Commercial in Confidence *24 March 2015*

Report for ARISTA Cereal Technologies Pty Ltd

Nutrition Activity 5.2 - First Human Ileostomy Study

RESISTANT STARCH CONTENT OF BREADS MADE FROM HIGH AMYLOSE WHEAT

The physiological resistant starch content of breads made from wholemeal and refined high amylose wheat flours was determined using the human ileostomy model.

Six proctocolectomised adults with conventional and well-functioning permanent ileostomies were recruited through consultation with the South Australian Ileostomy Association. These individuals reported that they had had minimal (<10 cm) small bowel resection, although the exact amount of terminal ileum removed at surgery is usually not known. The study protocol was approved by the CSIRO Food & Nutrition Human Research Ethics Committee. All volunteers, after receiving information on study objectives and protocol, gave written informed consent before participating.

Each volunteer was randomly assigned to consume breads made from either refined or wholemeal high amylose wheat flour (CS2F11) or corresponding products made from conventional wheat (placebo) in a crossover design. Hence, a total of 4 different tests were undertaken. The control breads were made using a 50:50 blend of varieties Sunstate and Chara whereas the other breads were made using flour from only high amylose wheat. Volunteers were instructed to consume two slices of the designated breads.

The breads were formulated and manufactured by Grain Growers Limited (North Ryde, NSW) in an accredited food manufacturing facility. The foods were certified fit for human consumption by the manufacturer.

The study comprised a randomised, crossover design involving were two intervention periods each of four consecutive days (Mon-Thurs) over two consecutive weeks separated by a weekend. The participants were on a controlled, low-starch background diet during both intervention periods. For some individuals the composition of the basal diet was tailored to meet individual preferences and appetite. However, for each individual, the same diet was eaten on each day of a given feeding period. Volunteers were asked to refrain from drinking alcoholic beverages during the feeding phases of the study. The weights of any uneaten foods were recorded. The order in which the four breads were assigned was randomised. Meals were eaten at specified times and the control and test breads were consumed at breakfast. Testing was carried out in the home or workplace of participants.

For the entire intervention period all ileostomy fluid was collected at regular, frequent intervals (every 2 hours between 0700-1100 h). Each collection was dispensed into sterile plastic pots which were then capped and placed immediately in a portable freezer. The frozen samples were collected regularly by CSIRO staff and delivered to the laboratory where they were stored at -20°C to await analysis.

Test and control foods were analysed for starch and other nutrients using standardised procedures. Ileal digesta collections were thawed, pooled within day, homogenised and aliquots analysed for moisture, starch and glucose using similar methods as for the foods. Ileal efflux of starch for each 24-hour period was measured and the resistant starch content of the test food was determined.

Values are expressed as means of 3 replicates for test breads and digesta samples from 6 individuals. The resistant starch content of a given test food was calculated as the difference between ileal starch output for the foods and that of the background (i.e. low starch) diet. Resistant starch values are expressed on both a food and total starch basis.

Results

The nutritional composition of the control and high amylose wheat breads consumed by the volunteers is shown in Tables 1 and 2.

The proximate composition was similar for all four breads (Table 1). As expected, starch content was greater in refined breads compared to the wholemeal products and the control breads contained more starch than those made from high amylose wheat. Simple sugar content was similar among the breads although levels in refined wheat products were marginally higher than those made from wholemeal flour.

Wholemeal breads contained more than twice the fibre of refined white bread and those made from high amylose wheat contained considerably more dietary fibre than the corresponding control bread (Table 2). However, the magnitude of the difference was method dependent.

The differences were also largely confined to the insoluble dietary fibre component. All four breads had similar concentrations of soluble dietary fibre. Similar trends were evident for neutral non-starch polysaccharide content of the breads.

Table 1. Proximates, total starch and simple sugars content of wholegrain and refined wheat breads made from high amylose and control wheat flours[1,2]

Bread Product[2]	Moisture	Ash	Protein	Total Fat	Total Starch	Simple Sugars
HAW Refined White[3]	36.6	1.8	8.3	2.7	35.1	3.0
HAW Whole Wheat [3]	43.8	2.1	8.0	3.3	25.6	2.6
Control Refined White[4]	29.2	2.0	5.7	2.9	42.1	4.4
Control Whole Wheat[5]	36.4	2.3	6.3	3.1	32.2	3.5

[1]*Values are means of singlet & duplicate determinations*

[2]*Wholegrain and refined high amylose wheat (HAW) and conventional wheat flours.*

[3]*Flour was 100% HAW*

[4,5]*Control wheat was a 50:50 blend of Chara and Sunstate.*

Table 2. Total and individual fibre contents of wholegrain and refined wheat breads made from high amylose and control wheat flours[1,2]

Bread Product[2]	Total Fibre (AOAC 985)	Soluble Fibre	Insoluble Fibre	Sum of Fibre (AOAC 991.43)	Total NNSP
HAW Refined White[3]	7.2	1.5	6.9	8.4	1.8
HAW Whole Wheat[3]	12.9	1.7	12.1	13.8	5.7
Control Refined White[4]	2.7	1.4	2.3	3.7	1.3
Control Whole Wheat[5]	5.4	1.2	6.7	8.0	4.5

[1]*Values are means of singlet & duplicate determinations*
[2]*Wholegrain and refined high amylose wheat (HAW) and conventional wheat flours.*
[3]*Flour was 100% HAW*
[4,5]*Control wheat was a 50:50 blend of Chara and Sunstate.*

All six volunteers successfully completed the feeding trial and compliance with the study protocol was high and on average 64 g of bread was consumed on a given test day. The quantity of bread consumed was similar for all products.

Daily wet and dry matter output of ileal digesta, and other characteristics of small intestinal function, were consistent with published data for similar studies in ileostomates. Average moisture content was 91.7% (8.3% DM) and daily digesta output on days when test and control breads were consumed was 603 g/24 hours (data not shown). Daily digesta output was higher for wholemeal than white bread (661 vs 546 g) where there was no consistent effect of high amylose wheat bread on stomal efflux.

That the basal diet contained negligible quantities of resistant starch, as intended, was confirmed by measuring starch digesta concentration. Average ileal starch excretion was only 0.25 g/d for the background diet whereas it was 10-fold higher in response to bread consumption. For high amylose wheat starch excretion increased >200-fold over background compared to about a 3-fold increase for the control breads (data not shown).

Table 3. In vivo resistant starch content of wholegrain and refined wheat breads made from high amylose and control wheat flours[1,2]

Bread Product[2]	Resistant starch	
	(g/100 g bread, 'as is')	(g/100 g starch)
HAW Refined White[3]	6.5	19.7
HAW Whole Wheat [3]	5.1	21.1
Control Refined White[4]	0.8	2.0
Control Whole Wheat[5]	0.6	2.0

[1]*Values are means of 6 observations (volunteers).*
[2]*Wholegrain and refined high amylose wheat (HAW) and conventional wheat flours.*
[3]*Flour was 100% HAW.*
[4,5]*Control wheat was a 50:50 blend of Chara and Sunstate.*

The resistant starch content of the control and high amylose breads is presented in Table 3. Both the refined and wholemeal high amylose breads, especially the former, had appreciable levels of physiological (in-vivo) resistant starch. Conversely, breads made from a blend of conventional wheat flours contained little resistant starch. Indeed there was nearly a 10-fold difference in the resistant starch content between the control and high amylose breads.

The study also showed that the digestibility of high amylose wheat was in the order of 80%. This is similar to starch digestibility coefficient (~0.7) reported by us for Hi-Maize®.