Investigation of rice starch molecular structure to slow starch digestion rate and lower glycemic response

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Executive Summary:

This one-year project examined the possibility of reducing glycemic index in US rice cultivars through a correlative study on starch amylopectin and digestion properties. The goal was both to identify low GI rice cultivars as well as gain a mechanistic understanding of how to develop new low GI cultivars.

Twelve rice cultivars with a narrow range of amylose contents were selected for further study based on their wide variation in Rapid ViscoAnalyzer (RVA) pasting breakdown to study the relationship between starch digestibility, and amylopectin fine structure and thermal and pasting properties. Rice samples were cooked under the same conditions for starch in vitro digestibility using the standard Englyst in vitro test. RVA and differential scanning calorimetry (DSC) were performed for pasting and thermal properties. Amylopectin was purified, debranched, fractionated and fine structure was analyzed using an HPSEC.

Results showed that rapidly digestible starch (RDS) was highly and negatively correlated (r=-0.86, p<0.01; r=-0.81, p<0.01) with FrI long and FrII intermediate debranched amylopectin linear chains, respectively, and positively correlated (r=0.79; p<0.01) with FrIII short linear chains. Slowly digestible starch was positively correlated (r=0.80, p<0.01; 0.76, p<0.01) with FrI and FrII, respectively, and negatively correlated (r=-0.76, p<0.01) with FrIII. RVA breakdown viscosity was positively correlated (r=0.88, p<0.01) with RDS and negatively correlated (r=-0.89, p<0.01) with SDS. RDS was negatively correlated (r=-0.62, p<0.05; r=-0.67, p<0.05; r=0.7, p<0.05) with DSC gelatinization temperature parameters of T₀, Tₚ and Tᶜ, respectively. SDS fraction was positively correlated (r=0.64, p<0.05; r=0.70, p<0.05; r=0.73, p<0.01) with T₀, Tₚ and Tᶜ, respectively. Results show a notable role of amylopectin fine structure on starch digestibility, and thermal and pasting properties, and apparently can be used to predict a slow digesting rice cultivar.

This study presents a molecular basis for slowly digestible starch in US rice cultivars, and could have real value in identifying slowly digesting
cultivars as well as developing a breeding strategy to produce low
glycemic index rices.

A further study relating growth location/environment with starch digestion
properties was conducted with only minor changes noted. This report is found
in the Appendix.

Amylopectin fine structure:

Amylose and total starch contents were similar among the cultivars.
Chromatograms of debranched amylopectin of all twelve starch cultivars
showed a bimodal linear chain molecular weight distribution. Fractions FrI
(DP>33), FrII (13<DP<33), FrIII (DP<13) were designated proportion of long,
intermediate/short, and very short linear chains, respectively.

Rice starch gelatinization related to amylopectin fine structure:

With regard to amylopectin chain length distribution, debranched amylopectin
fractions (FrI, FrII, and FrIII) were found to significantly correlate with DSC
gelatinization onset (T_o), peak (T_p) and conclusion (T_c) temperatures of rice
starches. FrI was positively correlated (r=0.69, p<0.05; r=0.74, p<0.01; r=0.76,
p<0.01) with T_o, T_p, and T_c, respectively. FrII was positively correlated (r=0.71,
p<0.05; r=0.74, p<0.01; r=0.66, p<0.05) with T_o, T_p, and T_c, respectively. FrIII
was negatively correlated (r=-0.86, p<0.01; r=-0.88, p<0.01; r=0.87, p<0.01)
with T_o, T_p, and T_c respectively. Similar observations were revealed in previous
studies. Very short (DP<12) amylopectin chains related negatively, while
somewhat longer (12<DP<24) amylopectin chains related positively to T_o, T_p
and T_c (Noda et al., 2003). Studies on rice starches from mutant (Wong et al.,
2003), transformant (Wang & Wang, 2002) and wild-type lines (Fujita et al.,
2003) affirmed these observations.

The positive or negative influences of amylopectin chains proportion on
gelatinization thermal parameters depend on the way these molecules are
packed into the lamellar structure in the starch granules. Short amylopectin
chains are not long enough to form double helices and most likely have a
reduced tendency to form double helices. The lack of double helices formation
may alter crystallinity in the lamellar structure by reducing the efficiency of
packing within the crystalline region of starch granules. Consequently, cultivars
with high proportion of short chains will most likely have lower gelatinisation
T_o, T_p, T_c. If the length of the crystalline region is 6 nm, which corresponds to
the length of 18 glucose residue, amylopectin chains of DP 18–21 can span the
complete crystalline lamella (Jane et al., 1999). High proportion of long chains
(FrI and FrII) could contribute to higher molecular order and an optimum
packing within the crystalline lamellae and correspondingly higher gelatinization
T_o, T_p, T_c values. Similar results were reported in aewx maize starches (Yao et
al., 1993). High proportions of longer amylopectin chains (DP>30 and DP>16) were related to high gelatinization temperatures. They suggested higher proportion of exterior intermediate chains to be responsible for the high $T_o, T_p, T_c$ and $\Delta H$. In our study, we found that long amylopectin and intermediate/short chain proportions (FrI and Fr II) are related to the high $T_o, T_p, T_c$ values.

**Pasting properties related to amylopectin fine structure:**

It is well known that RVA breakdown describes the viscosity behavior and the fragility of swollen starch granules. With increasing heat treatment, granular structure absorbs water and starts to swell. During the swelling, amylose and some amylopectin leached out. Swollen granules, as well as the leaching soluble components exuded from the granules, cause a rise in viscosity (Mohan et al., 2005). With increasing heat, swollen granules become more fragile and may break resulting in a decrease in viscosity (Whistler and BeMiller, 1997). The difference between the highest level of viscosity of swollen and gelatinized starch granules and their disintegration is recognized as paste breakdown.

The patterns of changes in viscosity of the starches brought out the differences in pasting behavior among starches of rice cultivars. BNGL, RU0301127, M202 and RU0201142 showed the highest breakdown and lowest FrI and FrII fractions. The lowest breakdown was shown for CHNR and CCDR cultivars with the highest proportion of FrI and FrII. Breakdown in the present study was highly and negatively correlated (r=-0.92, p<0.01; r=-0.86, p<0.01) with FrI and FrII, respectively. FrIII were positively correlated (r=0.96, p<0.01) with the breakdown. Cultivars with low breakdown had high pasting temperature and proportion of long and intermediate/short chains. RVA paste breakdown was previously related to proportion of amylopectin long chains (Han and Hamaker, 2001). They speculated that a higher proportion of long chain FrI may help to maintain the gelatinized starch granule structure. As explained above, high proportion of chains fraction FrI and FrII could extensively contribute to maintain gelatinized starch structure and reduce the breakdown.

**Amylopectin fine structure and starch pasting properties related to starch digestibility:**

Results show that RDS was highest in cultivars with high RVA breakdown, and with the lowest proportion of long (FrI) and intermediate/short (FrII) chains and highest proportion of very short chains (FrIII), and lowest gelatinization temperatures. In contrast, SDS was highest in cultivars with low RVA breakdown, highest proportion of long and intermediate/short chains and highest gelatinization temperatures. Correlation analysis showed that RDS was negatively correlated (r=-0.86, p<0.01; -0.81, p<0.01) with FrI and FrII, respectively, and positively correlated (r=0.79; p<0.01) with FrIII. SDS was positively correlated (r=0.80, p<0.01; 0.76, p<0.01) with FrI and FrII, respectively, and negatively correlated (r=-0.76, p<0.01) with FrIII. RVA
breakdown was positively correlated \((r=0.88, p<0.01)\) with RDS and negatively correlated \((r=-0.89, p<0.01)\) with SDS. RDS was negatively correlated \((r=-0.62, p<0.05; r=-0.67, p<0.05; r=-0.70, p<0.05)\) with \(T_o\), \(T_p\), and \(T_c\), respectively. The SDS fraction was positively correlated \((r=0.64, p<0.05; r=0.7, p<0.05; r=0.73, p<0.01)\) with \(T_o\), \(T_p\), and \(T_c\), respectively. As reported above by us, high proportion of short chains disturb the stability of amylopectin molecules in the starch granule and make it more vulnerable to the heating (DSC) and during heating and stirring (RVA). Our data strongly supports the role of amylopectin structure in determining starch digestion properties, but through physicochemical phenomena.

Overnight cooling of cooked starches allows amylose and amylopectin to retrograde. With narrow amylose content among the twelve starches using in this study, amylopectin retrogradation was the source of variation in starch digestibility. Correlation between amylopectin fine structure and pasting and thermal properties reported above shows the role of amylopectin fine structure on starch behavior during heat processing. It has been reported that long chains favor (Lin et al., 2001) and short chain inhibit (Vandeputte et al., 2003b) amylopectin retrogradation. Short chains \((\text{DP}<13)\) have less tendency to form double helices during retrogradation. Intermediate/short \((\text{FrII 13<DP<33})\), and long chains \((\text{FrI DP}>33)\) have the ability to form double helices. To emphasize, we found that starches with high FrI and FrII showed reduced RDS and high SDS.

Conclusions

The present study shows that variability in starch amylopectin fine structure causes physicochemical changes that significantly affect \textit{in vitro} starch digestion properties. The twelve rice samples used in this study were grown in the same location and under the same weather conditions and had a narrow range of amylose contents. This variability, therefore, cannot be explained by amylose content. However, amylopectin fine structure parameters were highly correlated to starch physicochemical characteristics and \textit{in vitro} digestion properties. Rice cultivars with high proportion of long and intermediate/short amylopectin linear chains had low RDS and high SDS, high gelatinization temperature and low paste breakdown. This novel finding has significant importance for the US rice industry. It not only shows that US rice cultivars can differ substantially in starch digestion properties and be predicted to have low glycemic response, but also demonstrates that easy physical (pasting breakdown) or thermal \((T_p)\) tests can be done to select rice breeding lines with slowly digestible starch \((\text{low GI})\). Therefore, we show the possibility that lines can be selected with high proportion of amylopectin long and intermediate/short linear chains for low RDS and high SDS.
References


