MOISTURE-TEMPERATURE RELATIONSHIP IN
STARCH GELATINIZATION

by

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INTRODUCTION

Widespread uses of starch are due mainly to two important properties, film and viscous paste formation. These two characteristic properties have been studied under varied conditions by many research workers. Formation of films and viscous pastes is altered by factors such as H-ion concentration, composition of the starch, structure of the granule, and time and temperature of cooking. These basic factors affect the final starch product in appearance, texture, yield, flavor, and digestibility. This investigation was undertaken to relate moisture and temperature to the gelatinization and pasting properties of various starches.

The effect of percent moisture and degree of heating as related to the percent gelatinization of the starch is a problem faced by manufacturers of starch-containing foods. The work reported here was done to clarify the effect of different moisture levels on starch gelatinization and pasting when subjected to two different temperatures, 100°C. and 200°C.

REVIEW OF LITERATURE

Starch Granule Structure

Raw starch is composed of minute cells or granules so small that it takes three quarters of a trillion to make one pound of corn starch (14). Starch granules from different sources show variety in shape and size and other superficial physical characteristics. The final shape of the starch granule depends to a

large extent on the surrounding environment during its formation
(30).

Internally, the granular structure of starch can be ex-
plained in terms of the attractive forces between the large carbo-
hydrate molecules. This concept, generally accepted today, was
formulated by K. H. Meyer as cited by Leach et al. (35). Accord-
ing to this theory, the intermixed linear and branched starch
molecules are arranged in the granule in a radial fashion. Where
ever linear segments of either branched or linear molecules par-
allel one another, hydrogen bonding forces pull the chains to-
gether into associated crystalline bundles or micelles. A long
linear chain may conceivably pass through a number of such micel-
lar areas while the outer fringe branches of the ramified mol-
ecule may participate in several separate micelles. Hence, the
crystalline areas hold the granule together, permitting swelling
in water at certain temperatures but preventing dispersion and
solution of the individual starch molecules. In the spaces be-
tween the micelles, the chains and branches are more disordered
and hence, less densely packed (35). The starch granule shows
the dark cross pattern of a sphero-crystal under the polarizing
microscope because the associated bundles of molecules represent
an essentially crystalline lattice. The associative forces be-
tween the molecules can be overcome if sufficient energy is
applied (10). Ponsler (40), in contrast, reported that the regu-
lar and uniform arrangement shown by starch when examined under
X-rays is destroyed when the granule is crushed, indicating that
the regularity is not that of a crystal structure.

As has been indicated, the starch granule is composed of two different fractions, namely the linear amylose and the branched amylopectin, each possessing a different molecular constitution. There has been some confusion about the naming of these two fractions and, furthermore, the existence of amylopectin was doubted for some time. The names alpha- and beta-amylose were used to distinguish the two fractions separated on the basis of their solubility characteristics. The term amylopectin was introduced by Maquenne and Roux (37), in 1905, as the name for the non-saccharifiable portion of starch (32). Samec and Haerdtl (45), in 1920, reported that starches consist of a highly viscous, electro-conducting fraction which precipitates on electrodialysis and a non-viscous, non-electro-conducting fraction which is not precipitated. The amounts vary according to the origin of the starch, their relative proportion influencing the viscosity, resistance to amylase, and water absorption. In 1926, alpha-amylose was considered the equivalent of amylopectin. Later, in 1933, the two terms were used synonymously (32). In 1934, Maquenne and Roux (37) described the concept of starch retrogradation.

The amylopectin molecule is considered to be made up of a large number of branches, each approximately 25 glucose units in length. The branches themselves are combined in such a way that the free reducing groups of the glucose unit of a branch are linked through the sixth hydroxyl group of a glucose in an adjacent branch (alpha 1-6 glucosidic linkage), thus forming a ramified structure. The evidence for a 1-6 linkage in amylopectin

has been well substantiated by the isolation of beta-D-isomaltose
as the crystalline acetoacetate from an acid-hydrolyzate of amy-
lopectin (16). The branched portion is thus a mixture of polymers
of unbranched chain molecules of varying length (19). Amylo-
pectin appears to contain a small amount of phosphoric acid as
part of the molecule. According to Czaja (12), amylopectin is
concentrated at the periphery of the granules. Czaja also de-
duced from the behavior during swelling of the starch granule
that amylopectin is mainly responsible for the optical activity
of the granule. Amylopectin is more resistant to attack by beta-
amylase since the action of this enzyme is limited to the open-
end chain of the branched molecule. Amylopectin yields, upon
partial acid hydrolysis, various amounts of glucose, maltose,
maltotriose, tetraose and pentaose, isomaltose, panose, and
nigerose.

Amylose, or the linear fraction, consists of glucose units
exclusively united through alpha 1-4 glucosidic bonds. It is hy-
drolyzed by both alpha- and beta-amylase, giving as products, a
mixture of glucose and maltose (54), and in addition, some malto-
triose, tetraose, and pentaose. Amylose appears to assume a ran-
domly coiled configuration in solution when it is complexed with
iodine, fatty acids, and higher alcohols. The amylose-amylopectin
ratio of different sources of starch are known to vary widely al-
though many common cereal starches, such as wheat and corn, usual-
ly have a 1:3 amylose-amylopectin ratio (19,20). It must be
recognized that the determination of amylose-amylopectin ratios
are dependent on the method of separation and that certain

artifacts might be introduced during the separation. Spark (51) found the X-ray diffraction pattern of amyloses depended more on the method of separation than on its source.

The properties of the isolated amylose differ from those of amylopectin in several respects (19,39,50). Amylose is slightly soluble in cold water but is soluble in boiling water to the extent of 7 to 10 percent, and the small amount that dissolves will rapidly retrograde from solution. It is completely hydrolyzed by beta-amylase. It has a great tendency to absorb iodine and produces a sharp, V-type X-ray pattern. There is evidence that amylose possesses a helical structure when complexed with iodine, high molecular weight fatty acids, or alcohols. On the basis of a starch-iodine complex, the blue color, on addition of iodine, may be explained adequately. The iodine molecules are assumed to be deposited within the amylose spiral, forming a complex which produces the blue color.

Amylopectin is quite soluble in water and forms a colloidal solution. Its tendency to absorb iodine is slight; it is degraded by beta-amylase only to the extent of about 55 percent, and it does not produce a V-type X-ray pattern. Amylopectin has a globular shape, showing increased dilation and consequent higher viscosity in most solvents (19,49).

The Nature of Starch Gelatinization

Native starch granules are relatively insoluble in cold water. However, when heated in the presence of water, the granules become increasingly soluble.

When an aqueous suspension of granular starch is heated, it is presumed that the more amorphous regions undergo progressive hydration and swelling, thus forming an expanded network which gives the swollen granule its elastic character and which is responsible for most of the viscous qualities of a cooked starch paste (35). It should be pointed out, however, that starch paste is a heterogeneous system, each constituent of which may react differently toward changes in the conditions of measured viscosity (8).

Heating starch in the presence of water opens the micellar bundles, thereby allowing water to penetrate into the granules (10). Sandstedt (46) reported that approximately ten or more times the weight of starch in water was absorbed during the gelatinization process. Although the micelles are disrupted during swelling, there is a persistent network that continues to hold the granule together even though greatly expanded. This network may be broken by supplying high levels of energy, such as by autoclaving a dilute starch suspension. The beginning of a significant swelling of individual corn and wheat starch granules commences at 50°C. Although gelatinization may not be in evidence until 65°C. is reached, gelatinization is rapid and may be completed at 67.5°C. The process is dependent on the source of starch (4,26,44). The mean temperature of complete swelling of the individual granules of corn starch was reported by Arzickowski (3) to be 60.9°C. plus or minus 0.10°C. Gelatinization of corn starch begins at 64°C. and continues over a range of 80°C., the shape of the curve resembling the curve for tapioca starch (22).

The short chain amylose molecules were dissolved out of the gran-
ule. Generally, the large granules of any starch gelatinize at
a lower temperature than the smaller ones (31). The difference
in gelatinization of the granules, however, takes place over a
range of temperature of about 10°C. (30,48). With the aid of
polarographic and amperometric investigations, Taufel and co-
workers (52) found that smaller granules dissolve more slowly
than the larger granules in spite of the larger specific surface.
He concluded that the structure of the large granules was less
compact.

McMasters (36) stated that the bright segments within the
four arms of the Maltese cross decreased in size and eventually
disappear entirely. They usually persisted longer at the periph-
ery of the granule. When all birefringence had been lost, the
granule was said to be gelatinized. MacMasters (36) also ob-
served that birefringence reappeared in the outer portion of the
gelatinized granule upon the addition of iodine solution. This
occurred with starch which had been heated in water to 10 or 15°C.
above the temperature at which birefringence was completely lost.
These observations suggested that disorganization of the starch
granule occurring during gelatinization was incomplete and that
some organization was persistent even where birefringence of the
granule was no longer microscopically observable.

Gel formation of a starch is related to the hydration
capacity of the starch (15,32). Starches derived from various
sources differ widely in hydration capacity. Even starches from
different wheat varieties vary somewhat in hydration capacity,

but not nearly so much as the values for the relative viscosities of the respective solutions would indicate. Continued heating of gelatinized starch pastes produces a rapid decrease in the hydration capacity. This change is much more rapid for potato starch pastes than for wheat starch pastes (17).

Starches which have predominantly linear fractions, such as wrinkled pea, gelatinize with great difficulty and do not dissolve even after prolonged autoclaving. At the opposite end of the scale, the absence of the linear fraction such as in waxy maize, causes the granule to swell rapidly and disintegrate with continued cooking. Between these extremes are the intermediate gelatinization behavior of corn and wheat starch. These differences are due largely to the content of the linear fraction and the micellar organization within the granule (10).

There is some disagreement on the temperatures at which different starches gelatinize because of the pre-treatment of the starch, difference in varieties of the same species, and also because of different subjective interpretations of the gelatinization point (1,43).

When starch granules are heated in an excess of water, the degree of swelling appears to be a function of temperature. If insufficient water is present, the swelling is greatly inhibited (42). The optimum swelling of the granule is dependent upon an adequate supply of water. Adequate adjustment of available water and the rate of heating may be sufficient to maintain complete control over starch gelatinization.

Anker and Geddes (2), citing Katz's studies of the viscosity changes which occur in corn and potato starch on heating and stirring, concluded that the heat gelatinization curve was a result of two opposing factors: the progressive swelling and hydration of the starch granule which increases viscosity and the breakdown of the granule which decreases viscosity.

Kawamura and Takahashi (29) reported that gelatinization was influenced more by temperature than by heating time. The rise of temperature from 65°C. to 80°C. had great influence on the extent of swelling measured at 90°C. Halick et al. (18) reported that swelling of the starch granule began well before the effect of increased viscosity became apparent. He showed that if the rice flour-to-water ratio was increased (100 grams of rice flour and 400 mls. water), the rise in viscosity was quite abrupt and the point of departure of the curve from the base line could be taken as a measure of gelatinization temperature. According to Anker and Geddes (2), the temperature of transition and paste temperature at maximum viscosity decreased with an increase in starch concentration. They concluded that starch swelling was a first order reaction process and that granule disintegration was a second or higher order process. It was shown by Anker and Geddes (2) that with wheat starch, the first indications of swelling are related to the temperature and the concentration of the starch suspension. The higher the starch concentration is, the lower the temperature at which swelling first occurs. Where a 7 percent wheat starch suspension was gelatinized in water, no indications of swelling were apparent until approximately 86°C. was reached,

as judged by the Brabender Amylograph. From 86°C. to a peak at 93°C., the viscosity increased rapidly, thereafter decreasing because of the mechanical action of stirring on the swollen granule. When a 10 percent wheat starch suspension was gelatinized, the first indication of swelling was evident at 71°C. The peak viscosity occurred at a slightly higher temperature. There was evidence of greater mechanical breakdown of granules with higher starch concentrations. With 10 percent concentration, swelling of the granules occurred from 71°C. to about 93°C., or a range of 22°C. Any further increase in starch concentration resulted in a viscosity greater than could be measured by the Amylograph. It was not possible to demonstrate two stages of gelatinization for wheat starch at concentrations of 10 percent or higher of starch. Two stages were observed by Katz and Rientsma (27) for wheat starch by means of the Roentgen spectrum.

The changes of the structure of the starch granule on heating in water takes place in three discernible phases. During the first phase, water is slowly and reversibly taken up, and limited swelling occurs. The viscosity of the suspension does not increase noticeably. The granule retains its characteristic appearance and birefringence, and upon cooling and drying, no obvious changes can be observed. At approximately 65°C. (the exact temperature being a characteristic of the particular variety of starch) the second phase of swelling starts. The granule suddenly swells, increasing many times in size, absorbing a large quantity of water, and losing its birefringence rapidly. The second phase of swelling is also marked by a rapid rise in the viscosity of the

starch suspension, and the granules upon cooling are altered in appearance, most of them having lost their structure and birefringence. A small amount of the starch has become solubilized. During the third phase of swelling at elevated temperatures, the granules become almost formless sacs and the more soluble part of the starch is leached out (30).

When the granule is heated, the Maltese cross gradually enlarges at the intersection of its arms, i.e., at the hilum of the granule (36). According to Hollo et al. (23), this factor, reported by MacMasters and others, has a bearing in the process of paste formation of the starch. The indication of a temperature of gelatinization has a meaning only if conditions of the measurements are specified. Some of the factors studied by Hollo et al. (23) were moisture, H-ion concentration, dextrinization, and the presence of enzymes.

An interesting practical aspect of starch gelatinization, as related to bread baking, was reported by Betchel (6). Betchel reported that the gluten bound nearly all the water, and the starch bound almost none until the temperature was raised to 125°F. After that temperature, gluten lost its water-holding ability, and starch began to absorb water readily. As the temperature was progressively raised, more water was absorbed by the starch, causing gelatinization. The starch granules did not swell to their fullest extent because of the limited amount of water present.

The characteristic gelatinization properties of starches have been applied to pre-gelatinized starches used as food

thickening agents and stabilizers (53). Products made with pre-
gelatinized starch are usually more uniform and stable to storage
and freezing. They offer a considerable saving of production
time (13).

Degradation of the Starch Molecules by Beta-amylase

Much study has been devoted to the degradation of the starch
fractions by beta-amylase.

Before the enzyme can act appreciably on the starch, it must
be gelatinized and its internal structure disorganized so that
beta-amylase has the open-chain and reducing ends available to
its action. Beta-amylase (M.W. = 150,000) acts on the non-
reducing terminal ends of either the linear or the branched frac-
tions, successively splitting off maltose until its action is
stopped by a branch point or other anomalous linkage (49). The
fission products of amylose are maltose and maltotriose, and those
of amylopectin are maltose and beta-amylase limit dextrin.
Amylose dextrins (chain length 16-30) may be hydrolyzed by soy-
bean and crystallized sweet potato beta-amylase (24,25). Preece
and Shadaksharaswany (41), using soluble starch as the substrate,
reported that sugar production (assessed by the ferricyanide
titration) by beta-amylase shows a linear relationship with time
up to 15 percent hydrolysis. As soon as beta-amylase is mixed
with starch, there is an immediate increase in optical activity
which is ascribed to the formation of an enzyme-substrate complex
(7). Cleveland and Kerr (9) stated that when the enzyme makes
contact with an amylose molecule, this chain is hydrolyzed to

maltose before the enzyme attacks another chain. This was shown
by hydrolysis of amylose with a limited amount of beta-amylase.
Only sugars and unconverted residues which have nearly the same
average size as the original sample were obtained. Bailey and
Whelan (5) reported that the action pattern of beta-amylase was
always intermediate between single and multichain.

The single mechanism can be explained by the reaction:

$$E + Sn \rightleftharpoons (E + Sn) \rightleftharpoons G_2 + (ESn - 2) \rightleftharpoons 2G_2 + (ESn - 4) \text{ etc.}$$

The multichain mechanism is explained as follows:

$$E + Sn \rightleftharpoons (ESn) \rightleftharpoons E + Sn - 2 + G_2$$

Where E = enzyme, S = substrate, n = number of glucose units,
and G = glucose.

Their results showed that the enzyme removes several maltose
units during each encounter with a substrate molecule. It was
suggested that these changes in the pattern of action can be ex-
plained by visualizing the enzyme having several active centers
or that multiple reactions can occur at a single active center.
Schoch (47) reported that beta-amylase must come into exact
juxtaposition with the appropriate alpha 1-4 linkage, which it
then splits by contact catalysis. It is calculated that one mole
of crystalline malt beta-amylase will hydrolyze 250,000 glucosidic
linkages per minute. If the starch paste is kept for a long time,
starch tends to retrograde and in this form amylolysis is greatly
retarded (37).

Starch Retrogradation

If a starch paste is prepared and left standing at room temperature, the solubility equilibrium shifts so that the starch loses solubility and is less hydrated. This tendency has been termed retrogradation and is exemplified by increased opacity and syneresis occurring during aging of starch pastes (27,28). Retrogradation may be considered as the reverse of swelling. The individual molecules in the paste come together to form a large insoluble aggregate with an internal structure similar to that of the original granule. The amylose fraction retrogrades rapidly because the chain molecules are flexible and can readily orient to form crystallites. Branched chain molecules stay in solution much longer. Waxy starch pastes retrograde slowly (21).

Khim (33) has defined retrogradation as those changes in the physico-chemical state of hydrophilic colloids that take place in the solutions, pastes, and gels on aging. The retrograded portion is the coagulum separated out in the clear liquid which contains the un-retrograded portion. The retrograded starch consists of shapeless masses, apparently composed of small, finely-divided granular particles. They do not show double refraction in polarized light and may be readily redissolved by heating at approximately 90°C.

Retrogradation is retarded by agents which tend to disperse the starch, and is aided by those agents that coagulate starch. Dilute starch pastes or solutions of low starch concentration retrograde to form a precipitate, whereas heavy pastes gel, retrograde, and undergo syneresis. At greater concentrations, starch

is deposited until an equilibrium is reached. The extent of
retrogradation appears to be governed by the molecular weight of
the fraction and by the colloidal structure (34).

MATERIALS AND METHODS

Five cereal starches including wheat, corn, waxy corn, non-
glutinous sorghum, and German rice were analyzed for beta-amylase
susceptibility and maximum hot-paste viscosity changes following
pre-heat treatment at two temperatures, 100°C. and 200°C., using
five different moisture levels.

Commercial wheat starch (Starbake) was used as a source of
wheat starch. German rice and unmodified corn starches were ob-
tained from Corn Products Co., Argo, Illinois. Waxy corn starch
was obtained from American Maize Products. Non-glutinous sorghum
starch was obtained from the Department of Flour and Feed Milling
Industries, Kansas State University.

The work was divided into two parts according to the tempera-
tures used. The first part comprised the beta-amylolysis and hot
paste viscosity determinations after pre-treatment of the starch
at 100°C. The second part comprised these same determinations
after pre-treatment of the starch at 200°C.

Part I

Beta-amylolysis. In preparation of the starches for the
beta-amylolysis determination, 1.1 grams of starch was mixed thor-
oughly with distilled water to provide starches with moisture
contents as follows: 29, 38, 52, 68, and 99 percent. The

starches were then heated in an autoclave at 100°C. for five min-
utes. After autoclaving and cooling, additional water was added,
and the paste was passed through a silk gauze (G.G.54) in order
to disperse the suspension. The resulting starch suspensions
were diluted to 100 ml. with water and blended for 15 seconds in
a Waring blendor to insure uniform dispersion. It should be
pointed out here that although the blending of the starch pro-
vided a uniform suspension, its use did not increase the starch
susceptibility to beta-amylase action in a consistent way as
shall be demonstrated in the discussion of results.

Forty ml. of these suspensions were pipetted into 125-ml.
flasks, five ml. of acetic acid-sodium acetate buffer (pH 4.60)
were added, and the suspension was placed in a water bath at 30°C.
Five ml. of a solution containing 4 mg. of beta-amylase[1] were
added to initiate the beta-amylolysis.

Five ml. of the starch-enzyme suspension were vacuum fil-
tered (medium-size sintered glass filters were used) immediately
after the addition of the enzyme. This sample was used as the
"zero" time sample. Three ml. of the filtrate were pipetted into
test tubes containing 10 ml. of 0.1 N solution of alkaline potas-
sium ferricyanide. The test tubes were immersed in a boiling
water bath for 20 minutes. At the end of the 20-minute boiling
time, the tubes were immersed in a cold water bath and brought to
room temperature. The contents of the test tubes were trans-
ferred to 125-ml. flasks with the aid of 25 ml. of acetic

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[1] Wallerstein Company, New York, New York.

acid-salt solution. The non-reduced ferricyanide was titrated
with 0.1 N sodium thiosulfate solution, using 1 ml. of potassium
iodide-iodine soluble starch reagent as the indicator. A sample
of the starch-enzyme suspension was taken every hour for three
hours.

Hot Paste Viscosity. The hot paste viscosity determinations
were made by using 45 grams of the starch (14% M.B.) and suf-
ficient water to obtain the same moisture content as employed in
the beta-amylolysis determinations. After pre-heating the
starches, they were suspended in 400 ml. of disodium phosphate-
citric acid buffer (pH 5.35). The maximum hot-paste viscosity
was recorded with the Brabender amylograph by heating at the rate
of 1.5°C. per minute. Maximum viscosity was taken as the perti-
nent measure of paste formation.

Part II

Beta-amylolysis. The starch suspensions for the beta-
amylolysis determination for pre-heat treatment at 200°F. were
prepared in exactly the same manner as for Part I under beta-
amylolysis. Ten grams of starch were used instead of 1.1 grams
and the amount of water adjusted to obtain the same moisture
levels in the suspensions as previously specified.

Stainless steel tubes (6¼ inches long and 0.5 inch in in-
ternal diameter) hermetically sealed by means of a steel cap with
a lead gasket were used as containers for the pre-heat treatment
of the starches. The heating at 200°C. was done in a Reed reel

oven[1] for five minutes. At the end of the heating time, the starches were transferred from the tubes and treated as described under Part I for beta-amylolysis. Because of the limited volume capacity of the tubes, 0.25 gram of starch was used for the 99.01 percent moisture level sample, and the water adjusted accordingly to obtain the desired moisture content.

Hot Paste Viscosity. The same stainless steel tubes as described above were used for the pre-heat treatment of the starches for this determination, except that the 45 grams of the starch-water mixture were equally divided between five tubes and heated at 200°C. in the Reed-reel oven for five minutes. At the end of the heating period the tubes were allowed to cool at room temperature. The starch paste was then transferred from the tubes and treated as described under Part I for hot-paste viscosity.

A control was prepared by mixing 45 grams of the raw starch with 400 ml. of disodium phosphate-citric acid buffer, and the maximum hot-paste viscosity determined with the Brabender Amylograph.

All these determinations were undertaken in order to measure the total effect of the moisture-temperature relationship on the starch-water system as a whole and not on each granule as an entity. It was recognized at the beginning that uniform swelling of all granules in the pre-heat treatment would not be attained in the system employed.

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[1] Serial No. HL 8 304. Bakers Engineering and Equipment Company, Kansas City, Kansas.

RESULTS AND DISCUSSION

The starches varied in appearance after the pre-heat treatment. The two starches with the lower moisture levels, 29 and 38 percent moisture, had a consistency similar to that of an agglutinated powder. The starch gels made with 52 and 68 percent moisture were hard, elastic pastes with a more or less dry core in the center. The starch gels made with 99 percent moisture were fluid. These properties of the starch gels existed for both the 100° and 200°C. pre-heat treatment.

The hard, dry core in the center of the starch gels made with 52 and 68 percent moisture levels was due to the uneven distribution of the water throughout the starch mass. As the heat penetrated from the outside of the container, the granules close to the walls of the container swelled and absorbed the water, thus removing the water from the center of the starch mass. This effect caused a non-uniform wetting of the granules in the gel throughout the system with only a percentage of the granules being fully gelatinized. The technique employed here was selected to measure the total moisture-temperature relationship on the entire system. This approach was taken in view of the practical applications of such findings to commercial food processing.

Difficulty in dispersing the starch gels was experienced early in this investigation. The pastes made with moisture levels of 52 to 68 percent were tough, elastic gels which defied dispersion in water or buffer by shaking or gentle stirring. It was felt that a uniform dispersion of the starch gel was necessary for

representative sampling as well as for having the starch readily
available to the beta-amylase. After investigating several tech-
niques, including the use of inert materials such as celite, glass
beads, and sand to reduce the gel strength, without success, the
use of the Waring blendor to disperse the starch was used. In
order to determine the effect of this procedure on the avail-
ability of the starch to beta-amylase, a series of wheat starch
gels was prepared varying in moisture. These gels were analyzed
for starch availability to beta-amylase with and without the use
of the Waring blendor to disperse the starch. The starch gels
partially dispersed in water were blended for but 15 seconds. The
results of this study are presented in Table 1. The use of the
Waring blendor to disperse the resulting pastes after pre-heat
treatment showed no consistent effects on the availability of the
starches to beta-amylase.

Table 1. Effect of the Waring blendor on the availability of
pre-heated wheat starch to beta-amylase.

Moisture level (%)	Treatment	Digestion time (hr.)		
		1	2	3
		Maltose equivalents (mg.)		
29	Blended	0.3	0.6	0.6
	Not blended	0.8	1.1	1.1
38	Blended	1.1	1.9	2.4
	Not blended	1.1	2.9	3.9
52	Blended	6.5	7.9	8.6
	Not blended	6.3	7.8	7.8
68	Blended	8.6	8.9	9.6
	Not blended	6.5	7.9	9.5

Availability of starch to beta-amylolysis increased with
each increment of moisture. The effect of the moisture-heat
treatment was characteristic for each starch as illustrated by
the data in Tables 2 to 6 and Figs. 1 to 5. Availability of the
starch to beta-amylase increased with each increment of moisture
whether heated at 100 or 200°C. However, heating at the elevated
temperature caused a significant decrease in availability of
starch at any given moisture level. No data are presented for
moisture levels below 29 percent because at lower moisture levels,
the effect of the heat-moisture treatment could not be demon-
strated by the technics employed. The results of pre-heat treat-
ment of wheat starch are shown in Tables 2 and 3 and Figs. 1 and
2.

Table 2. Maltose production by beta-amylolysis of wheat starch
after heating at various temperatures and at different
moisture levels.

Moisture level %	Pre-heat treatment °C.	Digestion time (hr.)			Maximum availability* %
		1	2	3	
		Maltose equivalents (mg.)			
29	100	0.30	0.60	0.60	6.10
	200	0.00	0.00	0.30	4.23
38	100	1.10	1.90	2.40	24.70
	200	0.00	0.20	0.50	7.05
52	100	6.50	7.90	8.60	88.60
	200	1.50	2.10	2.60	36.60
68	100	8.60	8.90	9.60	97.00
	200	3.90	5.20	5.50	77.50
99	100	8.60	9.20	9.70	100.00
	200	4.10	5.50	7.10	100.00

* Expressed as percent of available starch after three hours
digestion assuming 99 percent moisture starch as 100 percent
available to beta-amylase.

Table 3. Hot peak viscosities of the starches.

Moisture level %	Pre-heat treatment °C.	Wheat	Non-glutinous sorghum	Waxy corn	Un-modified corn	German rice
				B.U.		
Control 29	100	620	900	1000	900	1000
	200	520	720	1000	700	980
		480	680	1000	700	950
38	100	390	550	1000	565	975
	200	310	490	1000	520	947
52	100	341	415	470	338	960
	200	237	280	1000	180	580
68	100	290	220	200	315	840
	200	225	175	845	170	475

Wheat Starch

The hot paste viscosity is directly affected by the degree
of internal disorganization of the starch granule which in turn
is affected by temperature, available moisture, and amylose-
amylopectin ratio (10,30,29,6). Pre-heating of the starches at
100°C. resulted in a decrease in the hot-paste viscosity as the
available moisture was increased during pre-gelatinization. The
hot-paste viscosity of the starches after pre-treatment at 200°C.
was lower than that given by pre-treatment at 100°C.

As the moisture level was increased with heating to 100°C.,
the starch became increasingly available to beta-amylase. How-
ever, the increase was not linear for all levels investigated.
There was a difference in available starch of 63 percent between
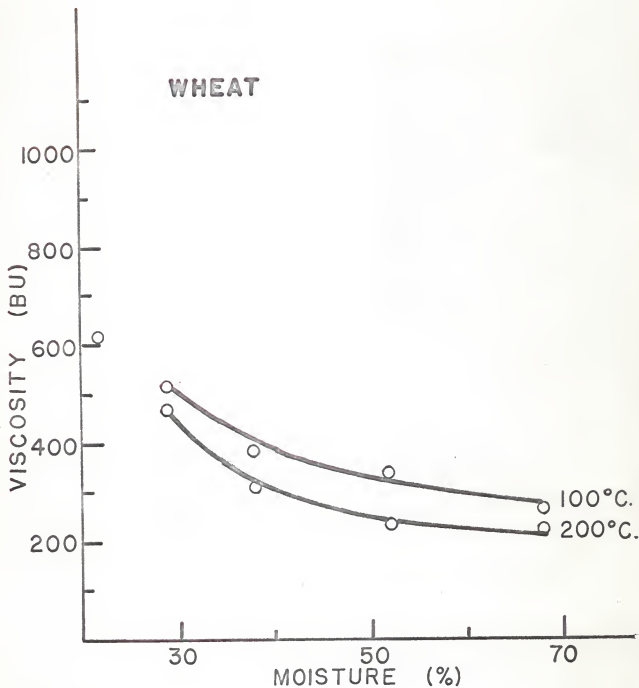the moisture contents of 38 and 52 percent; but, between the

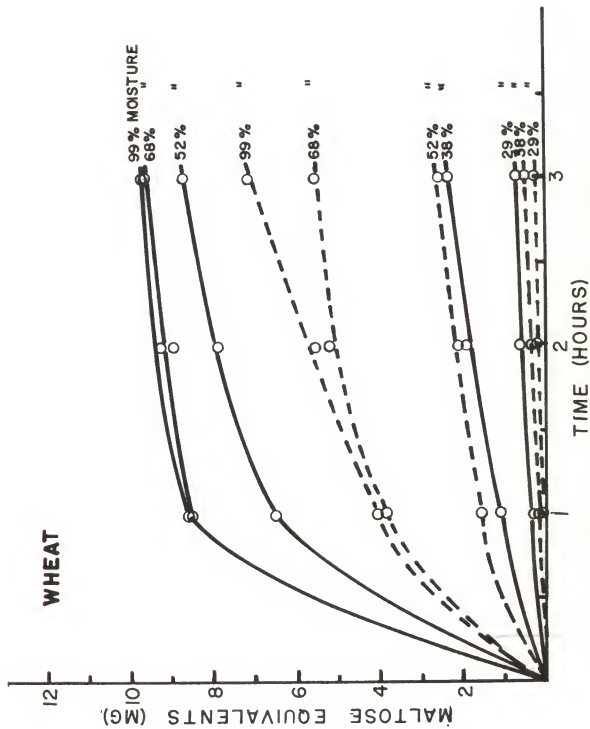Fig. 1. Effect of moisture and pre-heat treatment on hot-paste viscosity.

Fig. 2. Effect of moisture and heating at 100°C. (———) and 200°C. (- - - -) on beta-amylolysis.

68 and 99 percent moisture levels, the difference was only 3 percent in available starch as measured by maltose produced after three hours of enzyme digestion.

Compared to heating of the starch at $100^{o}C.$, heating at $200^{o}C.$ resulted in a decrease in availability of the starch to beta-amylase at all moisture levels. The availability of the starch at the 99 percent moisture level was 37.3 percent lower than that given by the same moisture level at $100^{o}C.$

Wheat starch decreased in hot-peak viscosity from 520 B.U. at 29 percent moisture level to 290 B.U. at the 68 percent moisture level. Pre-gelatinizing the starch at $200^{o}C.$ gave lower peak viscosity values and they were almost parallel to that of the $100^{o}C.$ treatment. Results are expressed in Table 3 and Fig. 2.

The beta-amylolysis data indicate that at a moisture level of approximately 65 to 70 percent, practically all of the starch granules were gelatinized and therefore generally available to beta-amylase. Furthermore, these data indicate that the degree of gelatinization is dependent on moisture levels.

Apparently, as the temperature is increased when water is limited, the starch molecules are not expanded and therefore are less vulnerable to the action of the beta-amylase. As the moisture becomes even more limited, the starch molecules are less extended and therefore even less available to attack by beta-amylase.

The Amylograph data also seem to support this postulate because pre-treatment of the starch at $200^{o}C.$ resulted in an even

lower hot-paste viscosity at higher moisture levels. This suggests that the formation of the new internal bonds prevents the starch molecules from forming the expanded network which is responsible for the higher viscosity of gelatinized starch pastes.

Unmodified Corn Starch

The effects of pre-heat treatment of corn starch are shown in Tables 3 and 4 and Figs. 3 and 4.

Table 4. Maltose production by beta-amylolysis of unmodified corn starch after heating at various temperatures and at different moisture levels.

Moisture level %	Pre-heat treatment °C.	Digestion time (hr.)			Maximum availability[*] %
		1	2	3	
		Maltose equivalents (mg.)			
29	100	0.43	0.66	0.66	5.08
	200	0.00	0.30	0.60	8.45
38	100	1.23	2.00	3.53	28.46
	200	0.00	0.50	1.00	14.05
52	100	6.06	7.65	7.90	63.54
	200	1.60	3.10	3.60	47.90
68	100	7.93	8.66	9.33	75.20
	200	2.90	4.20	4.90	65.40
99	100	10.06	11.93	12.40	100.00
	200	5.80	6.20	7.50	100.00

[*] Expressed as percent of available starch after three hours digestion assuring 99 percent moisture starch as 100 percent available to beta-amylase.

The unmodified corn starch gave higher maltose values than the wheat starch when gelatinized at the 99 percent moisture level. This occurred at both temperatures. It would appear that
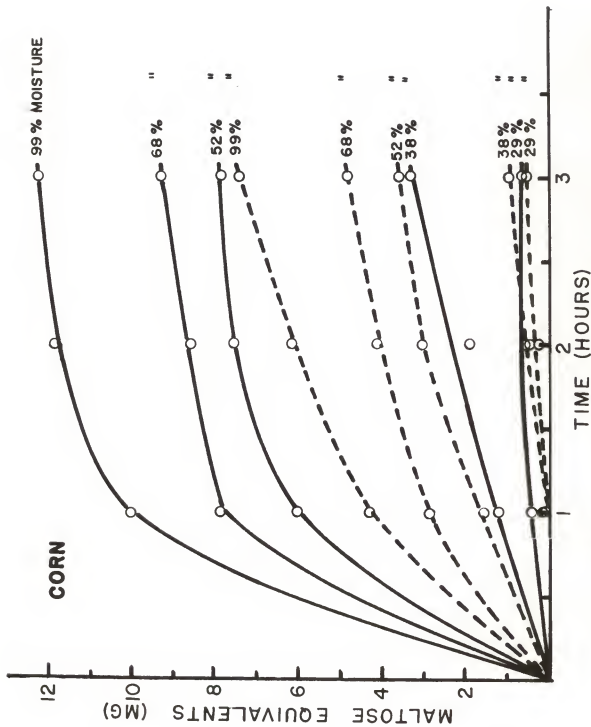
Fig. 3. Effect of moisture and heating at 100°C. (———) and 200°C. (------) on beta-amylolysis.
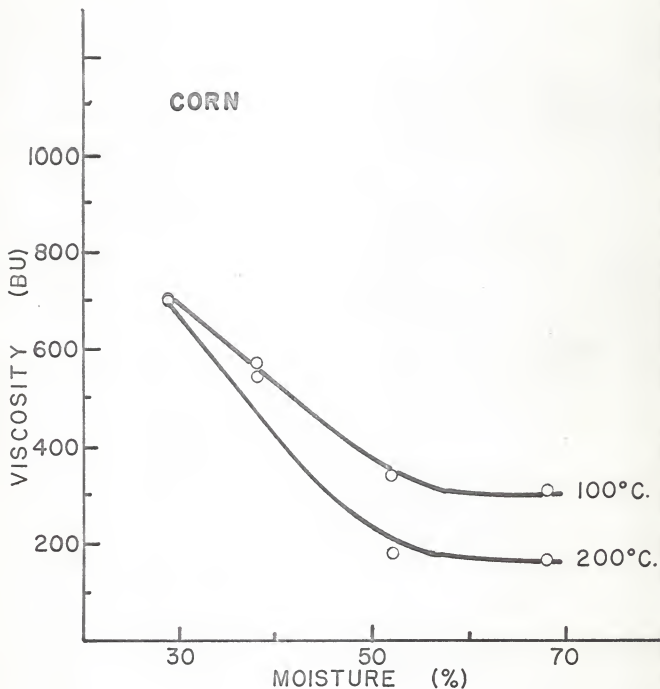
Fig. 4. Effect of moisture and pre-heat treatment on hot-paste viscosity.

corn starch required more water than other starches for an equiva-
lent amount of gelatinization. The maltose production of the
different concentrations at 100°C. varied considerably. The
smallest difference existed between the 52 percent moisture and
the 68 percent moisture levels. The 29 percent moisture starch
showed a slight increase between the first and the second hour
but showed no increase between the second and the third hour.
The other moisture levels tended to show a steady increase in
maltose production during the three hours of digestion. As with
wheat starch, pre-heat treatment at 200°C. decreased the avail-
ability to beta-amylase. In this case the difference for the 99
percent moisture levels at 100°C. and 200°C. was 39.5 percent.
The curves for all the moisture levels tended to increase
throughout the three hours of digestion.

Unmodified corn starch exhibited a very marked decrease in
hot-paste viscosity between the 29 and the 52 percent moisture
levels for both temperature treatments. However, the maximum
viscosity at 200°C. pre-treatment was lower. Beyond a moisture
level of 52 percent there was little effect of the elevated tem-
perature on reduction of the viscosity.

Corn and wheat starch behaved much in the same way although
the former gave higher maltose values and lower viscosity. The
general trend was more or less similar, indicating that the
molecular arrangement of the starches was similar. Some species
variation was observed and this is in agreement with the litera-
ture (1,10,43).

Waxy Corn Starch

The effects of pre-heat treatment of waxy corn starch are
reported in Tables 3 and 5 and Figs. 5 and 6. The three highest
moisture levels of this starch gave the highest maltose produc-
tion of all starches when heated at 100°C. The 99 percent mois-
ture level gave a maltose value of 12.70 mg. maltose equivalents
after three hours of digestion. The 68 percent moisture level
had 92.1 percent of the starch available, and 52 percent moisture
level had 87.5 percent of the starch available to beta-amylase
digestion. The 29 percent moisture level showed no increase
after the second hour of digestion. After the 200°C. pre-
treatment, the 99 percent moisture level had a 50.3 percent lower
starch availability than the same moisture level treatment at
100°C. All other moisture levels showed marked differences, with
only the 99 and the 68 percent moisture levels showing increased
amounts of available starch. The starch treated at 200°C. gave
lower maltose values than those treated at 100°C.

Waxy corn starch was the only starch that gave a higher
hot-paste viscosity for heat treatment at 200°C. as compared to
100°C. The treatment at 100°C. caused a large drop in hot-paste
with a moisture level of 68 percent compared to 38 percent. The
200°C. pre-treatment gave a straight line at 1000 B. U. until the
52 percent moisture level was attained. After that point it
dropped to 845 B. U. with pre-treatment with 68 percent moisture.

These results suggest that the molecular bonding in the
granule of waxy corn starch is weaker than that of the other

Table 5. Maltose production by beta-amylolysis of waxy corn starch after heating at various temperatures and at different moisture levels.

Moisture level %	Pre-heat treatment °C.	Digestion time (hr.) 1	2	3	Maximum availability* %
		Maltose equivalents (mg.)			
29	100	0.30	0.60	0.60	4.70
	200	0.00	0.20	0.30	5.18
38	100	3.20	3.70	4.20	33.00
	200	0.50	0.80	0.80	13.80
52	100	9.90	10.50	10.90	85.70
	200	3.10	3.60	3.70	63.90
68	100	10.70	11.30	11.70	92.10
	200	3.10	4.20	5.20	89.90
99	100	12.20	12.50	12.70	100.00
	200	4.50	4.70	5.80	100.00

* Expressed as percent of available starch after three hours digestion assuring 99 percent moisture starch as 100 percent available to beta-amylase.

starches used in this study. As a consequence, after gelatinization, beta-amylase can penetrate and act on the starch much easier. The second suggestion of these data is that the large amylopectin content of this starch promoted the formation of large numbers of cross bonds when the starch was heated at 200°C. This higher degree of bonding prevented beta-amylase from attacking the starch as extensively as it was when heated at 100°C. The hot-paste viscosity remained high when heated to 200°C., in spite of greater bond formation, because of the extensive network resulting from the high content of amylopectin.
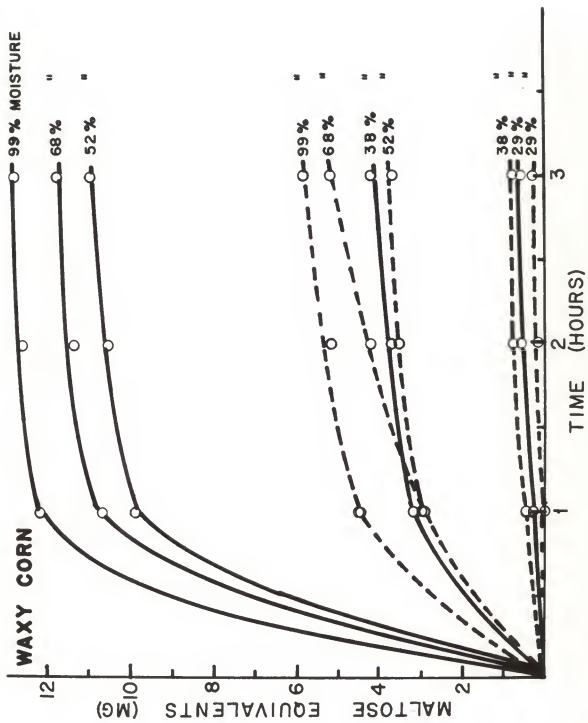
Fig. 5. Effect of moisture and heating at 100°C. (———) and 200°C. (----) on beta-amylolysis.
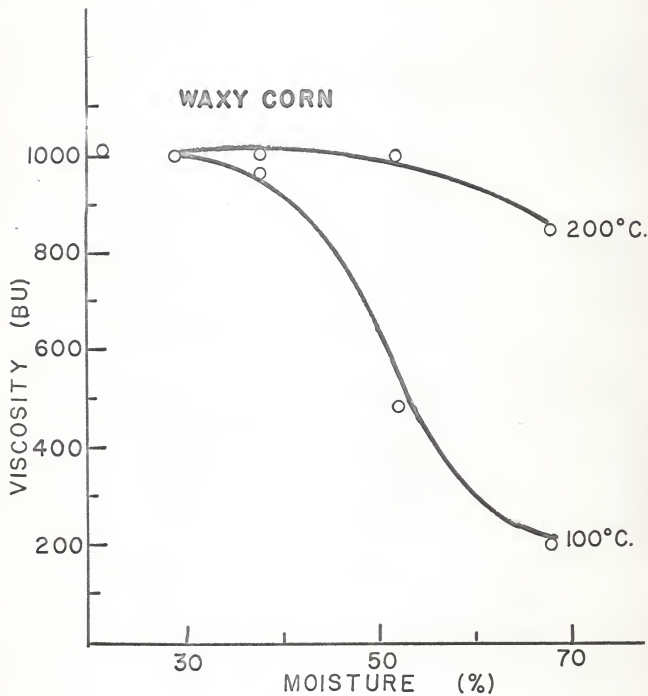
Fig. 6. Effect of moisture and pre-heat treatment on hot-paste viscosity.

Non-glutinous Sorghum Starch

The effects of pre-treatment of non-glutinous sorghum starch
are reported in Tables 3 and 6 and Figs. 7 and 8. The results
obtained after pre-treatment of non-glutinous sorghum starch at
100°C. were similar to those obtained for wheat starch although a
slight increase in maltose production was observed for the non-
glutinous sorghum starch. The beta-amylolysis of the non-
glutinous sorghum starch demonstrated an important difference in
moisture requirement for gelatinization. At moisture levels of
68 and 52 percent, maltose production was 83.76 and 72.27 percent
of the 99 percent moisture level while the two lower moisture
levels gave maltose equivalents of 26.73 and 8.91 percent of the
99 percent moisture levels, respectively.

Pre-heat treatment at 200°C. gave lower starch availability
at all moisture levels. A difference of 32.77 percent was found
between the 99 percent moisture levels treated at 100° and 200°C.
Starch availability increased at all moisture levels with the ex-
ception of the 29 and the 38 percent moisture levels which gave
almost identical results. The maximum hot-paste viscosity re-
duced steadily as the moisture level increased for the pre-heat
treatment at 100°C. Pre-treatment at 200°C. lowered the maximum
hot-paste viscosity values but the curve was almost parallel to
that for the 100°C. pre-heat treatment. Both the amylograph and
the beta-amylolysis data showed that the behavior of non-glutinous
sorghum starch was very similar to that of wheat starch, suggest-
ing a comparable internal arrangement of the granules.

Table 6. Maltose production by beta-amylolysis of non-glutinous
          sorghum starch after heating at various temperatures
          and at different moisture levels.

Moisture level %	Pre-heat treatment °C.	Digestion time (hr.)			Maximum availability* %
		1	2	3	
		Maltose equivalents (mg.)			
29	100	0.30	0.90	0.90	8.91
	200	0.00	0.80	0.80	12.15
38	100	1.73	2.50	2.70	26.73
	200	0.20	0.80	0.80	12.15
52	100	7.80	8.53	9.36	88.07
	200	1.80	2.80	3.70	56.90
68	100	8.96	9.86	10.06	94.50
	200	1.90	3.10	3.90	60.00
99	100	9.46	10.20	10.63	100.00
	200	5.00	5.80	6.50	100.00

*
Expressed as percent of available starch after three hours
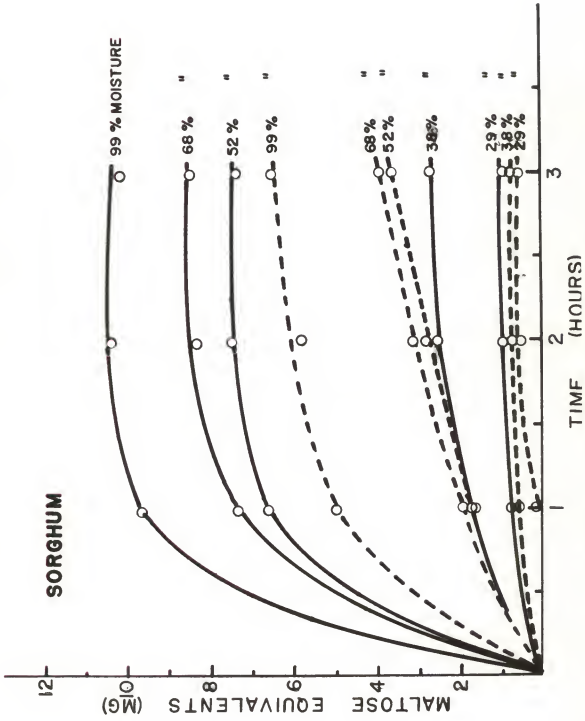digestion assuring 99 percent moisture starch as 100 percent
available to beta-amylase.

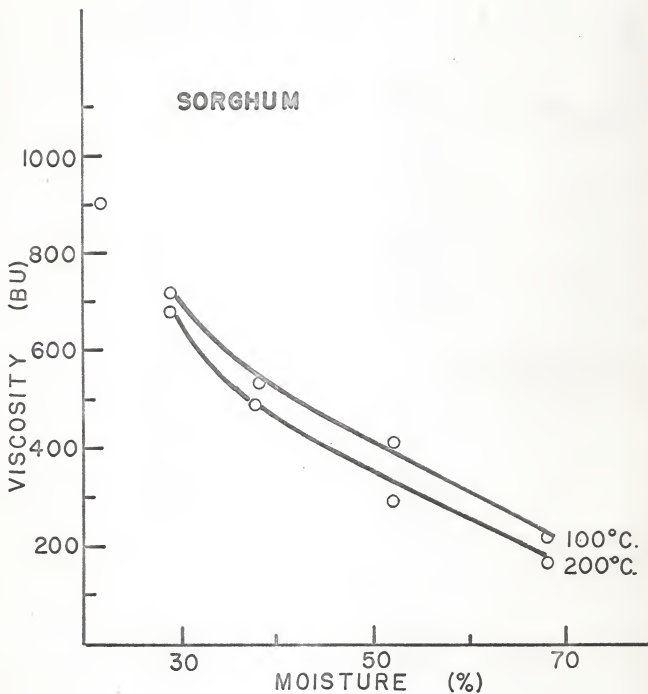Fig. 7. Effect of moisture and heating at 100°C. (———) and 200°C. (‒ ‒ ‒ ‒) on beta-amylolysis.

Fig. 8. Effect of moisture and pre-heat treatment on hot-paste viscosity.

German Rice Starch

The effect of pre-treatment of rice starch is reported in Tables 3 and 7 and Figs. 9 and 10. Starch availability after pre-heat treatment at $100^{\circ}C$. was large if sufficient water was present. At low moisture levels, while the starch availability was low, it was still higher than for other starches when compared at the same levels. Pre-heat treatment at $200^{\circ}C$. reduced the starch availability at the higher moisture levels but had little, if any, effect at the low moisture levels. This suggests that moisture has a distinct effect on bonding between molecules during the gelatinization process. With a starch such as German rice, bond formation after pre-heating was not high enough to inhibit the action of beta-amylase. This view is also supported by the maximum hot-paste viscosity. The hot-paste viscosity reduction as moisture increased was significant.

The data presented in this research work suggested that pre-heating the starch at $100^{\circ}C$. or $200^{\circ}C$., in the presence of water, opens the micellar bundles, causing the rupture of the original structure of the starch granule. The structural disorganization leads to the exposure of the open ends of the starch molecule, thus causing them to become vulnerable to the attack by beta-amylase. In addition, pre-heating the starches at $200^{\circ}C$. caused the formation of new bonds due to the interaction of starch molecules among themselves. This was particularly evident at the high moisture levels. When the moisture level was below 38 percent, insufficient water was present to cause bond rupture within the granule, thus causing small availability of the starch to beta-amylase.

Table 7. Maltose production by beta-amylolysis of German rice starch after heating at various temperatures and at different moisture levels.

Moisture level %	Pre-heat treatment °C.	Digestion time (hr.)			Maximum availability[*] %
		1	2	3	
		Maltose equivalents (mg.)			
29	100	1.60	2.13	2.76	25.90
	200	0.50	0.80	1.00	10.20
38	100	4.50	5.36	6.33	59.40
	200	0.50	0.80	1.10	11.70
52	100	7.80	8.53	9.36	88.07
	200	2.10	2.90	3.60	38.30
68	100	8.96	9.86	10.06	94.50
	200	4.10	6.00	7.30	77.86
99	100	9.46	10.20	10.63	100.00
	200	7.80	8.90	9.40	100.00

[*] Expressed as percent of available starch after three hours digestion assuring 99 percent moisture starch as 100 percent available to beta-amylase.

These findings are in agreement with other investigators (10,19, 22,30,35,42,46) who have found that starch gelatinization and bond rupture are intimately associated with the presence of water during heating.

Results of this investigation suggest that the amylose-amylopectin ratio of the starch has a direct relation to the effect of moisture on the gelatinization properties. As the amount of amylopectin increased in the ratio, the bonding between starch molecules in the granule became weaker. This conceivably permitted water to penetrate the granule more readily with subsequent swelling. Such granules became more available to beta-amylase action. In those starches having similar amylose-amylopectin
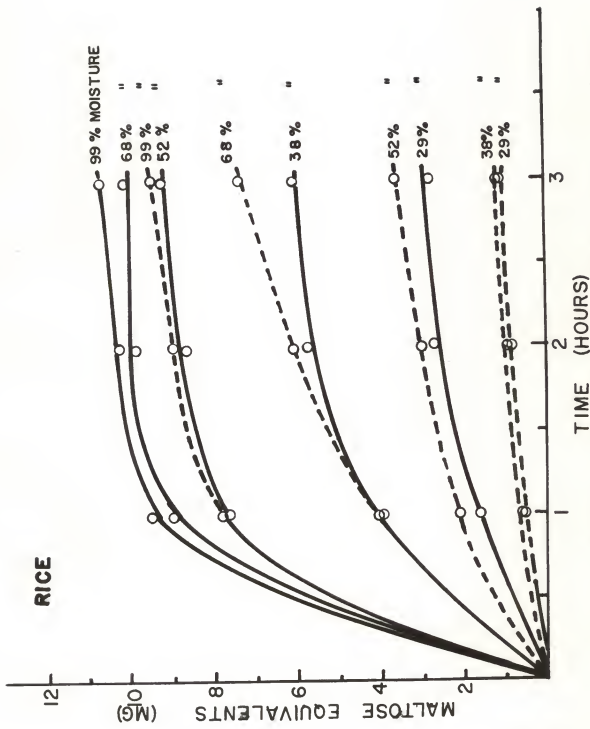
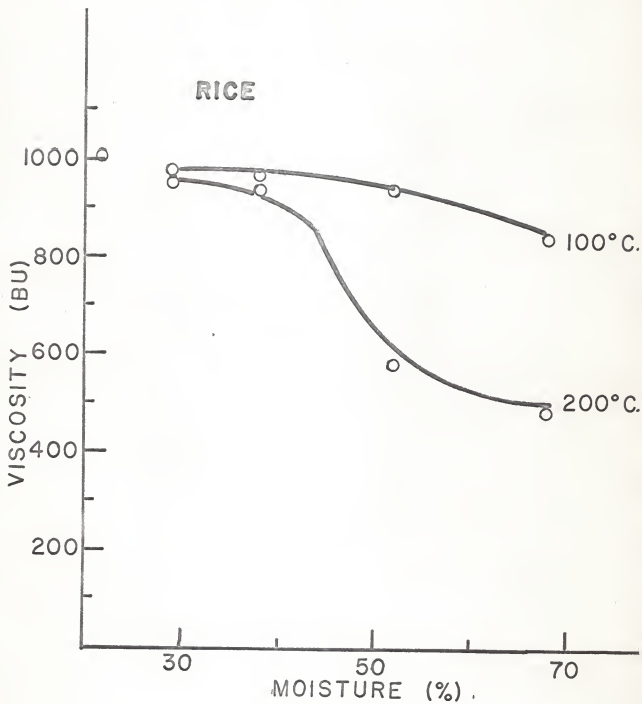Fig. 9. Effect of moisture and heating at 100°C. (——) and 200°C. (-----) on beta-amylolysis.

Fig. 10. Effect of moisture and pre-heat treatment on hot-paste viscosity.

ratios such as wheat, corn, and sorghum, the gelatinization char-
acteristics were almost alike but distinctly different from those
of the waxy corn and German rice starches.

It is reasonable to assume that pressure developed inside
the tubes when starch was heated at 200°C., and that such pres-
sure probably enhanced the formation of new bonds. These starches
were less available to beta-amylase action and had a considerably
lower hot-paste viscosity.

CONCLUSIONS

An investigation of the effect of moisture and temperature
on starch gelatinization has been conducted. Moisture may be a
limiting factor in starch gelatinization as shown by the increas-
ing availability of the starch to beta-amylase with an increase
in moisture. An increase in moisture resulted in a decrease in
maximum hot-paste viscosity. Heating had a greater effect on the
starch gelatinization as moisture became more available. With
sufficient available moisture, an increase in temperature de-
creased starch availability to beta-amylase and reduced hot-paste
viscosity. An insufficient amount of moisture during heating
caused an uneven gelatinization of the starch. The granules
closer to the surface absorbed most of the water present, leaving
the granules in the center of the system unaltered.

The amount of branching affected the degree of gelatiniza-
tion at 100°C. and therefore the availability of the starch to
enzyme attack. Granules with high amylopectin content such as
waxy corn starch appeared to have weaker intermolecular bonds.

Heating the starches at 200°C. in hermetically-sealed con-
tainers appeared to cause an intermolecular rearrangement with
the formation of new bonds which were responsible for the de-
crease of the starch availability to beta-amylase and maximum
hot-paste viscosity.

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MOISTURE-TEMPERATURE RELATIONSHIP IN
STARCH GELATINIZATION

by

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The effects of moisture and temperature treatment on gela-
tinization and pasting properties of the starches from wheat,
corn, waxy corn, non-glutinous sorghum, and German rice were
investigated. The starches, with different moisture levels,
were pre-treated at 100 and 200°C. and beta-amylolysis and
hot-paste viscosity measured to follow the changes in gelatini-
zation and pasting properties. Beta-amylolysis determinations
were made using the ferricyanide reduction method, and the
Brabender Amylograph was used to measure the maximum hot-paste
viscosity.

The results indicated that heating at both temperatures had
little or no effect on starch gelatinization if insufficient
water was present. In general, when the starches were heated at
100°C., the beta-amylase action on the starches increased, and
maximum hot-paste viscosity decreased as the moisture was in-
creased. As the temperature was raised from 100 to 200°C., both
starch availability to beta-amylase and maximum hot-paste
viscosity decreased for all starches except waxy corn. When
sufficient available water was present, pre-treatment at 100°C.
caused the internal molecular rearrangement of the starches. At
200°C. pre-treatment, the rearrangement of the starch molecules
was more pronounced and resulted in decreased starch availability
and reduced maximum hot-paste viscosity.