Physicochemical studies on starch fractions from Phalaris canariensis
by Jiun Guang Keng

Abstract:
Starch was prepared from the seed of phalaris canarlensls (canarygrass) "by a modified alkali process. Amylose and amylopectin were isolated from, the starch by a modified procedure which involved more drastic pretreatment with liquid ammonia and stronger dispersion conditions than normally used. The molecular size of the amylose was greater than that of corn amylose, based on the limiting viscosity and this was supported by ferricyanide number. Subfractionation of the amylose indicated that the dominant subfraction was twice the size of corn amylose. The average chain-length of the amylo-pectin was 23 glucose residues per end-group as determined by periodate oxidation. This value was supported by methylation, hydrolysis, and the determination of the methylated sugars.

The average molecular weight of the amylopectin determined by light scattering technique was 141 million in aqueous potassium chloride and 15% million in dimethyl sulfoxide. The radius of gyration of the canarygrass amylopectin calculated from the C=O line of the Zimm plot was 2310A in aqueous potassium chloride and 2790A, in dimethyl sulfoxide. These molecular weights are roughly twice that of corn amylopectin which would suggest a more highly compacted amylopectin molecule in canarygrass than in corn starch. Debranching of the amylopectin with pullulanase followed by gel filtration with Sephadex G~75 provided a two peak separation of linear chains. The average degree of polymerization ranged from 31 to 45 in the first peak and from below 12 to 17 in the second peak as measured by the wave length of peak light absorption in the iodine-stain. The area ratio of the two peaks was roughly 1:1 which corresponds to the 1:1 ratio of A chains to B chains in a ramified model of amylopectin. This would suggest very long branches for about one-half of the chains and rather short branches for the other half, which might explain the unusual properties of canarygrass starch.

PHYSICOCHEMICAL STUDIES ON STARCH FRACTIONS FROM PHALARIS CANARIENSIS

by

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ABSTRACT

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(canarygrass) by a modified alkali process. Amylose and
amylopectin were isolated from the starch by a modified
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INTRODUCTION

Starch, the reserve polysaccharide in plants, is a homoglucan which consists of two fractions, amylose and amylopectin. Amylose is a α-1,4-linked glucose polymer. Amylopectin, is a very large multiply-branched molecule consisting of short α-1,4-linked glucose chains joined to each other by α-1,6-bonds.

Starches occur naturally in the form of granules. The sizes and shapes of these granules depend on the botanical source from which the starch is isolated. Recently, interest has been shown in small-granule starches both from the standpoint of commercial application and in fundamental studies. There is also interest in the use of these starches for medical applications (1). The unusual properties of the small-granule starch from Saponaria vaccaria (2) and the large chunks from Amaranthus retroflexus (3) suggest that our present concepts of starch chemistry, which are based on cereal and tuber starches, may not be valid for starches in general. More comparative fundamental studies on a wider range of starch sources are needed for a better understanding of starch chemistry.

The starch from Phalaris canariensis (canarygrass) was first described by Reichert (4) but only the size and shape of the granules was reported. Since the size of this starch granule is greater than that of cow-cockle granules but

less than that of rice granules, this starch was considered
an excellent substrate to determine whether granule size has
any significant influence on the properties of starch. Goering
and Schuh (5) found that canarygrass starch has several
highly unusual properties. First, the high pasting temperature,
low swelling power and low solubility suggest that the starch
granule has strong bonding forces which are only observed in
high amylose starches; however, the iodine affinity of this
starch indicates that it has a normal amount of amylose.
Secondly, if existing concepts which are used to explain the
properties of potato starch are applied, the large amount of
esterified phosphate present would suggest that this starch
should have a low pasting temperature, high solubility and
high swelling power. Goering and Schuh (5) suggested that
the unique properties of canarygrass starch may be due either
to phosphate diester bonds between starch chains or to an
unusual structure in the amylose and amylopectin fractions.
Though phosphate diester bonds have not been reported in
natural starch, the possibility can not be excluded; however,
the phosphorus content in the starch reisolated for the
investigation reported herein was somewhat less than that
reported in the original work. Therefore, the postulation of
unusual molecular sizes of starch fractions was considered as
a more likely explanation for the unique properties of
canarygrass starch.

Although considerable work has been done on the structure of the starch granule, the molecular architecture within the starch granule is not clear. A granule structure model proposed by Meyer (6) is most generally accepted. According to this model, the two starch fractions are laid down in the granule in a radial fashion. Wherever linear segments of either branched or linear chains run parallel, hydrogen bonding forces can pull the chains together into associated crystalline bundles or micelles. A long linear chain may conceivably pass through a number of such micellar areas, or the outer fringe branches of a branched molecule may participate in several separate micelles. Hence, these crystalline structures are responsible for holding the starch granule together to permit swelling but prevent disrupting.

Very strong bonding forces in starch granules could be due to exceptionally long amylose molecules, amylopectin with unusual molecular size, and/or amylopectin with anomalous average chain-length. The longest amylose, which was reported to have an average degree of polymerization ($\overline{D.P.}$) of 4000, was isolated from potato by aqueous leaching (7). The anomalous amylopectin (36 unit-chain) was reported to exist in high amylose starch by several laboratories (8). Recent studies by Greenwood (9) suggested that this anomalous fraction was an artifact and that normal amylopectin could be separated by ultracentrifugation.

By conventional fractionation methods amylose can be
separated from amylopectin by forming an insoluble complex
of amylose with a polar, organic compound, provided complete
dispersion of the starch granules is achieved (10). Since
cereal starches are difficult to disperse in aqueous solutions,
it is necessary to pretreat the starch granules before frac-
tionation. Many inorganic and organic reagents have been used
for pretreatment but liquid ammonia is the most satisfactory
since it avoids degradation and yields more pure amylose and
amylopectin fractions (8, 11, 12). Complete dispersion by
autoclaving is efficient but yields degraded fractions (13).

In an attempt to establish the reasons for the unusual
physical properties of canarygrass starch the present investi-
gation was initiated to isolate the pure starch fractions and
to demonstrate whether or not they had unusual structures
using chemical, physical and enzymatic methods.

MATERIALS AND METHODS

Preparation of Starch

A modified alkali process was used for the preparation of
starch from canarygrass seed (CGS). The seed was steeped in
0.01 M mercuric chloride solution at 50°C for 24 hours, washed
with distilled water and homogenized in a Waring blender at
low speed for 1 minute. The pulverized material was screened
over a 60-mesh (297 micron opening) screen and the residue was
returned to the blender for another minute. The solids passing
through the 60-mesh screen were rescreened on a 320-mesh (44
micron opening) screen and the starch slurry was centrifuged
in a solid basket. The starch was removed and dried in a
convection oven at 50°C. It was resuspended in distilled water
and screened through a 320-mesh screen. The resulting suspension
was adjusted to pH 11 with alkali, stirred for 2 hours and the
starch was removed by centrifugation. This alkaline treatment
was repeated several times. The starch was then thoroughly
washed with water, separated by centrifugation and resuspended
in water; the pH was adjusted to 7.0 with hydrochloric acid
and the suspension was shaken with toluene overnight. The
toluene extraction was repeated three times and the final starch
was removed by centrifugation, washed with water and dried in a
convection oven at 50°C.

The corn starch used for a control was supplied by Corn

Products Company through the courtesy of Dr. T. J. Schoch.

Chemical Analysis of Starch

The protein content was determined by a modified Micro-Kjeldahl method (14, p. 643 using a conversion factor of 6.25). The distillate was titrated in 5% boric acid using methyl red-bromocresol green as the indicator. The sample was ashed according to the usual procedure (14, p. 284). Total free fat was determined by ether extraction (14, p. 287). Starch content was determined by the acid hydrolysis method with 3N sulfuric acid, and the glucose concentration in the neutralized hydrolysate was then determined by the alkaline ferricyanide method (15). The excess ferricyanide was titrated with iodine-thiosulfate (0.05N) instead of ceric sulfate. Sugar components in the starch hydrolysate were examined by paper chromatography using Whatman No. 1 paper and ethyl acetate:pyridine:water (10:4:3 v/v) as the solvent (16). Phosphorus was determined colorimetrically after digesting the starch with nitric and perchloric acids using a slight modification of the method of Allen (17).

Fractionation of Starch

The starch was defatted by extraction in a Soxhlet thimble with boiling 85% methanol for 24 hours as suggested by Schoch (18). The starch was then pretreated with liquid ammonia according to Hodge et al. (19). Pretreatment with dimethyl

sulfoxide as described by Foster (20) was also included in
the preliminary fractionation studies of CGS starch.

Canarygrass starch is extremely difficult to disperse
and many attempts were failures when the conventional aqueous
dispersion method under a nitrogen atmosphere was attempted
(11). Both n-butyl alcohol and n-amyl alcohol were used for
the initial precipitation of the amylose fraction from the
starch dispersion and the amylose was repurified by precipita-
tion as a butanol complex. The amylose isolated from different
preparations using the conventional techniques was impure as
judged by the low iodine affinity and the exceptional high
yield. Therefore, it was concluded that complete dispersion
of CGS starch was not achieved using conventional dispersion
methods.

The failure to isolate pure amylose suggested that a more
drastic pretreatment of the starch was necessary. CGS starch,
pretreated twice with liquid ammonia, was dispersed in boiling
water (0.5%) under a nitrogen atmosphere. The 0.5% hot starch
solution was stirred vigorously for 2 hours and then passed
through a Sharples continuous-flow supercentrifuge to remove
the cellular debris and impurities. The total centrifugate was
reheated to 90°C and sufficient n-amyl alcohol was added to
make the solution 5 to 6% by volume. The mixture was cooled
slowly overnight with constant stirring, refrigerated for 24
hours and collected with the Sharples supercentrifuge. The wet

amylose complex was redispersed in boiling water and recrystal-
lized by adding 10% by volume of n-butyl alcohol. Repurifi-
cation by n-butyl alcohol precipitation was repeated twice and
the amylose was then dehydrated in anhydrous n-butyl alcohol.
This dehydration treatment was repeated several times and the
product was dried at 95°C under vacuum.

The supernatant solution from the centrifuge was filtered
through a sintered-glass filter of medium porosity, concen-
trated at 50°C under vacuum, and treated with half its volume
of methanol. The mixture was vigorously stirred for several
minutes and allowed to stand overnight; the precipitated
amylopectin was dehydrated several times in methanol.

Characterization of Fractionated Products

A. Purity

The purity of amylose and amylopectin was determined from
the iodine affinity by potentiometric titration (13) using a
bright platinum and saturated calomel electrode (Leeds and
Northrup). The potentials were determined with a precision
potentiometer (Leeds and Northrup K-3 universal potentiometer)
equipped with a sensitive galvanometer. A calibration curve
relating the EMF readings to the amount of free iodine (Fig. 1)
was first prepared by titrating a solution consisting of
potassium chloride (0.05N) and potassium iodide (0.05N) with
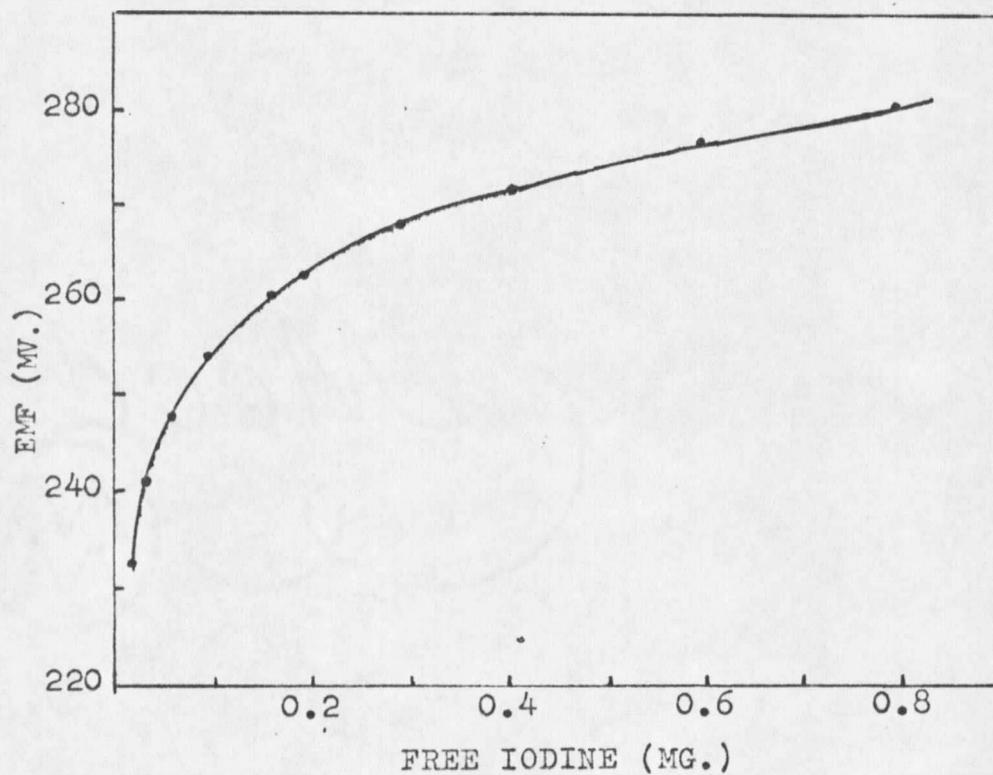standard iodine solution (2 mg./ml.) at constant salt concen-

Fig. 1.   Calibration curve of EMF against free iodine by potentiometric titration at 30°C.