lysine Corn. An unpublished Master's degree thesis. Kansas State University. (1969).

39. Schoch, T.J. and Maywald, E. Microscopic examination of modified starches. Anal. Chem. 28:382 (1956).

40. Haworth, W.N. The Constitution of Sugars. Arnold and Co., London. p. 94. (1924).

41. Samec, M. Some Recent Physicochemical Studies of Starch. J. Polymer Sci. 23:801 (1957).

42. Bois, E. and Vallicies, G. Crystallization of amylose. Can. J. Res. 23B:214-216 (1945).

43. Bourne, E.J. and Peat, S. The Amylose component of waxy maize starch. J. Chem. Soc. 5:9 (1945).

44. Barker, C.C., Hirst, E.L. and Young, G.T. Linkage between the repeating units in the starch molecule. Nature 147:296 (1940).

45. Greenwood, C.T. Aspects of the physical chemistry of starch. In Advances in Carbohydrate Chemistry, Vol. XI. Academic Press, Inc., New York, N.Y. (1956)

46. Dunbvow, B.A. and Beckman, C.O. Ultracentrifugal and viscometric studies of amylose acetates. J. Phys. Coll. Chem. 51:107 (1947).

47. Radley, I.A. Starch and its Derivitives. 4th ed. Chapman and Hall LTD., London. (1968).

48. Sullivan, J.W. Studies on Starch Gelatinization. An unplublished dessertation. Kansas State University, Manhattan, Kansas (1966).

49. Leach, H.W., McCowen, L.D. and Schoch, T.J. Structure of the Starch Granule. I Swelling and Solubility Patterns of Various Starches. Cer. Chem. 36:534 (1959).

50. Sandstedt, R.M. Photomicrographic studies of wheat starch III. Enzymatic digestion and granule structure. Supplement to Cer. Chem. 32:17 (1955).

51. Schwimmer, S. The role of maltose in enzymolysis of raw starch. J. Biol. Chem., 161:219 (1945).

52. Leach, H. W., and Schock,T.J. Structure of the starch granule II. Action of Various Amylases on Granular Starches. Cer. Chem. 38:34 (1961).

53. Sair, L. Heat-Moisture Treatment of Starch. Cer. Chem., 44:8 (1966).

54. Bultrose, M.S. Submicroscopic development and structure of starch granule in cereal endosperms. J. Ultrastructure Research 4:231 (1960).

55. Seib, P. Starch Gelatinization-Chemical and Physical. Feedstuffs, Vol. 43. No. 12. p. 44. (1971).

56. Bergland, R. Heat Processing of Grain for Beef Cattle Discussed at Pacific S.W. Conference. Feedstuffs, Vol. 39, No. 1, p.2 (1967).

57. Leach, H.W. Gelatinization of Starch. In. Starch: Chemistry and Technology. R.L. Whistler Ed., Academic Press, New York and London, 1965.

58. MacMasters, M.M. The return of birefringence to gelatinized starch granules. Cer. Chem., 30:63 (1953).

59. Watson, S.A. Methods of Carbohydrate Chemistry, R.L. Whistler, Ed. Academic Press, N.Y. (1964).

60. Jones, C.R. The production of mechanically damaged starch in milling as a governing factor in diastatic activity of flour. Cereal Chem. 17:133 (1940).

61. Atkinson, F.M. and Fuehrer, F. Effects of overgrinding on flour characteristics. Baker's Dig., 34 (3):49 (1960).

62. Sandstedt, R.M. and Mattern, P.J. Damaged starch: Quantitative determination in flour. Cereal Chem. 37:379 (1960).

63. Dadswell, I.W. and Gardner, J.F. The relation of alpha-amylose and susceptible starch to diastatic activity. Cereal Chem. 24:79 (1947).

64. Sullivan, B., Anderson, M.L., and Goldstein, A.M. The determination of starch damage in flour. Cereal Chem. 39:155 (1962).

65. Donelson, J.R. and Yamazaki, W.T. Note on a rapid method for the estimation of damaged starch in soft wheat flours. Cereal Chem. 39:460 (1962

66. Donelson, J.R. and Yamazaki, W.T. Note on the application of the Donelson-Yamazaki damaged starch procedure to high damage wheat flour and starch. Cereal Chem., 46:567 (1969).

67. Medcalf, D.G. and Gilles, K.A. Determination of starch damage by rate of iodine absorption. Cereal Chem. 42:546 (1964).

68. Williams, P.C., and Fegol, K.S.W. Determination of damaged starch in flour. Cereal Chem. 46:56 (1969).

69. Sung, A. Enzymatic evaluation of changes in processed grain. An unpublished Master's Thesis. Kansas State University, Manhattan, Ks. (196

70. Roth, M. Factors Affecting starch damage during the steam flaking of sorghum grain. An unpublished Master's Thesis, Kansas State University, Manhattan, Kansas. (1972).

71. Headley, V. and Pfost, H.B. Describing particle size. In Feed Manufacturing Technology. Ed. H.B. Pfost. American Feed Manufactures Association. Washington, D.C. (1970).

72. Clark, J.M. Jr. Experimental Biochemistry. W.H. Freeman. San Francisco. (1964).

73. Snedecor, G.W. and Cochran, W.C. Statistical Methods. The Iowa State University Press. Ames, Iowa (1967).

ACKNOWLEDGMENTS

I would like to thank Dr. Charles W. Deyoe, my major professor, for his guidance and assistance in the research and preparation of this mannuscript.

Appreciation is extended to Dr. William J. Hoover, head of the Department of Grain Science and Industry, for the use of the facilities and equipment to conduct this work.

Thanks are also given to Dr. P.A. Seib and Dr. E.E. Bartley for serving on the advisory committee and reviewing the manuscript.

I wish to thank Fred Anstaett and Mike Duncan, fellow students, for their assistance during preparation of this manuscript.

FACTORS AFFECTING STARCH DAMAGE AND ITS MEASUREMENT IN EXTRUSION-COOKED CEREALS

by

KEITH CHARLES BEHNKE

B.S., Kansas State University, 1968

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Grain Science and Industry

KANSAS STATE UNIVERSITY

Manhattan, Kansas

1973

Samples of feed grade yellow dent corn, sorghum grain and hard red winter wheat were obtained for trials to determine the effect of average particle diameter on the degree of starch damage sustained during controlled extrusion-cooking. Samples for the first trial were ground using different screens in two hammermills. Average particle diameter and moisture content were determined on each sample using standard methods.

Samples for the second trial were processed in a similar fashion except that the finer grind was obtained using a micro-pulverizer. Particle size and moisture content were again determined using standard procedures.

All samples for trials one and two were processed using an extrusion-cooker under controlled conditions. Starch damage was determined on each sample using the rate of attack by amylase and determining the reducing sugar concentration following enzyme hydrolysis.

A significant negative coefficient of correlation between average particle size and degree of starch damage was noted in both trials. This would indicate that, under controlled conditions, less starch damage would occur as average particle size of the raw material increased.

Two published methods of starch damage evaluation were compared with a colorimetric procedure developed during this investigation. One of the control methods measured the amount of gas produced by yeast and an enzyme over a three hour period to express starch damage. The second procedure used the rate of enzymatic attack and reducing sugars produced to indicate starch damage. The experimental colorimetric procedure measured the reducing sugars liberated by amylase using a reaction with dinitrosalicylic acid (DNS) and measuring the concentration colorimetrically.

It was found that all three methods were acceptable as quality control procedures with the colorimetric procedure requiring the least amount of time.

Coefficients of correlation between all methods were high significant. Line equations and regression analysis were provided to describe dispersion and to convert values from one procedure to those of a different procedure.

It was noted that moisture added prior to processing caused a tendency toward higher degrees of starch damage during controlled extrusion-cooking. Grain samples tempered to a higher moisture level prior to grinding produced a larger particle size but the degree of starch damage sustained was not significantly different than that observed when moisture was added during processing. This would suggest that total moisture content had an effect on the extrusion process but no additional starch damage was obtained from tempering grain prior to processing.