Resistant Starch in Micronesian Banana Cultivars Offers Health Benefits

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Abstract

Resistant Starch (RS) is a type of starch that is resistant to starch hydrolyzing enzymes in the stomach and thus behaves more like dietary fibre. RS has been shown to have beneficial effects in disease prevention including modulation of glycaemic index,, diabetes, cholesterol lowering capability and weight management, which are critically important for many people in the Federated States of Micronesia. Green bananas are known to contain substantial concentrations of RS and are a common part of the Micronesian diet. Therefore the aim of this study was to determine the RS content in banana cultivars from Pohnpei, Micronesia: Daiwang, Inahsio, Karat, Utin Kerenis and Utin Ruk, for which no such information was available. Utin Kerenis, Inahsio and Utin Ruk were found to contain the highest amounts of RS. The fate of RS after incorporation into a food product (i.e. pancakes) was also studied and a significant reduction in the RS content was found for each cultivar after cooking. Microscopy of the banana samples indicated that the overall morphology of the cultivars was similar. In conclusion, green banana, including these varieties, should be promoted in Micronesia and other places for their rich RS content and related health benefits including diabetes control. Further research is needed to more clearly determine the effects of cooking and food processing on RS.

Key words: *banana cultivars, resistant starch, diabetes, Micronesia, Daiwang, Karat, Utin Kerenis, Inahsio, Utin Ruk .*

Introduction

Bananas (Musa spp.) are well known as good sources of dietary energy (Arvanitoyannis *et al.,* 2008) and in the Federated States of Micronesia (FSM) are widely grown and form part of the staple diet (Englberger *et al.,* 2006). Some cultivars grown in Pohnpei, FSM, such as *Karat* have been shown to be rich in carotenoids as well as riboflavin (Englberger *et al.,* 2003a,b, 2006). Despite it being generally known that bananas are rich in starch and that green bananas have high levels of RS (Zhang *et al.,* 2005), little is known about the amount of starch and RS in the cultivars grown in Micronesia. Hence the aim of the present study was to investigate the starch in several banana cultivars grown in Micronesia.

Starch is comprised of two major polysaccharides, amylose and amylopectin (Birkett & Brown, 2007). Starch granules are generally 30% amylose and 70% amylopectin, and banana starch granules are known to contain similar levels (Torre-Gutierrez *et al.,* 2007). Studies by Berry (1986) and Englyst et al. (1982, 1992) for example have shown that starches can be classified according to their behaviour when incubated with pancreatic amylase and amyloglycosidase (AMG) enzymes. They can be classed as: rapidly digestible starch (RDS), slowly digestible starch (SDS) or resistant starch (RS) (Zhang *et al.,* 2005; Sajilata *et al.,* 2006).

RDS and SDS are expected to be completely digested in the small intestine by enzymes and converted to the constituent glucose molecules. RS is the fraction of ingested dietary starch that escapes digestion and absorption and instead enters the large intestine where it is partially or wholly fermented (Sajilata *et al.,* 2006). Hence RS can be determined as:

$$RS = \text{Total Starch (TS)} - (RDS + SDS)$$

There are four forms of RS: Type I (RS_1) is physically inaccessible starch found in whole and partly milled grains and seeds. Type II (RS_2) is granular starch, mainly found in green bananas and in raw potatoes. Type III (RS_3) is retrograded starch, formed in foods that have previously been cooked and then cooled and Type IV (RS_4) is chemically modified starch (Lehmann and Robin, 2007). RS is considered to be one of the components that make up total dietary fibre (TDF) of various foods (Birkett & Brown, 2007). The physiochemical properties such as high swelling and solubility power, viscosity increase, gel formation, water-binding and water-holding capacity of RS are desirable as they impart special characteristics not attainable in high-fibre foods, making it a functional and practical product. Commercial sources of RS2 and RS3 have the properties mentioned above, but also have good handling properties during processing, high gelatinization temperatures, good extrusion and film-forming qualities and lower water-holding properties (Sajilata *et al.,* 2006). This allows for production of low-bulk high-fibre products that have improved texture, appearance and mouth feel (better organoleptic qualities) with increased shelf-life.

RS may be beneficial in disease prevention, including modulation of glycaemic index (GI), diabetes, cholesterol lowering capability and weight management (Zhang *et al.,* 2005; Sajilata *et al.,* 2006). As RS passes through to the large intestine where it is bacterially fermented, it lowers the colonic pH as the resultant short-chain fatty acids (SCFAs) are produced and absorbed (Gordon *et al.,* 1997; Sajilata *et al.,* 2006). The three main SCFAs produced are acetate, propionate and butyrate (Sajilata *et al.,* 2006; Birkett & Brown, 2007). Butyrate is commonly in high concentrations and is the main energy substrate for colonic cells; it regulates intestinal cell function and growth by repressing tumour cells and reducing the proliferation of colonic mucosal cells, which is a risk factor in carcinogenesis (Johnson & Gee, 1996; Harris & Ferguson, 1999). Acetate and propionate are energy sources for the body, and are thought to play a role in carbohydrate (glucose) and lipid metabolism, particularly in the liver, muscle and adipose tissue, and influence weight management.

Evidence is increasing with regards to an inverse relationship between starch intake and colon cancer. Butyrate is thought to be associated with lower incidences of colon cancer and a lack of butyrate may increase the risk of some colonic pathologies and inflammatory diseases (Harris & Ferguson, 1999; Eliasson, 2004). RS also acts as a laxative as it reduces intestinal transit time and increases faecal bulk and can be used in products for coeliac disease. Recently RS has been classified as a prebiotic, as it beneficially affects the host by selectively stimulating the growth and activity of the microflora in the colon, and thus improving the host's health (Sajilata *et al.,* 2006).

Since RS is slowly digested, it influences the rate at which glucose is released. Slow release of glucose evokes a small increase in blood glucose (hypoglycaemic effect) as it is metabolised five to seven hours after consumption, whereas normally cooked starch is digested immediately. This slow digestion reduces postprandial glycaemia and insulinaemia (decreasing blood glucose, insulin and epinephrine levels) (Bjork, 2006). RS also has the potential for increasing the period of satiety by reducing the rate of gastric emptying and plays a role in providing improved metabolic control in non-insulin dependent type II diabetes (Bjork, 2006). Hypocholesterolaemic effects of RS diets have been reported. Such diets noticeably increase the faecal size, pool and absorption of SCFAs and lower plasma cholesterol and triglycerides. The hypocholesterolaemic properties suggest the use of RS in foods can improve cardiovascular health (Sajilata *et al.,* 2006).

In weight management, RS has two main roles with regards to energy metabolism and metabolic control (Birkett & Brown, 2007). Firstly, the digestible energy available from RS is reduced in comparison with a readily digestible starch and hence lowers caloric density. Secondly, the lower glucose and insulin impact of RS causes changes in lipid metabolism that favours lower lipid production, storage and increase fat burning. Important consideration is required in both choice of food and in the selection of specific ingredients to assist with targeting weight management regimes and improving overall health. RS is a versatile option for formulating high quality foods with added health benefits.

Materials and Method

Fruit material

The bananas (*Musa* spp.) obtained had been grown, harvested green but at the same maturity stage, sliced and dried in Pohnpei, Micronesia and imported into New Zealand under an import permit obtained from New Zealand MAF. The required FSM quarantine papers were also obtained.

Preparation of dried banana slices and banana powder (flour)

Immediately following harvest, the bananas were peeled and cut into 1.5-2.0 mm thick slices, and washed in cold water to minimise enzymatic browning. The slices were kept immersed in water (about 2 hours) and then arranged in a single layer on trays in a Harvest Maid forced air circular tray dryer (NESCO American Harvest Food Dehydrator, FD 60, The Metal Ware Corporation, Two Rivers, Wisconsin, USA) and dried at 60oC for 8-10 hours or longer until the moisture content was approximately 5%. The dried slices of each cultivar were then vacuum-packed in clear plastic bags, sealed and labeled.

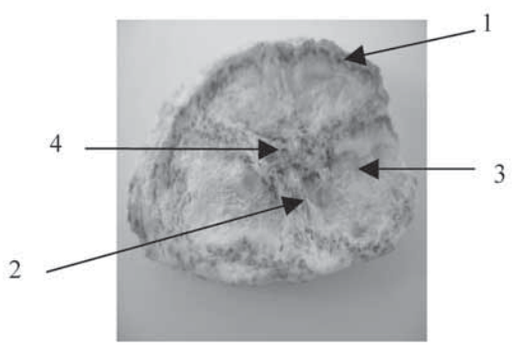
Upon arrival at the University of Auckland, banana powder (flour) was prepared from the dried banana slices of each cultivar. Approximately 30 g of the banana slices were ground into a fine powder to pass through a 2 mm sieve, using a coffee grinder (Kenwood Ltd, China). The powders were stored in re-sealable snap lock bags (Glad™ snap lock bags) at room temperature in a desiccator containing silica gel with as much of the air removed from the bag as possible to avoid moisture uptake by the powder. The AOAC (2000) vacuum oven method was utilized to dry and remove any moisture that the banana powder may contain prior to use in the RS and NRS analyses.

RS and NRS analysis of the banana flour

RS and NRS levels were analyzed following the procedure described in the Resistant Starch Assay Kit (K-RSTAR) which was purchased from Megazyme International Ireland Ltd (Bray Business Park, Bray, Wicklow, Ireland).

RS and NRS analysis of the banana pancakes =

Figure 1: A dried banana slice



Note: Arrows indicate zones from which sections were taken.

Banana pancakes were made by partial substitution of wheat flour with dried banana flour. Samples were prepared from these pancakes for each cultivar by cutting the pancakes into quarters. Two opposing quarters were taken from each pancake and ground using a laboratory blender (Warning Commercial, Connecticut, and USA) and dried to about 5% moisture content in an air dryer. Prior to RS and NRS analyses the banana pancake powders were dried and the moisture content determined accurately. RS and NRS analysis was carried out in the same manner as for the dried banana materials.

Measurement of residual glucose in banana powders and pancake material

Since measurement of starch using the Megazyme Assay Kit centers on the digestion of starch with enzymes followed by measurement of glucose, sample controls (in triplicate) of the Megazyme RS control, the banana powders and the banana pancakes were taken through all the procedures of RS and NRS analyses. This was done by excluding enzymes from the reagents. This precaution was taken to detect the presence of any residual glucose in the native fruit material and to check for any hydrolysis of sucrose in the pancakes which may contribute to the absorbance levels and erroneously increase the RS and NRS levels. Any glucose detected in the native material and pancake material was subtracted from the final RS and NRS levels.

Microstructure studies

To find out if there were any differences in morphology among the cultivars, sections from zones shown in Figure 1 were examined microscopically.

Bright field microscopy was carried out using a Leica DMLS microscope (Leica Microscopy and Scientific Instruments Group, Heerbrugg, Switzerland) fitted with a Leica DC 300F Digital camera (Leica Microscopy Digital Imaging, Cambridge, UK). Thin sections (1-3 cells thick) were hand-cut from the dried banana slices from each of the banana cultivars, using a double-edged razor blade. Transverse sections were taken from (1) the ovary wall, (2) the septum – partitions separating the three locules, (3) the locule (ovarian) cavity, and (4) the central fruit axis (Figure 1). Longitudinal sections were taken from (3) the locule only. Sections were stained with toluidine blue, which stains unlignified plant cell walls pink to purple and lignified cell walls

green with iodine in potassium iodide (Feder & O'Brien, 1968). The stained samples were studied together with unstained control sections which were mounted in the buffer for the stain, or water respectively.

Preparation of sections for environmental scanning electron microscope

Scanning electron microscopy (SEM) was carried out using a "FEI Quanta 200FEa-SEM microscope (FEI Ltd., Endhoven, Netherlands), operated in standard SEM mode. Thick transverse sections were taken from the dried banana slices of the *Inahsio* banana cultivar by splitting the banana slice horizontally manually to reveal a crude surface for viewing. Sections were taken from the four zones of the banana as for light microscopy. These sections were mounted on small metal stubs and coated with platinum for observation.

Statistical analysis

All statistical analyses were performed using the SPSS version 15.0 software for Windows. The Homogeneity of Variances test was applied to verify that if the samples followed normality and had equal variances on the dependent variable. Mean values of different cultivars based on one independent variable (or factor) were compared by One-Way ANOVA with a 5% significance level. The Post Hoc-Bonferroni test was run to see exactly which pairs of groups were significantly different.

Results and Discussions

Table 1: Residual glucose present in the dried banana and in the banana pancakes (dry weight basis)

	Measurement of glucose in the dried banana powder		Measurement of glucose in the banana pancake material	
Banana Cultivar	**RS (g/100g)**	**NRS (g/100g)**	**RS (g/100g)**	**NRS (g/100g)**
Megazyme RS Control	0.694 ± 0.060	0.590 ± 0.060		
Daiwang	0.208 ± 0.000	1.246 ± 0.000	1.248 ± 0.000	0.468 ± 0.000
Karat	0.138 ± 0.060	1.616 ± 0.060	1.937 ± 0.091	0.471 ± 0.000
Utin Kerenis	0.070 ± 0.061	1.540 ± 0.160	0.790 ± 0.274	0.211 ± 0.091
Inahsio	0.104 ± 0.000	1.319 ± 0.060	0.991 ± 0.090	0.209 ± 0.181
Utin Ruk	0.350 ± 0.061	2.450 ± 0.061	1.578 ± 0.158	0.684 ± 0.091

(Mean ± SD, n = 3)
Note: RS = resistant starch; NRS = non resistant starch

Table 1 shows the results where the Ð-amylase and amyloglucosidase enzymes from the RS and NRS assay had been excluded, it indicated that there was a small contribution to the glucose absorbance readings for the RS and NRS levels obtained for the cultivars from glucose present in the starting materials (i.e. sugars in the native fruit and sucrose in the pancakes). The actual RS and NRS values of the residual glucose were approximately < 2% of the total RS and NRS values obtained for the bananas and pancakes. Therefore the final RS, NRS and TS content for the particular bananas cultivars and their corresponding pancakes

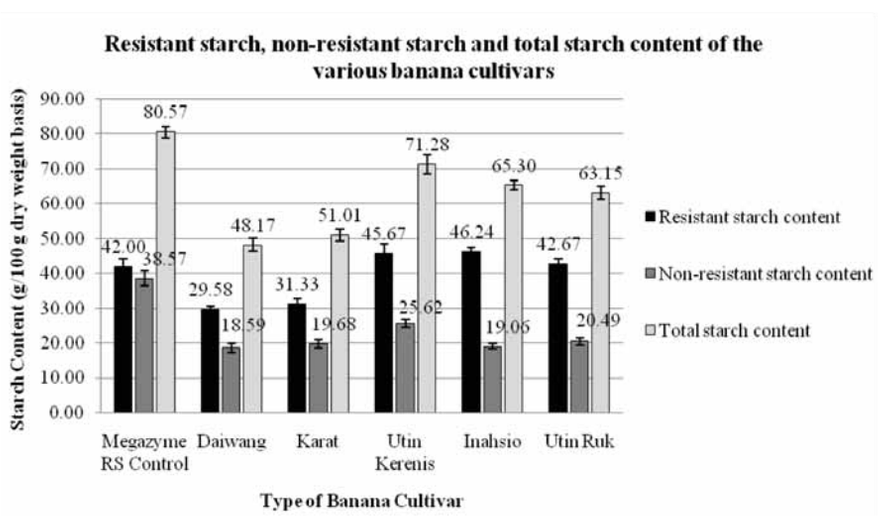
were adjusted to take into account the use of a 50/50 dilution of D-glucose and the equivalent residual glucose contribution from the starting materials to give an accurate sum of the starch levels in the fruits and pancakes.

RS, NRS and TS content of the dried bananas and banana pancakes for each cultivar

The Megazyme Resistant Starch Assay kit was a rapid and effective method for determining the level of RS, NRS and TS in these bananas. Significant differences were observed in the cultivars in their levels of the various starches. These differences may have been due to two factors (a) the compositional differences between the cultivars, (b) differences in the maturity of the different banana cultivars. Starch levels in the fruit (Figure 2) showed each cultivar contained higher levels of RS than NRS.

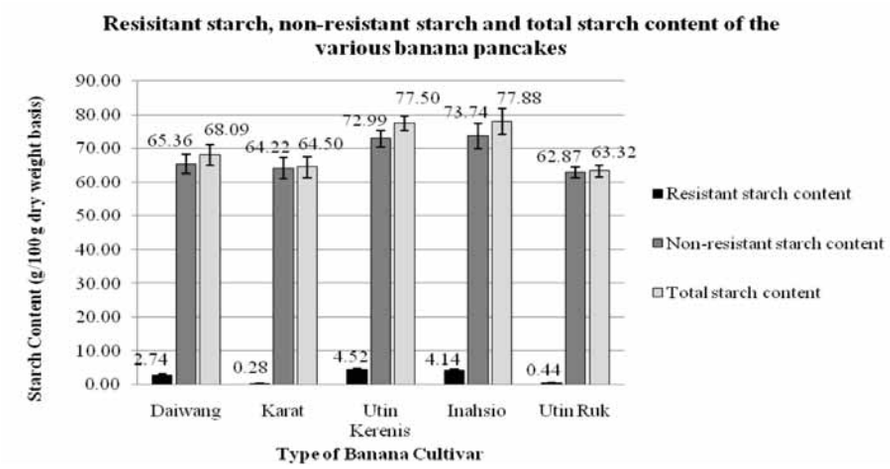
Comparisons of the RS levels made between the banana cultivars showed *Inahsio* to have the highest RS content (46.24%). With regards to NRS, *Utin Kerenis* had the highest content (25.62%) and overall it had the greatest TS content (71.28%). It is apparent that the RS levels had fallen significantly in the pancakes (Figure 3). Pancakes prepared from the *Utin Kerenis* cultivar contained the greatest amount of RS present (4.52%). Similar levels were found in *Inahsio*, but drastic reductions were seen in both *Karat* and *Utin Ruk*, resulting in almost negligible RS contents (0.28% and 0.44% respectively).

Figure 2: Starch content of banana cultivars (g/100 g)



A study by Akerberg et al. (1998) found green bananas to contain 72.1 ± 5.7 g/100 g of RS and 31.6 ± 3.2 g/100g of NRS. These values are notably higher than the values obtained in this study. The discrepancy might be related to the different degrees of maturity in the green stage of the bananas tested. The ripening process of bananas is known to consist of eight stages (Zhang *et al.,* 2005). During maturation the starch in bananas is converted into sugars. Unripe green bananas contain 20-23% starch which falls to just 1-2% in ripe yellow bananas and during this time the sugar content increases from less than 1% to 20%. This occurrence may affect the starch availability to enzymes, and hence the starch content (Zhang *et al.,* 2005). A ripe banana has been found to contain 5.1% RS dry weight basis (Elmstahl, 2002).

Figure 3: Starch content of banana pancakes (g/100 g)



However high levels of RS in the native fruit is explained by the large number of starch granules found in the raw green bananas, as seen from the microscopy studies. Granules are naturally very resistant to enzymatic hydrolysis due to their compact type-B crystalline structure, which limits the accessibility of digestive enzymes (Akerberg *et al.,* 1998). Although these starches are rather easily hydrolyzed after gelatinization reducing the RS content (Lii *et al.,* 1982; Faisant *et al.,* 1995; Zhang *et al.,* 2005), such an occurrence was seen in the pancakes.

The RS levels obtained for the ground native fruit samples would have been a combination of RS_1 and RS_2 starch, as physically inaccessible starch were made available. The susceptibility of the granule to amylases is known to influence RS content. Granule size plays a role in the level of starch available to enzyme attack. Bananas have been reported to have large starch granules as seen in these bananas (7.4 to 80 µm), indicating a small surface to volume ratio, and thus less starch available to enzymatic hydrolysis (Kayisu *et al.,* 1981; Lii *et al.,* 1982; Bello-Perez *et al.,* 2000). During ripening disappearance of the small granules is more rapid than larger granules, due to the action of enzymes (Lii *et al.,* 1982). The nature of the granules' surface also influences its susceptibility to amylases. Many SEM studies carried out on banana starch have shown the starch granules to have an adsorbed layer of non-starch material. In the current SEM study no preparative procedures were necessary as the material was already dried. Nevertheless some material can be seen adhered to granules (Figure 4a, b). Such deposits may effectively impede the action of the enzyme (Sajilata *et al.,* 2006). The amylose to amylopectin ratio appears to be correlated with high amounts of RS as mentioned in literature (Kayisu & Hood, 1981; Ling *et al.,* 1982; Garcia & Lajolo, 1988; Waliszewski *et al.,* 2003; Gonzalez-Soto *et al.,* 2007). Banana starches range in their amylose content. However those studied here seemed to have a large amount of amylopectin present in their starch as observed from red-purple staining with iodine in potassium iodide solution (Jensen, 1962) signifying amylopectin molecules.

The significant reduction in RS levels in the pancakes is likely due to gelatinization of the starch, in the presence of water and heat. This eliminated the crystallinity of the starch and made it susceptible to digestion and hydrolysis by amylases. Almost complete transformation of RS to digestible starch (NRS) occurred, hence the three-fold increase seen in NRS content. Amylose is known to have a greater tendency to retrograde than amylopectin under favorable conditions of temperature and starch-water ratio. A positive correlation exists between amylose retrogradation and formation of RS_3 and therefore the minute levels of RS seen in the pancakes would most presumably be retrograded amylose (RS_3) that formed upon cooling and only part of the RS1 and RS2 fraction would have been quantified (Gonzalez-Soto *et al.,* 2007).

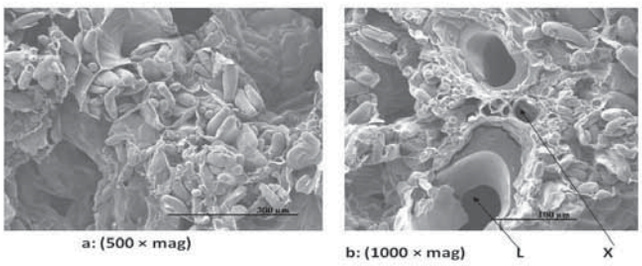


Figure 4a : SEM images of transverse sections taken from the ovary wall of the *Inahsio* banana cultivar showing starch granules

Figure 4b: SEM images of transverse sections taken from the ovary wall of the *Inahsio* banana cultivar showing Llatex vessel (L) cells and xylem vessels (X) (Figure 4b).

Processing conditions can influence the yield of RS formation in foods (Sajilata *et al.,* 2006). The RS content of foodstuff can significantly decreases when cooking under conditions of high moisture and temperatures, due to the disruption of the crystalline structure. On the other hand, RS levels can increase in foods, if specific conditions such as extrusion followed by cooling are carried out, inducing recrystallization and hence raising the RS content of a product. Chemical modifications also tend to increase RS levels (Sajilata *et al.,* 2006; Gonzalez-Soto *et al.,* 2007). Such factors are of great importance in the food industry; because it offers the possibility of increasing the RS content of processed foods and foodstuff, but the starches' functional and physicochemical characteristics need to be evaluated before its use as an ingredient in food formulations.

Microscopy

Observation of the banana sections indicated that the morphology was broadly similar among the cultivars. The parenchyma tissue, which made up the bulk of the fruits showed some variability in the shape and size of cells among the tissue zones within each cultivar.

Elongated cells were found in the region of the ovary wall (Zone 1, Figure 1), whereas towards the central fruit axis cells were more ovoid or spherical in shape. This is apparently due to less crowding and mechanical pressure in the inner regions (Von Loesecke, 1949). Generally the parenchyma tissue contained delicate unlignified, thin walled cells, which were tightly packed with numerous starch granules, some of which stained red with iodine and potassium iodide solution.

From the SEM images (Figure 4a), the starch granules seem to be closely clustered and intact within the cells that had retained their structure. The starch granules appeared to have a smooth surface, and were irregularly shaped, with many being long and elongated ovals and small spherical grains. These physical characteristics were also observed among the other banana cultivars, although Utin Kerenis and Utin Ruk appeared to have on average a slightly larger granule size.

Air spaces, lignified vascular bundles and xylem treachery elements were evident throughout the various regions of a banana when stained with toluidine blue. They were interspersed around parenchyma cells and considered more extensive in the tissue of the central fruit axis. Figure 4b shows large ovoids, which contain some amorphous material. These are likely to be latex vessels with latex deposited around inside of the vessels.

The surface of the deposits appears smooth as it may have solidified during the drying process of the banana slices. Latex contains tannins, other phenolic substances, variable amounts of oil, polyterpenes (rubber) and other secondary products (Kyamuhangire *et al.,* 2006).

Conclusion

Significant difference in the RS, NRS and TS levels were seen between the banana cultivars. Considerable reduction in RS levels was observed when the bananas were incorporated into pancakes. No marked differences in the morphology of the bananas were seen among the cultivars but differences were observed among the various zones within a cultivar. The data and information acquired from this research regarding the content levels of RS in these bananas and the characteristics analysed are fundamental and original. Such information would be of substantial importance in the food industry, especially in product development, as RS is known to have a number of applications due to its functional and beneficial health properties. The information can also be used to help promote these RS-rich banana cultivars for their health benefits. Considering the significant decline in RS content after cooking, further investigation into processing procedures of these bananas is required. The conditions at which high levels of RS can be maintained in products containing these bananas should be contemplated to impart the utmost benefit after processing.

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"Nobody stands taller than those willing to stand corrected."

William Safire