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Resistant starch content of Australian foods

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Summary The existing literature on resistant starch analysis in Australian foods has predominantly relied on older testing methods. Thus, our study aimed to address this gap by assessing the resistant starch content of selected Australian foods using the validated Association of Analytical Chemists 2002.02 testing method. Forty commonly consumed and available foods were tested in duplicate. The resistant starch content of foods varied from 0 to 13.72 g/100 g. Foods with the highest resistant starch were Hi-maize flour pancakes, red kidney beans, Lebanese bread, and Cornflakes cereal, which ranged from 2.30 to 13.72 g/100 g, respectively. The lowest resistant starch foods included beetroot, rice crackers, All Bran cereal, and Nutri-grain cereal, which ranged from 0 to 0.04 g/100 g, respectively. This is the first known Australian study to evaluate the resistant starch content of foods using the AOAC 2002.02 method. This data can be used to assess resistant starch consumption in the Australian population, inform gut microbiome research, and guide clinical practice recommendations for fibre intake.

Keywords Butyrate, fibre, gut microbiome, nutrition, prebiotics, resistant starch, SCFA.

Introduction

Dietary fibre encompasses a diverse array of non-digestible plant components. These components have gained substantial attention due to their observed capacity to mitigate cardiovascular disease, cancer and obesity risks (Gagnon *et al.*, 2023) while concurrently fostering gastrointestinal well-being and supporting immune function (Patterson *et al.*, 2020). Despite the well-documented health benefits associated with dietary fibre intake, fewer than 20% of Australian adults adhere to the recommended daily fibre intake to mitigate the risk of chronic disease (Fayet-Moore *et al.*, 2018). Australians consume, on average, 20.7 g per day (Fayet-Moore *et al.*, 2018), which falls short of the adult recommendation of 25 g for females and 38 g for males (National Health And Medical Research Council (NHRMC), 2013).

Research exploring the relationship between diet and microorganisms of the gastrointestinal tract, known as the 'gut microbiome', has demonstrated that dietary prebiotic fibre is a potent modulator of the gut microbiome and overall health (Ashwar *et al.*, 2016; Gagnon *et al.*, 2023), including chronic disease (McKeown *et al.*, 2022; Gagnon *et al.*, 2023). Dietary fibre can be broadly categorised into soluble and insoluble forms (Ashwar *et al.*, 2016; McKeown *et al.*, 2022) or be classified based on their functional and physiological effects (McKeown *et al.*, 2022). Although many fibre-rich foods exist, most individuals do not meet the recommended dietary servings, often opting for more processed varieties containing minimal fibre (McKeown *et al.*, 2022).

Resistant starch (RS) is a fraction of undigested starch fibre that resists enzymatic digestion in the small intestine (Bojarczuk *et al.*, 2022). The fermentation of RS in the colon may aid in preventing or treating many common gastrointestinal disorders (Bijkerk *et al.*, 2004; Higgins, 2004; Kendall *et al.*, 2004; Cione *et al.*, 2021). The Australian population consumes approximately 4–14 g of RS per day, which may be inadequate for optimal benefits (Genoni *et al.*, 2020). RS is divided into five sub-types based on physical and chemical properties: types I, II, III, IV, and V (Birt *et al.*, 2013; Raigond *et al.*, 2019). RSI is described as physically inaccessible starch in foods such as whole-kernel grains (Birt *et al.*, 2013). RSII is referred to as raw or ungelatinised granular starch and is found in foods such as high-amylose maize, raw bananas, and raw potatoes (Birt *et al.*, 2013; Raigond *et al.*, 2015). RSIII is retrograded resistant starch formed during cooking and cooling and is found in

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bread, potatoes, and cornflakes (Birt *et al.*, 2013; Raigond *et al.*, 2015). RSIV is a chemically modified starch and becomes resistant to digestion due to the addition of cross-links between chains using chemical reagents (Birt *et al.*, 2013; Raigond *et al.*, 2015). This type of RS is usually added to beverages and baked goods (Birt *et al.*, 2013; Raigond *et al.*, 2015). RSV starch is described as amylose-lipid complexes in foods with high amylose content (Raigond *et al.*, 2019). The RS values of these subtypes can be affected by milling, chewing, food processing/preparation, and *in vitro* digestibility (Maier *et al.*, 2017). Furthermore, the interaction of starch with different components present in foods can influence the formation of RS and its subsequent digestion (Raigond *et al.*, 2015).

Bacterial microorganisms' ferment 30% to 70% of RS in the colon (Sajilata *et al.*, 2006; Maier *et al.*, 2017). The variability in percentage may result from the potential malabsorption effects of the consumed starch (Sajilata *et al.*, 2006). The fermentation of RS by microorganisms results in the production of metabolites, including beneficial short-chain fatty acids (SCFAs) such as butyrate (Maier *et al.*, 2017; Teichmann & Cockburn, 2021). Studies on the colonic microbiome indicate that consuming RS as part of the regular diet confers essential health benefits, including reduced colonic inflammation, improved blood biomarkers such as blood glucose and cholesterol, increased mineral absorption, and enhanced gastrointestinal comfort (Nugent, 2005; Ashwar *et al.*, 2016; Teichmann & Cockburn, 2021). Recently, a narrative review of randomised controlled trials supports the important role of RS in improving cardiometabolic disease outcomes (Maiya *et al.*, 2023). While current research has focused on the contribution of RS to microbiome composition and metabolite production, there is currently a paucity of food composition data that limits research translation to the Australian population. An improved understanding of current RS intakes will guide future interventions and clinical recommendations.

The 2002.02 method measures RS in plant materials (Nugent, 2005) and has been deemed suitable for use in food regulation (Food Standards Australia New Zealand (FSANZ), 2017) and assessment of RS ranging from 1% to 75% (FSANZ, 2017). However, few studies have examined the RS content of local/traditional and internationally consumed foods using this method. Of these, a 2020 study from Costa Rica determined that foods with the highest RS content were green plantain flour, potato starch, and malanga flour, ranging from 50.41 to 56.59 g/100 g (Artavia *et al.*, 2020). The study also found that the levels of RS varied depending on processing, with flours showing a higher RS content. An Italian study using the same method explored variation in RS content in raw and cooked cereal-based foods and found RS varied depending on what they were made of; e.g., maize flour containing cereals revealed a greater diversity in RS content (Carcea *et al.*, 2009). Similarly to the Costa Rica study, these variations were potentially a result of differences in processing conditions.

Furthermore, limited food composition data for RS using the revised Association of Analytical Chemists (AOAC) methods exist, and current Australian dietary intakes of RS remain uncertain (FSANZ, 2017). In Australia, data from a National Nutrition Survey (NNS) (Roberts *et al.*, 2004) found that potatoes, bananas, and white bread were the country's primary sources of RS intake. The estimates of RS from the NNS were matched with values obtained from several published studies, including Englyst *et al.* (1982), determined through gas–liquid chromatography, and Gelroth & Ranhotra (2000), which used a modified method of prior commonly used processes. While these methods set the stage for future improvements in the analytical testing of RS, few methods have been validated and deemed credible in measuring the RS content of foods (FSANZ, 2017). Thus, applicability to current testing methods and dietary patterns is limited. As such, this research aims to assess RS content in selected starch-containing Australian foods using the updated AOAC 2002.02 (McCleary & Monaghan, 2002) testing method.

Materials and methods

Foods: Sampling

All products were purchased from major Australian supermarkets, and their raw and cooked contents were assessed (where appropriate). Hi-maize was obtained from a specialty bread manufacturer due to the small quantities required in this study. Forty Australian foods that were either untested or starch-tested using older testing methods were selected for RS analysis. The research team, comprised of dietitians and nutrition scientists, identified gaps in RS data for the tested foods, recognising the potential clinical significance (DeMartino & Cockburn, 2020) and the nutritional profile of these foods. Foods were selected based on overall total starch content (also likely to contain RS) and dietary consumption patterns in Australia (Roberts *et al.*, 2004; Australian Bureau of Statistics, 2022). A combination of packaged foods, raw, cooked, and cooked and cooled foods were prepared for testing. Standardised recipes and packaging instructions were used to prepare foods (Appendix S1). High-RS, low-RS, and blanks were employed as control standards for each batch evaluated per the Megazyme® Resistant Starch Assay Kit (K-RSTAR) protocol (Megazyme, 2019).

Foods: RS analysis

Dry foods were measured in 100 mg ± 5 mg portions with the exact weight recorded. To extract the required amount, part of the sample (e.g., portions of two tablespoons of baked beans, oats, quinoa, one slice of bread, two pieces of crispbread/crackers) was ground and homogenised using a mortar and pestle for a duration of 2 min. This step was conducted by the same researcher to ensure consistency. Dry foods (e.g. cereals) were ground to achieve a consistent powder, while wet foods like canned legumes were ground until homogeneous. Foods with high moisture content (>50%) were sampled at 500 mg ± 5 mg weights. All equipment was cleaned and sanitised between each sample preparation to prevent between-sample contamination. Analysis was conducted per the Megazyme® K-RSTAR method (Megazyme, 2019) purchased from Megazyme Ltd (Bray, Ireland). In brief, foods were measured in duplicate, with an additional inter-batch duplicate as per Megazyme® protocol (Appendix S2). The following modification (approved by Megazyme®) was applied to Step B of the Megazyme® protocol ii. 8 mL of 1.2 M sodium acetate buffer (pH 3.8) was added to all tubes while stirring with a magnetic stirrer, and then 0.1 mL of AMG was added intermittently to each tube, removed for vortexing, and placed in the water bath at 50 °C. Additionally, corn tortillas were measured at 11.5 mL, potentially affected by inherent laboratory variability during the measurement process.

Data collection

Sample weight and absorbance values were entered and analysed in Microsoft Excel (version 16.71 Microsoft Excel). The second and third researchers reviewed data entered for accuracy (AG & CT). Microsoft Excel was used to convert absorbance values to starch or RS values, which allowed the mean, standard deviation, and coefficient of variation between the duplicate samples to be calculated. Data was entered into the Megazyme®-specific RS calculation Excel sheets (downloaded from the Megazyme® website) to determine the concentrations of RS in grams.

Data synthesis

The RS values for each sample were determined using the formula in Fig. 1, which was integrated into the Megazyme® RS calculation spreadsheet.

Results

Assessed foods were categorised into food groups and included seven types of cereals, ten types of sliced bread, four grain and cereal products, two types of crackers/chips, three distinctive floured pancakes, five types of pasta, five different legumes, two types of vegetables, and two forms of seeds (Table 1). The measured RS content in the 40 sampled foods is shown in Table 1, including how foods were prepared (Appendix S1) and how much sample was required as per the protocol used. Variations among duplicate batches were detected for 12 out of the 40 sampled foods (Appendix S3).

The RS content of foods tested ranged from lowest at 0/100 g for beetroot to highest at 13.72 g/100 g for Hi-maize pancakes, respectively (Fig. 2). The foods with the highest RS content were Hi-maize pancakes, red kidney beans, Lebanese bread and cornflakes cereal, with a mean range of 2.30–13.72 g/100 g (Table 1). Conversely, foods with the lowest RS content were canned beetroot, rice crackers, All Bran cereal and Nutri-Grain cereal, with a mean range of 0–0.04 g/100 g. The high, moderate and low RS food thresholds were determined through consensus among authors to differentiate each food assessed and

Resistant Starch (g/100 g sample) (samples containing > 10% RS):

$$= \Delta E \text{ x } F \text{ x } 100/0.1 \text{ x } 1/1000 \text{ x } 100/W \text{ x } 162/180$$

$$= \Delta E \text{ x } F/W \text{ x } 90$$

Resistant Starch (g/100 g sample) (samples containing < 10% RS):

$$= \Delta E \text{ x } F \text{ x } 10.3/0.1 \text{ x } 1/1000 \text{ x } 100/W \text{ x } 162/180$$

$$= \Delta E \text{ x } F/W \text{ x } 9.27$$

Figure 1 The formula used to calculate the RS values of each sample.

Table 1 Comparison of resistant starch contents (g/100 g) for novel and commonly consumed Australian foods

Food groups	Sample weight (mg)	Preparation	Resistant starch (g/100 g)	SD	AGHE serve (g)	Resistant starch (g/per serve)
Cereals						
Cornflakes	100.5	Raw	2.30	0.091	30	0.69
Uncle Toby's Plus Antioxidant	101.0	Raw	0.91	0.042	30	0.27
Weet-Bix[†]	100.1	Raw	0.90	0.110	30	0.27
GF Weet-Bix[†]	100.1	Raw	0.70	0.092	30	0.21
Special K	100.2	Raw	0.13	0.006	30	0.04
Nutri-grain[†]	100.1	Raw	0.04	0.023	30	0.01
All Bran[†]	100.2	Raw	0.03	0.036	30	0.01
Breads						
Bread (Lebanese, white)	502.9	Raw	2.31	0.095	40	0.92
Corn tortilla (GF, White)	501.9	Raw	1.05	0.006	40	0.42
Bread (wholemeal)	499.3	Raw	1.00	0.024	40	0.40
Bread (multigrain/mixed grain, oats)	502.5	Raw	0.73	0.012	40	0.29
Bread (dark rye sourdough – 35 h)	500.7	Raw	0.69	0.007	40	0.28
Bread (mixed seed sourdough – 35 h)	500.0	Raw	0.64	0.018	40	0.26
Bread (white)[†]	500.2	Raw	0.61	0.187	40	0.24
Bread (GF, white)	500.6	Raw	0.45	0.001	40	0.18
Bread (GF, sourdough, grain & seed)	501.4	Raw	0.38	0.035	40	0.15
Bread (low-GI high fibre)	499.3	Raw	0.37	0.011	40	0.15
Grains/cereal products						
Ryvita crispbread	100.6	Raw	2.13	0.017	35	0.75
Instant oats (Dry, uncooked)[†]	100.2	Raw	0.25	0.026	30	0.08
Instant oats (with water)[†,‡]	500.0	Cooked as directed	0.15	0.058	120	0.18
Instant oats (with milk)[‡]	502.1	Cooked as directed	0.11	0.008	120	0.13
Crackers/chips						
Chips (corn, original)[†]	100.6	Raw	1.15	0.188	30	0.35
Cracker (rice, plain)	100.1	Raw	0.00	0.000	30	0
Flours						
Pancake (hi-maize flour)[‡]	501.6	Cooked as per recipe	13.72	0.296	60	8.23
Pancake (green banana flour)[‡]	501.4	Cooked as per recipe	1.78	0.038	60	1.07
Pancake (buckwheat flour)[†,‡]	501.2	Cooked as per recipe	0.21	0.095	60	0.21
Pastas						
Gnocchi	504.4	Cooked as directed	1.00	0.080	75–120	0.75–1.20
Pasta (pulse, chilled)[‡]	501.1	Cooked, chilled overnight	0.82	0.067	75–120	0.62–0.98
Pasta (Pulse, cooked)[‡]	502.0	Cooked as directed	0.70	0.068	75–120	0.53–0.84
Pasta (GF, rice, chilled)[‡]	502.5	Cooked, chilled overnight	0.28	0.009	75–120	0.21–0.34
Pasta (GF, rice)[‡]	502.4	Cooked as directed	0.25	0.003	75–120	0.19–0.30
Legumes (canned, cooked)						
Beans (red kidney)	502.4	No additional preparation	3.23	0.085	150	4.85
Lentils (brown)	501.3	No additional preparation	2.16	0.138	150	3.24
Peas (chick)	500.2	No additional preparation	2.06	0.149	150	3.09
Beans (cannellini)	500.1	No additional preparation	1.99	0.006	150	2.99
Beans (baked)	502.2	No additional preparation	1.48	0.131	150	2.22
Vegetables (canned)						
Corn (sweet)[†]	500.9	No additional preparation	0.08	0.023	75	0.6
Beetroot[†]	500.4	No additional preparation	0.00	0.000	75	0
Seeds						
Seed (quinoa)[†,‡]	501.7	Cooked as directed (water)	0.60	0.078	75–120	0.45–0.72
Seed (chia)[†]	102.4	Raw	0.07	0.066	30	0.02

AGHE, Australian Guide to Healthy Eating (NHRMC, 2013); SD, standard deviation.
[†]Variations <10% between duplicate batches.
[‡]Recipe in Appendix S1.

facilitate comparisons. RS content was categorised into low (0.00–0.60 g), moderate (0.61–1.00 g) or high (1.01–14.00 g). A colour-coding system was implemented to further distinguish between the three levels of RS, with low RS in red, moderate RS in orange, and high RS in green (Fig. 2).

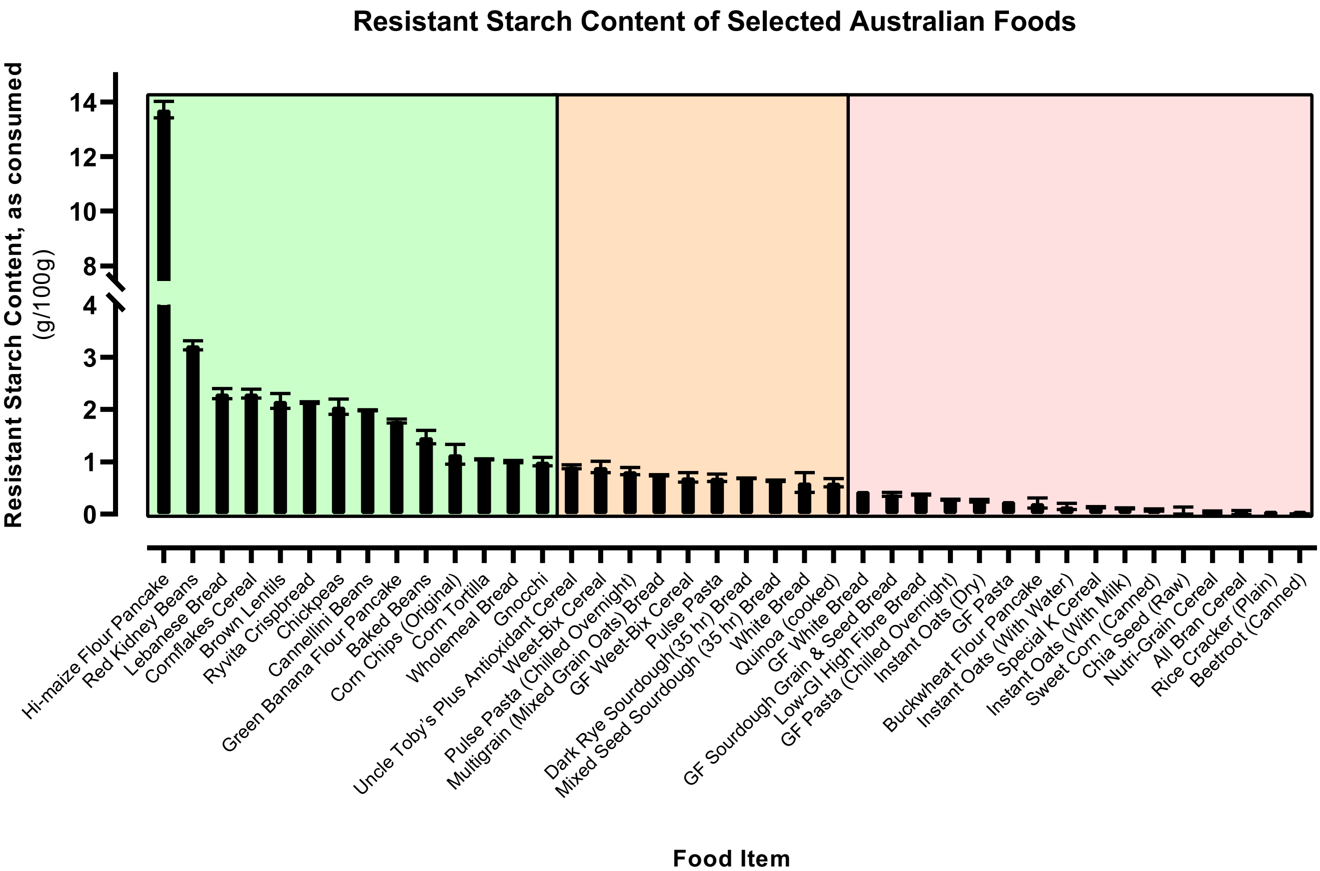

Figure 2 Resistant Starch content in selected Australian foods. Resistant starch (RS) content assessed by Association of Analytical Chemists 2002.02 testing methods using Megazyme® Ltd standardised kits (K-RSTAR) and approved by Food Standards Australia.

Discussion

RS is commonly present in starch-rich foods such as cereals, bread, grains, legumes, pasta and starchy vegetables (McKeown *et al.*, 2022). The accessibility of commercialised kits employing validated methods has facilitated the quantitative analysis of RS in these foods. With the growing scientific focus on enhancing gut health, deepening our understanding of food composition and prebiotic content has become imperative. This knowledge is essential for making well-informed dietary recommendations to improve gut and health outcomes. However, current RS intakes in the Australian diet using newer analytical methods remain undetermined. Therefore, our study aimed to address this gap by utilising the AOAC 2002.2 testing methods to measure RS content in Australian foods.

The main finding from our research highlights that maize-derived foods and legumes contain the highest amount of RS. Additionally, bread and pasta also contain high quantities of RS. However, inconsistencies were found across different varieties. Seeds, various cereals, and canned vegetables revealed the lowest RS contents. The clinical application for each food group is discussed in detail.

Grain/cereal products

Grain-based foods (bread, pasta, breakfast cereals, oats and tortillas) are the primary source of RS in most carbohydrate-based dietary patterns (Patterson *et al.*, 2020). Studies using diverse analytical methods have reported rolled oats to contain 7.70–11.30 g/100 g of RS when uncooked, compared to 0.10–0.20 g/100 g when cooked (Murphy *et al.*, 2008; Patterson *et al.*, 2020). In contrast, our findings indicate that raw/uncooked wholegrain instant oats contain a significantly lower amount of RS (0.25 g/100 g) than this but higher than their cooked form (0.13 g/100 g). Our findings also indicate that cooking oats with water resulted in slightly higher RS levels than cooking with milk. Variations in RS concentrations in oats can be attributed to factors such as oat type, harvest location, growing conditions (Kaur *et al.*, 2022) and differences in milling, storage and cooking processes.

Our study focused on analysing quick oats (or instant oats) in both their uncooked and cooked states, commonly consumed in Australia. Processing stone-cut oats into a rolled whole or quick-cooking flakes involves rolling, steaming, and changing the water content (Decker *et al.*, 2014). Oats differ in flake thickness and water content, with quick oats being cut into thinner flakes to increase water absorption and reduce cooking time (Decker *et al.*, 2014). Steaming and drying processes can also modify the starch, causing it to become partially pre-gelatinised, further contributing to differences in the amount of water absorbed and cooking time, and resultant RS content (Decker *et al.*, 2014). Oats also contain about 60% starch and 4% beta-glucan, a soluble fibre that contributes to the low digestibility of oats and gut health benefits (Henrion *et al.*, 2019). Although we did not measure beta-glucan contribution to the oat slurry prior to analysis, future studies could address this. Whilst RS content in oats is low, according to our results, this is consistent with a recent study (Karunarathna *et al.*, 2024), which used the Megazyme rapid-resistant starch assay kit. Future research is warranted to explore how different cooking and cooling methods affect RS content in various oat products, including steel-cut, overnight rolled and instant oats.

Corn-/maize-based foods

Cornflakes had the highest RS content compared to other cereals. This finding aligns with previous literature that employed various analytical methods, which reported RS levels ranging from 1.80 to 6.30 g/100 g (Murphy *et al.*, 2008; Patterson *et al.*, 2020), thereby supporting the high RS content of corn and corn products. Other evaluated corn-based food products included Hi-maize pancakes (containing approximately 27%–30% Hi-Maize flour), corn tortillas, sweetcorn (canned), and corn chips. From this study, canned sweetcorn had the lowest RS content. Furthermore, corn chips and corn tortillas (despite measurement at a slightly higher volume) reported similar results, and predictably, Hi-maize pancakes yielded the highest RS content. Hi-maize flour, a high amylose corn starch that contains 46%–53% RS, is typically used in smaller doses to replace a portion of the flour in baking (Le Leu *et al.*, 2009). Thus, the differences between Hi-maize pancakes and corn tortilla values are not unexpected. Typically, tortillas and other corn-derived food products like corn chips and taco shells are prepared by a traditional nixtamalisation process (TNP), which involves cooking maize grains in the lime-water mixture for 30–60 min, then steeping in the same solution for 12–14 h (Santiago-Ramos *et al.*, 2015). The resulting product, nixtamal, is rinsed to remove lime residue and ground into masa for tortilla preparation (Méndez *et al.*, 2013). This process enhances RS content and nutrient bioavailability in maize. An alternative method called ecological nixtamalisation, which substitutes calcium hydroxide with calcium salts, has also been found to boost RS content at each processing stage (Santiago-Ramos *et al.*, 2015). This underscores the significant impact of processing and preparation methods on RS formation.

Canned sweetcorn is commonly consumed in Australia; however, currently published data measuring RS content is lacking. This study showed that canned sweetcorn contained 0.08/100 g of RS. While the values here are lower than the corn chips, it should be noted that measurement is on an as-is-consumed basis and does not account for the considerable variation in moisture content. Therefore, the large amount of water in canned sweetcorn may have resulted in lower as-consumed RS values despite the retrogradation of RS (cooking and then cooling).

Bread and flours

Whilst bread-making techniques differ globally, common ingredients such as flour, water, salt, and yeast remain the same (Roman & Martinez, 2019). Cooking with different flours, such as banana and buckwheat, has increased over time, adding other nutrients and flavours and supporting individuals with gastrointestinal conditions with susceptibilities to conventional flours (Roman & Martinez, 2019). The demand and commercialisation for banana flour (RSII) have particularly been popular across Costa Rica, Canada, the United States, and Australia (Roman & Martinez, 2019). Various methods in assessing RS levels of banana flour (dry) have shown that the flour contains a substantial amount of RS, typically 40%–50% (Roman & Martinez, 2019). Two studies using the 2002.02 method reported banana flour RS levels ranging from 40.09–58.5 g/100 g (Tribess *et al.*, 2009; Giau *et al.*, 2023). Giau *et al.*, 2023 reported RS waffles made from green banana flour, potato starch and wheat flour and found that partial replacement of wheat flour with banana flour and potato starch increased the RS content of the waffles. The highest replacement recipe at 7.5% green banana flour, 12.5% potato starch and 80% wheat showed RS content of the batter at 4.26 g/100 g before baking and at 3.41 g/100 g post-baking, highlighting baking reduced RS content (Giau *et al.*, 2023). The RS content of banana flour pancakes reported in this study is lower than the raw and cooked values reported in the literature (Giau *et al.*, 2023). It is important to note that adding starches in the waffle and variations in recipe/methods may have contributed to increased levels of RS. Consequently, a direct comparison cannot be accurately made. However, it can be postulated that RSII from green bananas is best consumed uncooked due to its thermal instability and partial degradation (Roman & Martinez, 2019). As observed in this study and consistent with prior literature, cooking with banana flour may lead to a loss of RS content (Roman & Martinez, 2019; Giau *et al.*, 2023).

Due to the widespread consumption of white bread containing additives like sugar, bread fortification with RS has become increasingly popular (Roman & Martinez, 2019). Englyst & HudsoN (1996) showed that white, wholemeal, and rye bread contained 0.60–3.20 g/100 g of RS as eaten (Englyst & HudsoN, 1996). More recent literature using the 2002.02 method showed that wholemeal bread had 1.2% RS of dry matter (Carcea *et al.*, 2009), 0.87 g/100 g as eaten, and 0.87 g/100 g as eaten for white bread (Landon *et al.*, 2012). Grey literature showed that rye bread had 1.35 g/100 g of RS, almost double our findings (Landon *et al.*, 2012). Our findings are lower for white bread, higher for wholemeal bread, and incomparable for rye bread. The disparities between the literature and current findings could be attributed to differences in the types of rye bread being tested, the baking process and duration of fermentation, and the methods used for analysis. We tested a commercially available rye loaf; more traditional methods of bread production using rye flour may impact these findings (Landon *et al.*, 2012).

Gluten-free (GF) foods

GF bread and GF pasta had the lowest RS content compared to other bread and pasta forms tested. All purchased GF breads were derived from modified tapioca starch and rice flour. The GF pasta was also produced from rice flour. Tapioca starch is obtained from the cassava root, which contains natural forms of RS (Pereira & Leonel, 2014). As a cassava derivative, tapioca is high in amylopectin content (low amylose) (Utsumi *et al.*, 2021), thus containing less natural RS due to gelatinisation in the presence of heat (Pereira & Leonel, 2014). White rice flour has been used as a GF substitute in many products, particularly pasta (Foschia *et al.*, 2017). It is a suitable alternative due to its hypoallergenic and high digestibility properties; however, this type of flour lacks specific nutrients, including low amounts of dietary fibre (Foschia *et al.*, 2017). In line with the literature, cooked and chilled overnight GF pasta may have resulted in higher RS content, possibly from retrogradation (Robertson *et al.*, 2021). However, there is limited research assessing the RS content of GF foods to compare to the findings of this study.

Legumes and associated products

Literature on gnocchi and RS are limited. Gnocchi is a starchy food, a type of Italian potato-based pasta (Merlino *et al.*, 2022). Compared to pulse and GF rice pastas (both cooked, cooked and chilled) gnocchi yielded the highest levels of RS. Potatoes have been shown to have considerable levels of RS (Nolte Fong *et al.*, 2022). The RS content of gnocchi may be influenced by the ingredients, preparation, and cooking methods, i.e., the type of potatoes used, how it is cooked (boiled, roasted or baked), and what is added to the dough. The concentration can rise depending on how they are prepared, i.e., cooked and chilled potatoes often contain larger quantities of RS than cooked and non-chilled potatoes. This may result from retrogradation, as observed in the pulse pasta when refrigerated overnight (Nolte Fong *et al.*, 2022). Compared to other pasta types, the potato added to the flour may enhance the RS level further. In this context, homemade recipes may have an impact on RS levels.

Pulse-based flours derived from legumes, vegetables, and seeds are primarily used for their nutritional and functional properties (Bresciani *et al.*, 2022). We showed here that pulse pasta chilled overnight had the second-highest level of RS compared to all other pasta tested and in line with the high RS content of legumes. There is limited literature comparing RS content variations regarding pulse-based products.

Red kidney beans reported the highest levels of RS, and baked beans reported the lowest values of RS. Studies using various analytical methods found that cooked and canned kidney beans contain an average of 2.00–3.80 g/100 g of RS (Murphy *et al.*, 2008; Patterson *et al.*, 2020). Conversely, another study using the 2002.02 method showed that red kidney beans contained 9.54 g/100 g of RS (Moongngarm, 2013). In this study, the RS content of red kidney beans was 3.23 g/100 g, consistent with outcomes from two prior studies conducted in 2008 and 2020 (Murphy *et al.*, 2008; Patterson *et al.*, 2020). Compared to a study in 2013 (Moongngarm, 2013), the RS content was significantly lower. It is postulated that variations in RS can be due to different stages of processing leading up to distribution to consumers (Moongngarm, 2013). There is currently limited literature published on baked beans. Based on a singular report using the same method, grey literature indicated that baked beans contain 1.40 g/100 g of RS (Landon *et al.*, 2012), which coincides with our results at 1.48 g/100 g. Adding this food provides a valuable contribution to a typical Australian diet, emphasising its importance (Kadyan *et al.*, 2022).

Seeds such as quinoa and chia contain good sources of protein, healthy fats, and fibre (Kakkar *et al.*, 2023) and are now increasingly consumed as part of the

Australian diet. As a result, edible seeds have been increasingly popular due to their benefits for various chronic conditions (Slavin, 2013). Our results showed a small amount of RS in quinoa once cooked and a very minute amount in chia seeds when tested raw. Despite edible seeds being reported to contain RS1 (Slavin, 2013), few studies have measured its RS content with more recent and updated methods revealing higher dietary fibre content (Pastell *et al*., 2019). Dietary fibre can differ between samples due to the different varieties and environments where they are harvested, processed, and produced (Pastell *et al*., 2019). Nevertheless, as an added ingredient, seeds have the potential to support the daily recommended servings of fibre (Polmann *et al*., 2022). Given these factors, overnight chia pudding may be considered a potential option for future sampling.

Strengths and limitations

One of the study's strengths is testing commonly consumed Australian foods using robust analytical techniques. Three key factors drove this: (i) the need to assess RS in foods believed to benefit gut health but lacking prior RS composition data; (ii) the prevalence of these foods in the Australian diet; and (iii) their ready availability.

Due to time limitations, total starch was not quantified, and foods were measured on an as-consumed basis. Further testing may wish to include both total starch and dry weight contents in the analytical process. To control for variation, the same food brands were used for consistency, and the same sample was used for duplicate testing for homogeneity. These factors helped reduce the variations between the batches. Despite these efforts, the coefficient of variations showed some variability between samples (duplicates of the two different samples of foods used to obtain an average value), which may have reflected slight differences in the food matrix between duplicates.

Furthermore, Due to the varied nature of the foods, using a particle sieve was impractical, and particle size could not be reported. Attempts to use a bead homogeniser were also ineffective due to the required liquid volume and moisture content differences. Thus, measuring sample homogeneity was not possible under the current lab conditions.

Conclusions

Our study pioneers the comprehensive assessment of RS in Australian food products, utilising innovative analytical techniques. This advances the field by revealing RS content in everyday foods, enhancing food composition data. Our dataset now offers a guiding framework for tailoring personalised dietary regimens to strengthen gut microbiome health through the increased consumption of RS-rich foods. Furthermore, it lays the groundwork for future investigations into the optimal threshold at which the inclusion of these foods may alleviate gastrointestinal symptoms.

In addition to these, our research also has broader implications. By leveraging microbiome data, healthcare practitioners and researchers can now explore potential correlations between RS intake and health outcomes. Nevertheless, it is imperative to acknowledge the inherent variability in RS content among foods, even within identical samples, compared to existing literature. Thus, future assessments and composition studies should consider factors influencing nutritional content, including processing, preparation times and methods. The ongoing expansion of studies utilising updated and validated methods for food composition analysis will pave the way for clinical applications, enabling informed decision-making and nutritional management practices for various health conditions.

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Author contributions

Laima W. Hareer: Conceptualization; investigation; writing – original draft; methodology; validation; writing – review and editing; formal analysis; project administration; data curation; visualization; software. **Christine Tran:** Methodology; writing – review and editing; formal analysis; data curation; writing – original draft. **Hayley M. O'Neill:** Supervision; project administration; writing – review and editing; conceptualization; methodology; formal analysis; funding acquisition. **Angela Genoni:** Conceptualization; investigation; supervision; writing – review and editing; funding acquisition; methodology; validation; formal analysis; project administration; data curation.

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Conflict of interest statement

The authors declare no conflict of interest. The funders had no role in the design of the study, in the collection, analyses, or interpretation of data, in the writing of the manuscript or in the decision to publish the results.

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Recipes.
Appendix S2. Step-by-Step Assaying Protocol.
Appendix S3. Variation of >10% between duplicate batches of food items.

