161

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The interaction between barley protein and starch structure: effects on *in vitro* digestion of starch

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Introduction

Though barley protein is usually considered as one of the most important factors that can affect the degradation of starch granules because of its interaction with starch granules which is also related with the level of modification of starch during malting. However, the mechanism underlying is still un- known. By studying the *in vitro* digestion rate of barley starches using the first- order kinetics and other combined techniques including confocal microscopy, we can know how protein in barley affects the starch digestibility. This can provide new knowledge about the effects of protein-, enzyme-, starch interactions in barley and related effects on starch degradation in both the brewing and food industries.

The aim of this study

To characterize the influence of barley protein on starch digestibility and to deduce a possible mechanism

Materials

Three cultivars of barley grains from the 2013 Qld National Variety were grown in Emerald (Queensland, Australia), as listed in table 1. 10 g barley seeds were ground using a cryo-grinder (Freezer/Mill 6850 SPEX, Metuchen, NJ, USA) with liquid nitrogen (2 cycles, 5 min/cycle, cooling for 1 min between each cycle). Raw barley flour were stored at room temperature for future use. Pepsin (P-6887, from gastric porcine mucosa) and porcine pancreatic α-amylase (A-6225, from porcine pancreas) were purchased from Sigma-Aldrich.

Table 1. Chemical composition barley varieties [a]

Genotype	Locations	Raw barley flour [b]			Purified starch content
		Amylose content	Starch content	Protein content	
Grout	Emerald	29.35 ± 0.98	52.47 ± 1.33	13.57 ± 0.07	75.77 ± 2.21
Commander	Emerald	33.76 ± 0.78	53.45 ± 1.03	15.24 ± 0.1	73.11 ± 0.71
Hindmarsh	Emerald	29.99 ± 1.66	51.79 ± 0.21	14.52 ± 0.12	73.99 + 1.41

a: based on duplicate measurements; b: based on dry weights.

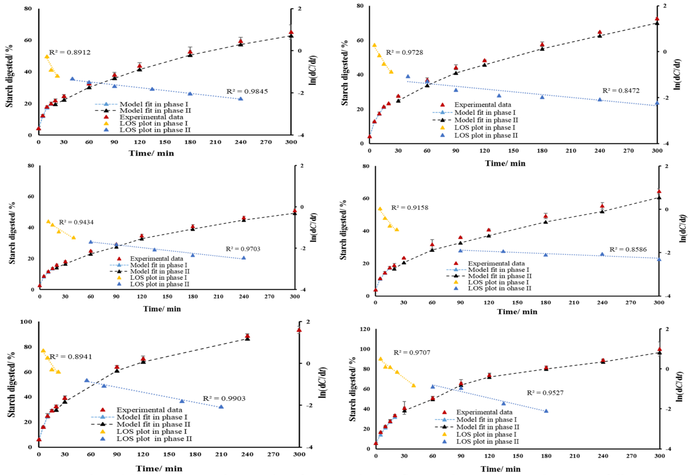

Figure 1. Typical model- fit curves and LOS plots from raw barley flour with pepsin treated (a), top, following pepsin hydrolysis; middle, following no pepsin hydrolysis; bottom, raw pure barley starches. The left was Grout while the right was Commander.

As shown in Figure 1. The *in vitro* digestion of all raw barley samples including purified starches showed a discontinuity, suggesting that there is a fraction of starch (less than 20%) that can be rapidly digested than the remainder starch. Meanwhile, compared with barley samples that without being pre- treated by pepsin hydrolysis, as shown in Figure 2, the digestion rate of starch was significantly higher when being pre- treated with pepsin solutions, indicating **the negative effects of barley protein on starch digestibility**.

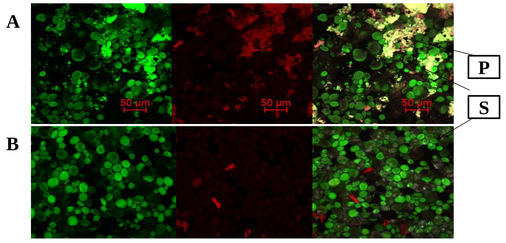

Figure 3. Confocal scanning laser microscopy of raw barley flour following or not following pepsin hydrolysis. A) samples were only steeped with water: B), samples were pre-treated with pepsin solutions; the samples were stained with FITC and Rhodamine B and the starch granules (S) and protein network (P) are shown in green and yellow, respectively

Meanwhile, as shown in Figure 3, the confocal results showed that, when steeped with water, the protein around starch granules aggregated together which can be removed when mixed with pepsin solutions. This indicates that during the *in vitro* digestion, **starch granules were entrapped with protein resulting to slower digestibility**[1].

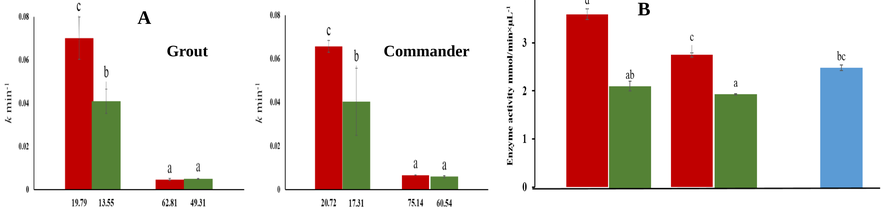

Figure 2. A), Value of starch digestion rate constants (k min^{-1}) at each phase and corresponding estimated percentage of starch digested (C_∞ %); B), the enzyme activity of α-amylase at 10 min of *in vitro* digestion. Starch digestion following pepsin hydrolysis are shown in red, starch digestion following no pepsin hydrolysis are shown in green. The left was Grout while the right was Commander barley variety. (one U of α-amylase activity was defined as the amount of enzyme required to release 1 mmol of reducing sugar in one minute at 37 ° C, pH= 6.0).

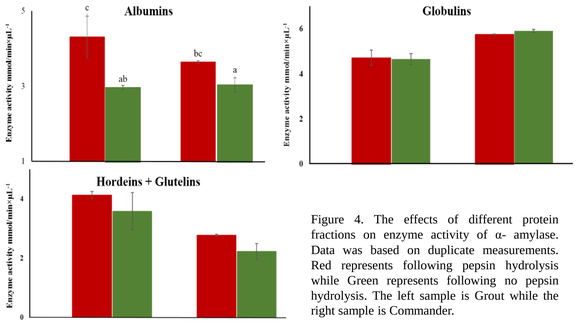

Figure 4. The effects of different protein fractions on enzyme activity of α- amylase. Data was based on duplicate measurements. Red represents following pepsin hydrolysis while Green represents following no pepsin hydrolysis. The left sample is Grout while the right sample is Commander.

What the Figure 3 B) shown is that, during the digestion experiments, the enzyme activity was significantly higher being pre-treated with pepsin, **indicating that the existence of barley protein can slow down the enzymatic activities**. Meanwhile, compared with the rest protein fractions, the water- soluble protein (albumin) is responsible for the slowed enzymatic activities.

Hypothesis

- It is highly possible that there is endogenous starch hydrolytic enzymes been released when protein has been hydrolyzed by pepsin during the digestion experiments, and then increases starch digestibility.
- α- amylase activity has been reduce by barley albumins, possibily, because of the existence of enzyme inhibitors.

Conclusions

- **The existence of barley protein can slow down the degradation of starch**
- **The protein matrix reduces the enzymatic degradation rate of starch through inhibiting the susceptibility of starch granules while the enzyme activity has also been reduced resulting to slower starch digestibility.**

References

1. Zou, W., et al., *Combined techniques for characterising pasta structure reveals how the gluten network slows enzymic digestion rate*. Food Chemistry, 2015. **188**: p. 559-568
2. Bhattarai, R.R., S. Dhital, and M.J. Gidley, *Interactions among macronutrients in wheat flour determine their enzymic susceptibility*. Food Hydrocolloids, 2016. 61: p. 415-425.

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