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## INTRODUCTION

Australian health authorities agree that including fibre in the diet provides positive health outcomes. This message has reached both consumers and manufacturers, leading to a range of products with increased fibre levels and types. Accurately measuring both the fibre content and fibre type in foods is critical to making substantiated benefit claims, whether simple nutrient content claims or health maintenance claims. It is also critical to the accurate preparation of national food composition tables, upon which population intakes are assessed and consumption targets set for both maintenance of health and prevention of chronic disease. While the Food Standards Code provides direction on analytical methods to use for total dietary fibre for the purpose of meeting regulatory purposes such as the nutrition panel information, no such direction is given for resistant starch.

This report presents an overview of the different types of resistant starch, the health benefits associated with particular forms of resistant starch and methods of analysis, together with a comprehensive table of analytical values for foods from the Australian food supply. The purpose of this report is to provide a practical resource and update for the food industry, health and regulatory authorities and health professionals to support consistent and optimal food labelling and the development of dietary-based strategies to encourage greater resistant starch intakes.

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## WHAT IS RESISTANT STARCH?

Resistant starch is defined as the total amount of starch and the products of starch degradation that resists digestion in the small intestine of healthy people (Asp 1992). Starches that resist small intestinal breakdown are fermented by the resident bacteria in the large intestine, producing a variety of endproducts including short chain fatty acids (SCFA) that in turn provide a range of physiological benefits (Topping D & Clifton P 2001).

To support the physiological definition of resistant starch and take into account the characteristics of the various resistant starches which exist, resistant starch categories have been developed:

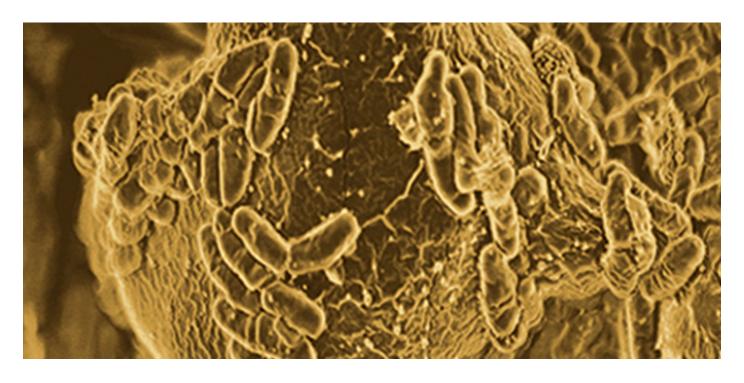
- RS1 The starch is physically inaccessible to digestion due to intact cell walls, e.g. grains
- RS2 Native starch granules protected from digestion by the structure of the starch granule
- RS3 Retrograded starch e.g. cooked then cooled potato, rice or pasta; RS3 forms as it cools
- RS4 Chemically modified starches do not occur naturally but are created to be resistant to digestion

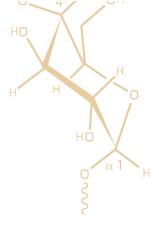
(Adapted from Topping D & Clifton P 2001)

While the above classifications are useful for categorising the origins of

various forms of resistant starch, a recent review suggests these resistant starch classes are not strongly related to physiological function or particular health benefits such as prebiosis (Bird AR & Topping D 2008), highlighting the importance of specific research on resistant starch sources to confirm particular health effects.

It is noteworthy that most scientific research, and specifically the fermentative qualities of resistant starch, has focused on the RS2 high amylose maize starch (HAMS) source. Other forms of resistant starch have been less well researched, with few published human studies (Bird AR & Topping D 2008).





# THE MEASUREMENT OF RESISTANT STARCH

Today there are many methods for measuring all types of soluble and insoluble fibres, resistant starches, maltodextrins and dextrins. For nutrition labelling purposes, Foods Standards Australia New Zealand (FSANZ) considers dietary fibre as the material isolated using AOAC Method 985.29, 991.43 or 2001.03 (FSANZ 2012). While methods are stated for inulin, fructooligosccharides and polydextrose, no methodological direction is provided for resistant starch.

The presence of a starch fraction resistant to enzymatic hydrolysis was first recognised by Englyst and others in 1982 during their research on the measurement of non-starch polysaccharides (Englyst HN & others 1982). This work showed that the resistant starches measured under the conditions of these methods were much higher than actual resistant starch confirmed by studies with healthy ileostomy subjects. These studies were followed by other methods estimating resistant starch levels by subtracting the sum of readily digested starch plus slowly digested starch from total starch. Such methods yielded good results but were laborious and gave poor reproducibility.

In this present study, the method of AOAC 2002.02 and AACC 32-40 was used. This method has undergone a comprehensive investigation on the effect of pH, enzyme concentration, the importance of the maltose inhibition of the alpha amylase, the need for amyloglucosidase inclusion and problems

of recovering the resistant starch pellet. The results of these studies have been published elsewhere (McCleary B.V. & Monaghan D. 2002) and these, combined with the collaborative study that followed (McCleary B.V & others 2002), lead to the acceptance of the method under AOAC Official Method 2002.02 and AACC Method 32-40. This method was described as a robust and reliable method that reflected in vivo conditions and yielded values that were physiologically significant.

The principal of the method involves hydrolysing the starch by incubation with  $\alpha$ -amylase and amyloglucosidase, extracting the hydrolysed product with 50% ethanol and discarding it. The resistant starch is then solubilised with 2N potassium hydroxide and hydrolysed with amyloglucosidase. The glucose is measured with a glucose oxidase/peroxidase (GOPOD) analysis kit.

This is not the only method currently available for resistant starch analysis however. Most foods in the US containing resistant starch are analysed and marketed as total dietary fibre (TDF), hence TDF methods like AOAC Method 991.43 or AOAC 985.29 have been commonly used (Finocchiaro ET pers coms). Newer methodology such as the AOAC method 2009.01 has been developed as a more accurate measure of RS but the issue of quantifying physiologically relevant amounts of dietary resistant starch is complicated by the diverse physico-chemical properties

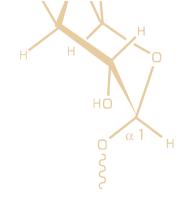
of the various forms of RS. There are six in-vitro methods that are of interest in quantifying resistant starch in foods and the selection of the method requires careful consideration of regional labelling regulations. Note some of these methods are approved as fibre methods while others are approved as resistant starch methods. Table 1 (page 5) details these six methods along with their advantages and disadvantages. They all use enzymatic digestion steps, can be complicated to run, take a full day to do, have to be done in duplicate and need to be carefully timed. Consequently, the skill of the laboratory performing the assay is paramount.

A seventh, relatively new method has been developed by the CSIRO Food Futures Flagship (Bird A. pers coms) and has undergone a pilot inter laboratory study establishing the operational performance of the method. Several human ileostomy studies have provided resistant starch reference values for establishing the accuracy of the laboratory assay. The method has been automated and high throughput instruments for analysing for resistant starch are due for commercial release later this year.

TABLE 1: PRIMARY IN-VITRO ANALYTICAL METHODS FOR RESISTANT STARCH, TOTAL DIETARY FIBRE AND RESISTANT DEXTRINS

Method	Principle	Advantages	Disadvantages
AOAC Official Method 2002.02 / AACC Approved Method 32-40 for Resistant Starch	Sample is hydrolysed by alpha-amylase and amyloglucosidase (AMG) for 16hr and the resistant starch is recovered as a pellet Resistant Starch (RS) present in the residue pellet is solubilised in KOH, then hydrolysed to glucose with AMG. Glucose is then quantified with a glucose oxidase/peroxidase (GOPOD) analysis kit.  Provides direct measurement of RS.	Provides results comparable to those in in-vivo studies with ileostomates.  Good research tool  It is primarily useful for screening ingredients.  Measures RS2 and RS3 starches.	Needs to be further proven for high amylose corn starches.  Analysis shows consistently low and variable RS contents in various food products.  RS result does not necessarily relate to final dietary content of foods and food products.
Englyst Method for Resistant Starch	Sample is hydrolysed with pancreatic amylase, pepsin and AMG and the amount of glucose released is measured with glucose oxidase. Rapidly digested Starch (RDS) is the amount of starch converted into glucose within 20 minutes of enzyme digestion and slowly digested starch (SDS) is the amount of glucose after an additional 100 min. Resistant Starch is calculated as the amount of starch not digested after 120 min. or the difference between the total amount of starch and the sum of RDS and SDS.  Provides indirect measurement of RS.	Provides results comparable to those in in-vivo studies with ileostomates.  Adapted to a variety of substrates, such as minced or fresh samples.  Good research tool.  Measures digestion profiles Shows higher results for RS with more consistent RS data vs. 2002.02 method (Modified Englyst method using currently available enzyme sources)	Has not been validated through collaborative study.  Can be difficult to perform.
AOAC Official Method 985.29 / AACC 32-05 for Total Dietary Fibre (TDF)	Sample is hydrolysed by alpha amylase, protease and AMG, followed by 78% ethanol precipitation. Residue-filtered, dried and corrected for ash and protein.	Early version of TDF method	Does not recover all fibre sources as defined in the Food Standards Code.
AOAC Official Method 991.43/ AACC 32-07 for Total Dietary Fibre (soluble and insoluble)	Sample is hydrolysed by alpha amylase, protease and AMG, followed by 78% ethanol precipitation. Residue-filtered, dried and corrected for ash and protein.	Updated version of AOAC 985.29 (organic buffer system) Simple and easy to run.  Recovers all non-starch polysaccharides and lignin and part of RS and non-digestible oligosaccharides (NDOs).  Most precise and accurate in food products.	Does not recover all of RS and NDOs.
AOAC Official Method 2001.03 for Resistant Dextrins	Sample is hydrolysed by alpha-amylase, protease and AMG followed by 78% ethanol precipitation. Supernatant is analysed for oligosaccharides by HPLC. Residue filtered, dried and corrected for ash and protein.	Provides NDO values in addition to conventional total dietary fibre values.	Time consuming.  Requires highly trained personnel.  Does not recover all of RS components.  Tends to give higher TDF than actual due to buffer interactions.
AOAC Official Method 2009.01 for Total Dietary Fibre	Sample is hydrolysed by alpha-amylase and amyloglucosidase (AMG) for 16hr, followed by protease digestion. Alcohol precipitation, filtration and HPLC steps yields total dietary fibre which includes RS and NDO.	Integrated method modelled on AOAC methods 2002.02, 991.43 and 2001.03	Expensive to run  May not capture all forms of RS.

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# HEALTH EFFECTS OF RESISTANT STARCH

A significant body of research has been developed demonstrating digestive and colonic health benefits of High Amylose Maize Starch (HAMS) resistant starch as well as research showing metabolic advantages (Nugent AP, 2005).

# Resistant Starch and Digestive Health

When resistant starch reaches the bowel, it is broken down (fermented) by the resident bacteria, generating a range of beneficial changes. These can impact digestive health in a number of ways:

- Beneficially increasing stool bulk, giving a mild laxative effect which promotes 'regularity' (Phillips J & others 1995).
- Encouraging the growth of healthy bacteria in the bowel the 'prebiotic effect' (Topping D & others 2003).
- Producing short chain fatty acids (in particular butyrate) which promote intestinal health (Weaver GA and others 1992).
- Reducing intestinal pH and the production of potentially harmful secondary bile acids, ammonia and phenols (Birkett A & others 1996).
- Preventing the degradation of the mucous layer within the colon. The mucous layer is believed to protect colon cells (Toden S & others 2006).

#### **Short Chain Fatty Acids**

Scientific interest has focused on the benefits of short chain fatty acids and in particular, butyrate. In addition to being a favoured energy source of colon cells (Schwiertz A & others 2002), butyrate has also been shown to facilitate other important physiological changes including:

- The capacity to reverse neoplastic changes in vitro (Ferguson L & others 2000).
- Nutritive effects on the colonic epithelium (Mentschel J & Claus R 2003).
- Inducing apoptosis (programmed cell death) of damaged cells (Mentschel J & Claus R 2003).

Importantly, resistant starch is one of the best substrates for the production of butyrate – producing levels twice that of wheat fibre and four times that of pectin (Champ MJ 2004).

#### Resistant Starch as a Prebiotic

The researchers responsible for originally developing the term prebiotic have updated the definition such that a prebiotic is now defined as "a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health" (Gibson & others 2004).

Thus, to qualify as a prebiotic, a food ingredient must be shown to: escape digestion and absorption in the small intestine, where reaching the large bowel it changes the composition or activity of the microbiota selectively, which results in demonstrable health benefits for the consumer (Gibson & others 2004).

A recent review investigating the potential for resistant starches to act as prebiotics revealed that not all resistant starches function as prebiotics and that the second and third points from the previous paragraph can be difficult for some forms of resistant starch to demonstrate (Bird AR & Topping D 2008).

The review concluded that HAMS are capable of modulating the composition of the colonic microbiota in ways considered conducive to host health (Bird AR & Topping D 2008) thereby satisfying the prebiotic criteria.

## Resistant Starch and Colon Cancer

Understanding the factors that may lead to or prevent colon cancer is a complex and as yet unresolved field of science. However, there is growing recognition of the important connection between diet and bacterial metabolism in the colon and specifically how the interaction can impact diseases like colon cancer (Ridlon JM & Hylemon PB 2006).

Research with dietary components, such as resistant starch, supports a direct link between diet, colonic bacteria and colon cancer, with recent animal studies indicating high resistant starch diets may prevent colon carcinogenesis (Ridlon JM & Hylemon PB 2006).

Some of the evidence pointing to a potential role for resistant starch in colon cancer risk reduction is includes:

Population Studies - an international comparison of dietary consumption and colorectal cancer incidence found a linear correlation between starch intake (and thus resistant starch) and reduced risk of colorectal cancer but did not find a correlation between dietary fibre intake and colorectal cancer (Cassidy A & others 1994).

A South African population consuming high levels of cooked and cooled corn in the form of a maize porridge, containing high levels of retrograded RS3 and low levels of dietary fibre had significantly lower levels of colorectal cancer compared to another South African population consuming higher levels of dietary fibre, but lower levels of resistant starch. The authors suggested that the fermentation of resistant starch (RS3) could be responsible for the observed protection (Ahmed R & others 2000).

Clinical Studies resistant consumption increases short chain fatty acid production in the colon (Topping D & Clifton P 2001). While mechanisms for the anti-neoplastic effects butyrate have not been fully established, butyrate has been demonstrated to affect many processes that are critical to tumorigenesis (Williams EA & others 2003). In addition, ammonia and other nitrogen-containing compounds related to increased colon cancer risk (Visek WI 1978, Bingham SA, 1988) are decreased by resistant starch consumption (Birkett A & others 1996, Muir JG & others 2004).

#### Resistant Starch and **Glycemic Control**

The ability for resistant starch to reduce glycemic response has been extensively examined and although investigation methods vary, the evidence supports the ability for resistant starch to reduce glycemic response (Behall KM & others 1988, vanAmelsvoort JMM & Westrate 1992, Noakes & others 1996, Behall KM & Hallfrisch 2002, Nilsson AC & others 2008).

In addition, a number of other studies (Behall KM & others 1988, Behall KM & others 1989, vanAmelsvoort JMM & Westrate 1992, Hospers II & others 1994, Behall KM & Howe JC 1995) have demonstrated the capacity for resistant starch to dampen insulin response.

The European Food Safety Authority substantiated a health claim relating to the ability for resistant starch to reduce postprandial glycemic responses when replacing digestible starch in high carbohydrate foods (EFSA 2011).

A body of evidence is also developing for RS2 High Amylose Maize Starch (HAMS) to improve insulin sensitivity (Robertson MD & others 2003, Robertson MD & others 2005, Johnston KI & others 2010, Maki & others 2012) with the most recent study indicating a 72% improvement in insulin sensitivity when 30g of RS2 HAMS was added to the diets of men at risk of pre-diabetes. An improvement of 56% in insulin sensitivity was also observed when 15g of RS2 HAMS was added to the diet (Maki & others 2012).

#### Resistant Starch and Satiety

Recent studies indicate resistant starch may have a role in enhancing both short term (Willis & others 2009, Anderson & others 2010) and long-term satiety (Nilsson & others 2008, Bodinham & others 2010), with the most recent study (Anderson & others 2010) demonstrating a dose response relationship between resistant starch content and satiety. The highest resistant starch ingredient (66% RS2 HAMS) reduced cumulative food intake by 15% (Anderson & others 2010).

Whilst the mechanism behind these findings is not fully understood, it is postulated that colonic fermentation may play a role. Animal studies show that the expression of satiety hormones PYY and GLP-1 is influenced by resistant starch consumption (Keenan MJ & others 2006, Zhou J & others 2006, Zhou J. & others 2008). Both gene expression and plasma protein levels of these hormones were increased in response to dietary resistant starch and remained elevated for 24 hours. The authors postulate that fermentation of resistant starch is responsible for the hormone regulation as short chain fatty acid production in the large intestine was associated with

PYY and proglucagon gene expression (Zhou J. & others 2008).

Up-regulation of GLP-1 by short chain fatty acids has been previously documented in response to dietary fibre intake in animal studies (Reimer RA, McBurney MI 1996, Cani PD & others 2005). Additional studies are needed to confirm this mechanism and to validate this response in humans.

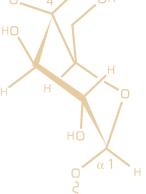
The evidence that resistant starch and specifically RS2 High Amylose Maize Starch (HAMS) makes a positive contribution to a variety of important health markers is considerable and developing. Emerging areas of interest for resistant starch and the product of resistant starch fermentation, short chain fatty acids, include inflammatory conditions and immune function (Maslowski KM & Mackay C 2011). The potential for resistant starch to also play a role in enhancing the effectiveness of oral rehydration therapy is attracting scientific interest.

#### The Australian Paradox

In Australia, attention is now being given to the mix of dietary fibres currently consumed, specifically the low intake of resistant starch relative to the two other major forms of dietary fibre, non-starch polysaccharides (NSP) and soluble fibre.

Despite the fact that Australian dietary fibre intakes are approaching the levels advised by the National Health and Medical Research Council (around 27g per day for men and 21g per day for women), conditions such as bowel cancer, the second most common form of cancer in Australian men and women, remains stubbornly high. It has been postulated (Bird AR & others 2010) that the mix of fibres consumed may need to change in order to increase the resistant starch component towards the 20 grams per day level thought to be required for optimal bowel health (Baghurst K & others 1996).

Public health nutrition messages that not only advocate higher fibre intakes but emphasise resistant starch may facilitate improved public health outcomes.



# SOURCES OF RESISTANT STARCH IN THE AUSTRALIAN FOOD SUPPLY

In 2004 (Roberts JR & others 2004) the resistant starch intake was approximated in the Australian diet using the 1995 National Nutrition Survey (NNS95). This work, acknowledging the limitations of resistant starch methodologies, focused on compiling a set of maximum and minimum values for starchy foods from in vitro methods published in the literature from 1986 to 2000 based on the Australian and international food supply.

The authors concluded that when the maximum values were used, potatoes and bananas accounted for nearly half the total resistant starch intake in Australia, whereas when minimum values were used, bananas and white bread accounted for nearly one third of the intake. These findings were consistent with earlier estimates (Baghurst KI & others 1996) where the top three sources of resistant starch in the Australian food supply were listed as vegetables (26.1%), breads/crackers (24.7%) and fruit/fruit juice (22.3%).

The present investigation has taken the table of foods originally examined by Roberts JR & others (2004) and produced a new set of resistant starch values based on analysis of foods purchased during April–May 2012 in Sydney, NSW. As such, the 2012 research provides data using a single analytical method on the resistant starch content of foods in the Australian food supply that are currently being consumed.

The actual food selection was based on the description of the food from

the NNS95, with the addition of green bananas (1651-G) to the testing to illustrate the impact of ripeness on resistant starch levels. Where the NNS95 description contained multiple food types, a single food was selected and noted in Table 2 (page 12). For example, chickpeas were selected for mature legumes and pulses - lentil, soy, spilt peas (2411) and hummus (a dip that is based on chick peas) was tested as the sample for product of legumes and pulses (2421) to explore the impact of further processing on the resistant starch levels. Noting that the nature of the food supply has changed from 1995 when this list of foods high in starch was compiled, breakfast bars (1277) no longer exist as a defined category and cereal bars (other than muesli bars) were selected. These bars consist of puffed grains and/or coextrusion.

Foods were purchased in triplicate based on market share as published by *Retail World* (2011) with analysis conducted in duplicate on composite samples. Where market share data was not available, common varieties were purchased, for example, Sebago potatoes. Data relating to price, pack weight, serve size, ingredients and nutrition information was also collected to allow further analysis at a later point.

#### Materials and Methods

Analysis of 52 foods was conducted by Grain Growers Ltd using the AOAC 2002.02 method. Megazyme's Resistant Starch kit (K-RSTAR, Megazyme International, Bray, Ireland) was used. All foods were analysed as eaten, so some preparation was required to replicate typical consumer consumption practices. A 1550W microwave was used when cooking instruction indicated. Microwave cooking was the method of choice if available over any other instructions. A fan forced oven at 180°C was used for baking potatoes and cooking pizzas and hash browns.

The duplicates of each sample were tested either on the same day or on separate days, depending on the nature of the sample and preparation required. Samples requiring preparation and testing of freshly prepared samples were analysed on the same day, while samples that required drying were analysed on separate days.

Samples were grouped according to the nature of pre-analysis treatment required:

#### Breads and baked goods

This included bread (1221-5), Lebanese bread (1243), croissant (1341), English muffins (1241), crumpets (1242), cheese and bacon rolls (1244), pancakes (1361), pizza (1351), doughnut (1332) and cakes (1331). These samples were dried in a commercial dryer at 40°C overnight then ground using Van Gelder grinder with a 0.5 mm sieve. Samples were defatted when fat content of the fresh samples was higher than 6.5 % or when fat content of dried samples was >10% (food categories 1244, 1331, 1332,1341 and 1351).



#### Biscuits, cereals bars and savoury snacks

This included biscuits (1311-4, 1321-4), muesli bars (2721), breakfast/cereal bars (1277), other snacks (2542), pretzels (2541), potato crips (2511), corn chips (2521) and popcorn (2522); all were defatted prior to testing.

#### Grains

Barley (1211), rice (1261), pasta (1251) and noodles (1253) were cooked according to instructions provided on packages. For barley, 100g of three different brands were cooked separately according to cooking instructions to make a porridge like product, then 100g of each cooked sample was combined to one sample that was tested, with minimal cooling, for resistant starch.

#### **Vegetables**

Potatoes (2311) were washed and boiled (covered with water, lid on, for 23 minutes) or dry baked (fan forced oven at 180°C for 75 minutes). For sample 2331,

the parsnip and carrots were peeled, cut and boiled with water for 10.5 and 16.5 minutes respectively, whilst the sweet potatoes were dry baked. The composite was then prepared from 100g samples of each and tested. All vegetables were tested with minimal cooling.

#### Other

Potato dishes (2313), banana (1651, 1651-G) and chickpeas (2411) were mashed using a high speed homogeniser and analysed.

Relevant correction factors were taken into account in the preparation of the final reported values, shown in Table 2 along with the 2004 values (Roberts J & others 2004).

Green bananas (1651-G) contained the highest amount of resistant starch, with 8.5g per 100g. This is in contrast to a ripened banana, as is typically consumed (1651), with 1.23g of resistant starch per 100g. Looking at the remaining foods, those with at least 1g of resistant starch per 100g (range 1.01 to

2.10g per 100g) were; bread - unspecified (1226), chickpeas (2411), bread - white fibre increased (1225), breakfast cereal - single grain (1233), taco shells (1245), baked beans (2422), bread - rye (1224), breakfast/cereal bars (1277), breakfast cereal - bran (1232), breakfast cereal wheat based (1234), hash browns (2312) and English muffins (1241).

The 2012 values are more closely aligned with the lower resistant starch values that Roberts J & others (2004) determined, with over half of the 2012 values near or below the minimum estimate range.

The impact of repeated cooking and cooling on RS3 formation is illustrated with the rice and potato based samples. White rice (1261) cooked and immediately tested reported a value of 0.37g of resistant starch per 100g, compared to rice based noodles (1253) at 0.77g per 100g which have undergone cycles of cooking and cooling during the manufacturing process prior to final consumption. Potatoes (2311)



cooked and immediately tested reported a value of 0.59g of resistant starch per 100g, compared to hash browns (2312) at 1.07g per 100g that are parcooked, frozen and cooked in the home prior to consumption.

The impact of further processing on resistant starch levels is evident in chickpeas (2411) where the resistant starch value decreased from 2.08g resistant starch per 100g for chickpeas to 0.66g per 100g in hummus (2421). It is anticipated the reduction in resistant starch content occurs as a result of dilution brought about by the presence of other ingredients in hummus, such as olive oil and tahini.

In order to more accurately assess the relative merits of one food versus another as a source of resistant starch in the food supply, the 2012 results should be considered in the context of national consumption data as per Roberts J & others 2004. However, it is of little value to undertake such an analysis at this time given the NNS95 is nearly two decades old. It is proposed this analysis be initiated when new data from the 2011/2013 Australian Health Survey is released. In the absence of contemporary dietary intake data, the authors have considered relative sales as a crude surrogate for dietary consumption data. In doing so, the billion dollar categories of biscuits, bread and breakfast cereals contrast sharply with significantly smaller categories of legumes, fruit and vegetables, highlighting the importance of the higher selling categories as sources of resistant starch in the Australian food supply. For example, the bread category is valued at \$2.2 billion, compared to bananas at \$400 million or canned legumes at \$50 million (Retail World 2011, ABGC 2008).

Breads fortified with RS2 HAMS

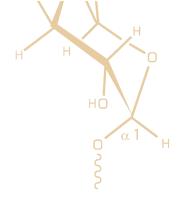
(Hi-Maize starch) were introduced into the Australian market in 1994 and held 8% of the entire bread market in 2003 (Roberts J & others 2004), which is consistent with current market share (personal communications Goodman Fielder Baking 2012). Despite the fact that in the years since the NNS95, RS2 HAMS (Hi-Maize starch) has been introduced to additional foods, such as baked goods like crumpets and English muffins, the sale volumes are small relative to breads fortified with RS2 HAMS.

Roberts J & others (2004) estimated that in 1995 such foods (food category 1225 fibre increased white bread and rolls) provided less than 2% of the total resistant starch intakes, suggesting this food category would be unlikely to increase butyrate excretion to any significant extent. Such estimates need to be revised once new data from the 2011/2013 Australian Health Survey is released, noting that only one brand in this category (1225) utilises RS2 HAMS (Hi-Maize starch) in the Australian market. Other high fibre white bread and rolls make use of soy fibre, oat fibre or RS4 chemically modified maize starch to substantiate their fibre status.

Another way to consider the foods shown in Table 2 as a source of resistant starch in the food supply is to view their resistant starch content on the basis of serve size, reflecting how they are typically consumed. The data from Table 2 has been represented in Table 3 (page 16) on per serve basis, using the current Australian Guide to Healthy Eating (Commonwealth of Australia 2008) as a guide to serve sizes. Where these were not possible, such as with mixed meals for the pizza (1351-8) then the serve size was based on the sample foods purchased.

green bananas provide the highest amount of resistant starch with 12.75g per standard banana (approximately 150g), the resistant starch drops by 85% to 1.84g when the banana is ripe, emphasising the importance of the degree of ripeness on the level of RS2 present. Considering the remaining foods in terms of resistant starch content per serve, only a limited number provide at least 1g per standard serve (range 1.06 to 1.84g). It is evident that the number of foods providing at least 1g of resistant starch is considerably less when viewed on a per serving basis. Further, the profile also changes when resistant starch per serve is considered. Foods providing at least 1g of resistant starch per serve include: grains - barley (1211), chickpeas (2411), pasta/egg noodles (1251), noodles (1253), bread unspecified (1226), baked beans (2422) and bread – white fibre increased (1225).

The values for barley, pasta and noodles indicate these foods can make a valuable contribution to resistant starch intake based on standard serves. As the analysis was carried out on the cooked hot sample, values could be expected to increase due to RS3 formation if allowed to cool prior to consumption, for example pasta salad. To what degree the RS3 content may increase cannot be estimated, as the increase in RS3 observed for cooked potato (2311) to potato salad (2131) was smaller than anticipated.



# RECOMMENDED INTAKES

The current Nutrient Reference Values (NRVs) for dietary fibre were set in 2006 by the NHMRC (NHMRC 2006) for both adequacy and chronic disease prevention. These specifically included a resistant starch component, acknowledging the science supporting its positive impact on health. An Adequate Intake (AI) was set at 30g/day for men and 25g/day for women. An AI is the average daily intake level based on observed or experimentally-determined approximations or estimates of intake by a group (or groups) of apparently healthy people that are assumed to be adequate, i.e. it represents the average amount required for adequate laxation and gastrointestinal function. However, recent evidence suggests a significant proportion of the Australian population suffers from disrupted laxation regularity (Peppas G & others 2008). In addition, the AI does not take into account the other potential benefits of fibre such as blood glucose control and cholesterol lowering, which are specifically included in the current FSANZ definition of fibre (FSANZ 2012). A suggested dietary target (SDT) for dietary fibre was also set. This is 38g/ day for men and 28g/day for women, reinforcing the need for substantial increases in fibre consumption. Unlike the AI, a SDT is based on the upper 90th percentile of intake that may help prevent or lower the risk of chronic disease.

Both the AI and SDT figures were derived from national nutrition surveys (FSANZ 2010) and as such, are dependent on the data within food

composition databases at the time. Such databases were analysed by methods that do not fully measure resistant starch and therefore do not fully account for the resistant starch in the diet. NHMRC attempted to address this at the time by including an allowance of slightly more than 4g per day for men and slightly less that 3g per day for women in the AI figure. Specific consideration of resistant starch, however, was not made in setting the SDT for fibre. These targets were based on levels present in the diet of prominent cardiovascular risk studies of the time that had brought about major improvements in cardiovascular risk. These were 29g and 35g per day for men and 23g per day for women. The values from these studies were then related to intakes of the Australian and New Zealand population at the time and the median intake of the highest quintile used; 38g per day for men and 28g per day for women. It therefore appears that no direct consideration was given to the benefit of fibre, especially resistant starch, in relation to a lower risk or potentially the prevention of chronic disease relating to the gastrointestinal system.

The way in which resistant starch is considered within the NRVs and dietary fibre analysis was raised by FSANZ in a consultation paper during 2010 looking at NRVs overall (FSANZ 2011). While most of the responses to the consultation paper supported a review of the current NRV, submitters' views were wide ranging on the selection of a suitable test method for resistant starch. Based on concern

from a public health perspective, there was support for maintaining fibre levels of at least 30g and possibly 33g per day, e.g. the midpoint between the SDT for men and women. Submitters recommended that approved method(s) of analysis for resistant starch should be determined separately from any proposal to revise the NRVs. Several relevant methods were suggested (AACC, AOAC 2005 and McCleary), along with the recognition that if the preferred approach indicated by FSANZ was ultimately adopted there would be a requirement to update food composition databases for fibre to provide accurate information.

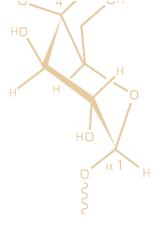
The review of the NRVs is currently on hold as FSANZ is now considering how to progress this work in light of the release of the Australian Government's Labelling Logic - Review of Food Labelling Law and Policy in January 2011, review of the Dietary Guidelines for Australians (underway at time of writing) and the progress on P293 - Nutrition, Health and related claims. Based on the current status of official recommended intakes for dietary fibre and noting the current NRVs do not adequately or consistently reflect dietary targets for resistant starch, the authors revert to the 1996 target for resistant starch set by Baghurst KA & others (1996). Baghurst KA and others (1996) suggest, "intakes in the order of 20g per day resistant starch may be needed to obtain some of the bowel related benefits".

TABLE 2: ANALYSED RESISTANT STARCH CONTENT (G PER 100G AS EATEN) OF STARCH RICH FOODS

Food Code	Food name	Food detail	Roberts et al <sup>a</sup> g/100g		2012
			Highest RS	Lowest RS	RS/100g
1211	Grains (other than rice) e.g. millet, barley, raw oats	Barley	16.1	1.6	0.81
1221	Bread, rolls, white		0.91	0.55	0.87
1222	Bread, rolls, mixed grain		0.9	0.9	0.97
1223	Bread, rolls, wholemeal		1.5	0.52	0.87
1224	Bread, rolls, rye, black, pumpernickel	Rye	4.5	3.2	1.35
1225	Breads, rolls, white, fibre increased		2.7	0.9	1.77
1226	Breads, rolls, major flour not stated		1.7	1.7	1.17
1232	Breakfast cereal, bran, processed		1.1	0.6	1.22
1233	Breakfast cereal, low sugar, puffed, flakes, extruded single cereal		3.1	2.3	1.53
1234	Breakfast cereal, wheat based biscuits and shredded wheat		2.3	1.6	1.12
1235	Breakfast cereal, wheat, low added sugar, puffed, flakes		6.2	1	0.85
1241	English style muffins		1	1	1.01
1242	Crumpets		1	1	0.99
1243	Flat breads: lavish, naan, chapati, pitta	Lebanese bread, white	1.6	1.6	0.40
1244	Fancy breads; focaccia with cheese, fruit, veg	Cheese and bacon roll	1.2	1.2	0.43
1245	Tortilla, taco shells, corn bread	Taco shell	0.85	0.85	1.44
1251	Pasta and egg noodles	Dry	1.5	0.9	0.88
1253	Noodles, Asian style	Shelf stable, rice	0.45	0.45	0.77
1261	Rice	White, plain	3.1	0	0.37
1262	Rice flavoured, puffed, crackers	Plain flavour	0.96	0.7	0.32
1271	Breakfast cereal, muesli, untoasted	Almond and apricot variety	4.3	4.3	0.47
1274	Breakfast cereal, grain mixtures		0.9	0.9	0.72
1275	Breakfast cereal, grain/fruit/nut mixtures		0.8	0.8	0.83
1277	Breakfast/cereal bars	Cereal bar other than muesli	0.2	0.2	1.29
1281	Breakfast cereal, hot porridge type		0.37	0.1	0.17
1311	Sweet biscuits, plain or flavoured		1.3	0.96	0.42

1312	Sweet biscuits, plain with fruit, nut	Fruit paste filled, laminated or co-extruded	1.9	0.94	0.35
1314	Cream filled biscuit		0.86	0.63	0.32
1321	Savoury biscuits, plain, low-med fat	<16g fat	0.6	0.6	0.50
1322	Savoury biscuits, plain, high fat	>16g fat	0.4	0.4	0.58
1323	Savoury biscuits, fibre increased or bran		4.3	2.8	0.79
1324	Savoury biscuits, flavoured	Chicken flavoured	0.55	0.55	0.50
1331	Cakes, cake mixes, buns, scones, slices	Madeira style cake	0.7	0.4	0.30
1332	Buns and yeast products	Yeast doughnut - iced/glazed	0.7	0.4	0.52
1341-5	Pastry, croissant, pie crust	Croissant	0.7	0.4	0.71
1351-8	Pizza, sandwich, rolls, pasta, rice dishes	Margherita style pizza	0.7	0.4	0.68
1361-5	Pancakes, crepes		0.7	0.4	0.61
1651	Banana raw, average ripe		5.4	2.4	1.23
1651-G	Banana raw, average green				8.50
2311	Potatoes - baked, boiled, mashed, instant, fries (hot)	Sebago-boiled nothing added or baked dry	2.7	0.16	0.59
2312	Potato products - gems, wedges, hash browns, patty (cold/reheated)	Hash brown, oven baked	0.8	0.17	1.07
2313	Potato dishes - salads, canned, scallops (cold/reheated)	Potato salad	2.4	0.46	0.63
2331	Sweet potato, root vegetables	Sweet potato (orange)/carrot/ parsnip	2.1	1.1	0.08
2351	Peas and edible-podded peas		2.1	0.92	0.77
2352	Beans - butter, broad	Green/broad	2.6	1.2	0.14
2411	Mature legumes and pulses - lentil, soy, split peas	Chickpeas	6.6	2.3	2.08
2421	Product of legumes and pulse	Hummus	4.1	4.1	0.66
2422	Dishes with legumes - baked beans	Baked beans	5.6	1.5	1.40
2511	Potato crisps	Plain flavour	4.5	4.5	0.21
2521	Corn chips	Original flavour	0.7	0.7	0.87
2522	Popcorn		0.3	0.3	0.39
2541	Pretzels		1	1	0.74
2542	Other snacks	Extruded salty snacks	0.53	0.53	0.39
2721	Muesli bars	Apricot flavour	0.2	0.2	0.35

<sup>(</sup>a) Data from Roberts  $\mathcal{J}$  & others 2004



# ESTIMATES OF DIETARY INTAKES IN AUSTRALIA

In the late 1990s, estimates (Baghurst KA & others 1996) of resistant starch intake in the Australian and New Zealand populations were made based on national nutrition surveys from 1988 and 1993. These showed an mean figure of 5.3g per day for men and 5.0g per day for women in 1993, rising from 4.9g per day for men and 4.4g for women in 1988.

In early 2000 revised estimates were made (Roberts J & others 2004), again based on the national nutrition survey current at the time. Their daily resistant starch intakes ranged from  $3.8\pm0.04$ g to  $10.7\pm0.11$ g for men and  $3.0\pm0.03$  to  $8.2\pm0.08$  for women, with the population estimate from  $3.4\pm0.03$ g up to  $9.4\pm0.07$  per day.

The 2012 values reported in this study (Table 2) give the impression of dietary intakes that are more in keeping with the conservative minimum estimates made in 2004 (Roberts J & others 2004), with over half of the 2012 values near or below the lowest estimate range. Based on a daily target of 20g of resistant starch, the 2012 figures suggest an estimated Australian dietary intake of resistant starch between 17% and 47% of that required, leaving considerable scope for an increase in resistant starch consumption across the population.

One way to support increased resistant starch intake through education is to ensure consumers and health professionals have access to information on the benefits associated with a combination of fibres in the diet, optimal levels of intake and which foods provide good sources of resistant starch. Published data on the

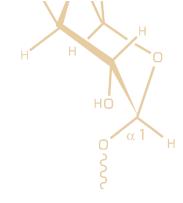


resistant starch content of everyday foods, in combination with scientific evidence supporting the health advantages of that form of resistant starch, could then be applied in the form of simple content statements on packaged foods, indicating that a food "contains" or is "high" in resistant starch.

Foods providing at least 5% of the AI (1.5g) in a serve are able to claim to be a "source" of fibre, while those with at least 10% of the AI (3g) in a serve can claim to be "good source" or to be "high" in fibre. Finally, those with at least 20% of the AI (6g) in a serve can claim to be an

"excellent source" or to be "very high" in fibre.

Following this model, and considering the levels of resistant starch indicated in Table 2, it is suggested that foods with at least 1.0g per serve of resistant starch should claim they "contain" or are a "source" of resistant starch, while those foods with at least 2.0g of resistant starch per serve may claim to be "high" in resistant starch, thus representing 5% and 10% of the 20g of resistant starch per day considered by Baghurst & others (1996) as beneficial.



# **OPPORTUNITIES AND** NEEDS FOR THE FUTURE

This report provides a practical resource to enable the food industry, health and regulatory authorities and health professionals to develop dietary-based strategies that encourage greater resistant starch intakes and support optimal food labelling initiatives. With new dietary data from the 2011/2013 Australian Health Survey soon to be released, key groups have an opportunity to facilitate the following steps to promote better health outcomes for Australians.

#### Industry and plant breeders

- · Ensure resistant starch analysis method or methods meet all needs, to satisfy regulatory compliance or substantiation of structure/function claims from clinical trials.
- Develop more products with useful levels of resistant starch, including published substantiation for the health benefits of the form of resistant starch used.
- · Selective breeding of strains naturally high in amylose.
- Develop consumer promotions to raise awareness of RS benefits and increase consumption.

#### **Health Authorities**

The capacity for resistant starch to improve colonic health is now established. The National Health and Medical Research Council (NHMRC 2006, NHMRC 2011) has specifically included resistant starch in the dietary fibre advice and recognised it as part of the mechanism by which grain (cereal) foods improve health outcomes via the provision of a more protective environment in the colon.

Similarly, the ability for resistant starch to improve glycemic control is well documented and is supported by a positive opinion from the European Food Safety Authority.

Emerging science is now demonstrating the ability for particular forms of resistant starch (RS2 HAMS) to enhance satiety, increase insulin sensitivity and potentially to mediate inflammatory-related immune responses via short chain fatty acid production, therefore reinforcing the value of resistant starch in the Australian diet.

Health authorities now have an opportunity to:

- Prepare estimates of resistant starch intake of the Australian population by analysing the new national nutrition survey and revising the AI to reflect these findings.
- · Expand the NRVs to look beyond cardiovascular health in the revision of the SDT for fibre and consider gastrointestinal health and the specific contribution of resistant starch.
- Ensure the consumer tools supporting the revised AGTHE address sources of resistant starch in the diet.

#### Regulatory Bodies

AOAC Official Method 2002.02/AACC Approved Method 32-40 is currently the most widely used and approved method for resistant starch analysis and should be readily cited as such within Australia. Although time consuming, it provides a direct measurement of resistant starch and results that are comparable to in-vivo studies with ileostomates. Its most notable limitation, however, is that it needs to be further proven for HAMS.

Regulators have an opportunity to:

- Update the national food tables to include data on resistant starch content produced from analysis of Australian foods via AOAC 2002.02.
- · Amend Standard 1.2.8 to cite AOAC 2002.02/AACC 32-40 as the required method for the determination of resistant starch for food labelling purposes.
- Provide scope for the inclusion of additional resistant starch measurement techniques once accreditation is satisfied.

#### **Health Professionals**

Health Professionals are encouraged to:

- Educate and support patients to increase their awareness of the health benefits of resistant starch.
- Ensure specific food choice advice is based on analytical values of foods from the Australian food supply, using the most appropriate method.

Credible science supporting the health benefits of resistant starch has developed significantly over the past decades. The opportunity now exists to translate this body of evidence into practical measures designed to facilitate higher resistant starch intakes. Public health messages, in tandem with food regulatory frameworks encouraging resistant starch intake that are underpinned by credible scientific substantiation, are worthy of consideration.

**TABLE 3**: ANALYSED RESISTANT STARCH CONTENT (G PER SERVE AS EATEN) OF STARCH RICH FOODS

			Serve size as	2012 values
Food Code	Food name	Food detail	per AGTHE (grams)	for RS g/ serve as eaten
1651-G	Banana raw, average green		150	12.75
1211	Grains (other than rice) e.g. millet, barley, raw oats	Barley	230	1.86
1651	Banana raw, average ripe		150	1.84
2411	Mature legumes and pulses - lentil, soy, split peas	Chickpeas	80	1.66
1251	Pasta and egg noodles	Dry	180	1.58
1253	Noodles, Asian style	Shelf stable, rice	180	1.38
1226	Breads, rolls, major flour not stated		60	0.70
2422	Dishes with legumes - baked beans	Baked beans	80	1.12
1225	Breads, rolls, white, fibre increased		60	1.06
1224	Bread, rolls, rye, black, pumpernickel	Rye	60	0.81
1351-8	Pizza, sandwich, rolls, pasta, rice dishes	Margherita style pizza	100	0.68
1261	Rice	White, plain	180	0.67
2312	Potato products - gems, wedges, hash browns, patty (cold/reheated)	Hash brown, oven baked	60	0.64
1233	Breakfast cereal, low sugar, puffed, flakes, extruded single cereal		40	0.61
1241	English style muffins		60	0.61
1242	Crumpets		60	0.60
1222	Bread, rolls, mixed grain		60	0.58
2351	Peas and edible - podded peas		75	0.57
1223	Bread, rolls, wholemeal		60	0.52
1221	Bread, rolls, white		60	0.52
1232	Breakfast cereal, bran, processed		40	0.49
1323	Savoury biscuits, fibre increased or bran		60	0.48
2313	Potato dishes - salads, canned, scallops (cold/reheated)	Potato salad	75	0.47
1234	Breakfast cereal, wheat based biscuits and shredded wheat		40	0.45
2311	Potatoes - baked, boiled, mashed, instant, fries (hot)	Sebago-boiled nothing added or baked dry	75	0.44
1245	Tortilla, taco shells, corn bread	Taco shell	30	0.43
1281	Breakfast cereal, hot porridge type		230	0.39
1277	Breakfast/cereal bars	Cereal bar other than muesli	30	0.39
1243	Flat breads: lavish, naan, chapati, pitta	Lebanese bread, white	89	0.36
1235	Breakfast cereal, wheat, low added sugar, puffed, flakes		40	0.34
1275	Breakfast cereal, grain/fruit/nut mixtures		40	0.33
1361-5	Pancakes, crepes		50	0.30

Food Code	Food name	Food detail	Serve size as per AGTHE (grams)	2012 values for RS g/ serve as eaten
1271	Breakfast cereal, muesli, untoasted	Almond and apricot variety	65	0.30
1274	Breakfast cereal, grain mixtures		40	0.29
1341-5	Pastry, croissant, pie crust	Croissant	40	0.28
2421	Product of legumes and pulse	Hummus	40	0.26
2521	Corn chips	Original flavour	30	0.26
1244	Fancy breads; focaccia with cheese, fruit, veg	Cheese and bacon roll	60	0.26
2541	Pretzels		30	0.22
1332	Buns and yeast products	Yeast doughnut - iced/ glazed	40	0.21
1322	Savoury biscuits, plain, high fat	>16g fat	35	0.20
1324	Savoury biscuits, flavoured	Chicken flavoured	35	0.18
1321	Savoury biscuits, plain, low-med fat	<16g fat	35	0.17
1311	Sweet biscuits, plain or flavoured		35	0.15
1331	Cakes, cake mixes, buns, scones, slices	Maderia style cake	40	0.12
1312	Sweet biscuits, plain with fruit, nut	Fruit paste filled, lami- nated or co-extruded	35	0.12
2542	Other snacks	Extruded salty snacks	30	0.12
2522	Popcorn		30	0.12
1314	Cream filled biscuit		35	0.11
2721	Muesli bars	Apricot flavour	30	0.11
2352	Beans - butter, broad	Green/broad	75	0.10
1262	Rice flavoured, puffed, crackers	Plain flavour	25	0.08
2511	Potato crisps	Plain flavour	30	0.06
2331	Sweet potato, root vegetables	Sweet potato (orange)/ carrot/parsnip	75	0.06



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