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Resistant starch in cassava products

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Abstract

Found in different foods, starch is the most important source of carbohydrates in the diet. Some factors present in starchy foods influence the rate at which the starch is hydrolyzed and absorbed *in vivo*. Due the importance of cassava products in Brazilian diet, the objective of this study was to analyze total starch, resistant starch, and digestible starch contents in commercial cassava products. Thirty three commercial cassava products from different brands, classifications, and origin were analyzed. The method used for determination of resistant starch consisted of an enzymatic process to calculate the final content of resistant starch considering the concentration of glucose released and analyzed. The results showed significant differences between the products. Among the flours and seasoned flours analyzed, the highest levels of resistant starch were observed in the flour from Bahia state (2.21%) and the seasoned flour from Paraná state (1.93%). Starch, tapioca, and sago showed levels of resistant starch ranging from 0.56 to 1.1%. The cassava products analyzed can be considered good sources of resistant starch; which make them beneficial products to the gastrointestinal tract.

Keywords: products; flour; digestion.

1 Introduction

Starch is the basic source of energy for the majority of the world's population. It plays a major role in supplying the metabolic energy that enables the body to function. Starch can be divided into three categories based on nutritional classification: rapidly digestible starch, slowly digestible starch, and resistant starch. The term resistant starch (RS) refers to the sum of starch and products of starch degradation which have not been absorbed in the small intestine of healthy humans (Englyst et al., 1992).

Some studies suggest that slowly digested starch and resistant starch have significant implications for human health. Resistant starch fraction passes on to the colon, where it is fermented by the microorganisms producing mainly short chain fatty acids (SCFA). Due to this fact, RS has functional properties and positive effects on diabetes, some kinds of cancer, cardiovascular diseases, colonic health, obesity, and osteoporosis (Lunn & Buttriss, 2007; Nugent, 2005; Sajilata et al., 2006; Morales-Medina et al., 2014). Resistant starches have been shown to have equivalent and/or superior impacts on human health similar to that of conventional fiber-enriched food ingredients. It will, in turn, improve the health status of consumers. The potential health benefit of RS varies greatly with the study design and difference in the source, type, and level of RS consumed (Buttriss & Stokes, 2008).

Resistant starch has been categorized into four main types: type 1, RS is physically entrapped starch within whole plant cells and food material; type 2, RS includes granules from certain plant sources (e.g., raw potatoes, green bananas, and high-amylose corn); type 3, RS comprises retrograded starch from cooked and cooled starch and starch-containing foods; and type 4 RS includes chemically modified starches that are used by food manufacturers to improve the functional characteristics of the starch. Different processing conditions can alter RS content in the starch and starch-based foods (Chung et al., 2011).

In addition to the structural factors which can influence the amount of RS present due to the presence of water and the chemical structure of starch, other factors intrinsic to starchy foods can affect α-amylase activity and therefore starch breakdown. These include the formation of amylose-lipid complexes, the presence of native α-amylase inhibitors, and also non starch polysaccharides; all of which can directly affect α-amylase activity.

From a technological standpoint, resistant starch type 3 is the most important since their formation is a result of food processing. The amylose content, temperature, physical form, the degree of gelatinization, cooling, and storage affect its contents. The content of resistant starch in foods can be changed by relatively simple processing techniques, influencing the rate and expected extent of starch digestion in the human intestine. This changes can be used beneficially by either the consumer, through the maintenance of good health, or by the food industry, which uses a source of fiber that do not cause organoleptic changes as pronounced as those caused by the sources traditionally used in products such as brans.

The presence of resistant starch has been detected in various foods such as white bread, breakfast cereals, biscuits, corn mashed, potatoes, and legumes. The key challenge to the food industry is the production of consumer-friendly foods, which contain enough resistant starch to result in a significant improvement in public health (Chung et al., 2011).

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Cassava (*Manihot esculenta* Crantz) is the sixth most important food crop globally in terms of annual production (Food and Agriculture Organization of the United Nations Statistics Database, 2013), and it is a staple food for approximately 800 million people. Cassava is a major source of calories in the tropics, where its roots are processed into several foods.

Products derived from cassava are produced and consumed throughout Brazil. Brazilians with lower family income consume more cassava flour in their different types and starch. Products with higher added value such as frozen products are sold in supermarkets and purchased by consumers with higher household income (Barros, 2004).

The cassava flour has large variations due to differences in processing of the root. It is consumed in large quantities but with high inequality in per capita consumption between different regions in Brazil. The national average is 7.8 Kg per capita per year. Rural areas appear as the main consumers of flour, with an average of 20.6 Kg per capita annually. This is four times higher than the average of urban dwellers, from 5.1 Kg per capita per year (Barros, 2004).

Considering the importance of resistant starch to health, the consumption of cassava products by Brazilians, especially those who have little access to high-value products, as well as, the differences in processing methods for obtaining products of cassava, this study aimed to evaluate the content of total starch, resistant starch, and digestible starch present in cassava products from different regions in Brazil.

2 Materials and methods

Thirty-three cassava products (Table 1) usually consumed, produced, and sold in different Brazilian states were evaluated for total starch, resistant starch and digestible starch. For each product, three packages from different batches but produced and packaged by the same manufacturer were acquired and analyzed.

Table 1. Cassava products, origin, and classification.

Products		Brazilian states	Classification			
			Group	Class	Color	Type
Flours	F1	São Paulo	Dry	Fine	White	1
	F2	São Paulo	Dry	Flocked	Yellow	-
	F3	Paraná	Dry	Fine	Yellow	2
	F4	São Paulo	Raw	Fine	-	-
	F5	Bahia	Dry	Fine	White	1
	F6	Rio Grande do Norte	Dry	Fine	Yellow	1
	F7	Pará	Water group flour	-	-	-
	F8	Pará	Dry	Coarse	Yellow	1
	F9	Bahia	-	-	-	-
	F10	Minas Gerais	Torrada	Coarse	Yellow	1
	F11	Rio Grande do Norte	Dry	Medium	White	1
	F12	Maranhão	Water group flour	Fine	White	1
	F13	Maranhão	Water group flour	Fine	Yellow	1
	F14	São Paulo	Dry	Coarse	White	1
	F15	São Paulo	Dry	Fine	White	2
	F16	São Paulo	Dry /Toasted	Fine	Yellow	1
	F17	São Paulo	Dry	Flocked	Yellow	-
	F18	Mato Grosso	Dry	Fine	White	1
	F19	Acre	Dry	Medium	Yellow	1
	F20	Minas Gerais	Dry	Fine	Yellow	1
	F21	Pará	Water group flour	Medium	Cream	1
	F22	Pará	Dry	Coarse	White	1
	F23	Pará	Dry	Fine	Yellow	1
	F24	Pará	Dry	Coarse	Yellow	1
	F25	Sergipe	Dry	Fine	White	1
Seasoned flour	SF1	Paraná	Seasoned flour	-	-	-
	SF2	São Paulo	Seasoned flour	-	-	-
	SF3	Minas Gerais	Seasoned flour	-	-	-
Tapioc	T1	Paraná	-	Granulated	White	1
	T2	Pará	-	Granulated	-	1
Sago	ST	São Paulo	-	-	-	1
Starch	S1	Paraná	-	-	-	-
	S2	São Paulo	-	-	-	-

The classification of cassava flour is established on the basis of its identity and quality requirements. Cassava flour is classified into groups, classes, and types. According to the technological process used in its production, cassava flour is classified into three groups: a) dry flour, product obtained from roots of healthy cassava that were properly cleaned, peeled, mashed, grated, ground, pressed, dismembered, sieved, and dried to the proper temperature, which could be sifted again and processed; b) water group cassava flour (known as *farinha d'água* in Brazil), predominantly fermented product, obtained from healthy cassava roots that were macerated, peeled, crushed or ground, pressed, broken down, sieved, and which could be dried at appropriate temperature and sieved again; c) flocked flour, low density product obtained from healthy roots of cassava that were cleaned, shelled, grated, ground, pressed, broken down, sieved at the proper temperature, to form irregular and light flakes (Brasil, 2011).

The cassava flour of the dry group is classified into three classes according to its particle size: a) fine flour - 100% of the product pass through the sieve with a mesh aperture of 2 mm, b) medium flour - when more than 10% of the product is retained even on the sieve with an aperture of 2 mm, and c) coarse flour - when more than 15% of the product is retained on the sieve with an aperture of 2 mm (Brasil, 2011).

The water group cassava flour is classified into three classes according to its particle size: a) fine flour- when the product is retained up to 10% on the sieve with a mesh aperture of 2 mm; b) medium flour - when over 10 to 15% of the product is retained on the sieve with an aperture of 2 mm; c) coarse flour - when over 15% of the product is retained on the 2 mm-mesh sieve (Brasil, 2011).

Total starch (TS) content was determined according to Goñi et al. (1997). The samples (50 mg) were suspended in 2M KOH to disperse starch and shaken at room temperature for 30 min. The samples were then incubated (60 °C, 45 min, pH 4.75) with amyloglucosidase (1 mL (300 U/mL), Sigma A-7255) to hydrolyze starch. The free glucose was determined using glucose oxidase, peroxidase, and ABTS assay (Bergmeyer & Bernet, 1974). The total starch was calculated as glucose × 0.9. The wheat starch (Sigma S-1514) was used as reference standard.

Resistant starch (RS) content was analyzed using the methodology proposed by Goñi et al. (1996). The samples were subjected to: incubation (40 °C, 60 min, pH 1.5) with pepsin (0.1 mL (10 mg/mL), Sigma P-7012) for protein removal; incubation (37 °C, 16 h, pH 6.9) with α-amylase (1 mL (40 mg/mL), Sigma A-3176) to hydrolyze digestible starch; residue treatment with 2M KOH for solubilization of resistant starch; incubation (60 °C, 45 min, pH 4.75) with amyloglucosidase (80 mL (140 U/mL), Sigma A-7255) to hydrolyze the resistant starch solubilized; and determination of glucose, as described for determination of total starch. The value of digestible starch (DS) was calculated by the difference between TS and RS.

Results were expressed as means of values of three separate determinations. Comparison of means was performed by one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test.

3 Results and discussion

Table 2 shows the amount of total starch, resistant starch, and digestible starch in cassava products, expressed in dry weight. Statistical analysis showed differences in these components in the cassava products analyzed.

As for the starch content in cassava flours, Brazilian regulations establish that for the dry and water group flours, the content of starch (wet weight) must be ≥ 86.0% in type 1, ≥ 82.0% in type 2, and ≥ 80.0 in type 3. As for the flocked flour, the starch content must be ≥ 80.0%. Thus, the various flours analyzed in this study with labeled type 1 do not fall within the recommended limits (total starch in wet basis ranged from 78.80 to 89.92% in cassava fours) (Brasil, 2011).

The levels of resistant starch in the cassava flours ranged from 0.19 to 2.21% (dry weigh). Among the analyzed cassava flours the highest content of resistant starch were found in the flour sold in bulk (F9), and the lowest levels in the dry flour

Table 2. Total starch, resistant starch and digestible starch in cassava products (dry weight).

Products	Total starch (%)	Resistant Starch (%)	Digestible starch (%)
F1	92.41jk	1.92b	90.49jk
F2	92.98i	1.58e	91.4h
F3	92.97j	1.65d	91.32i
F4	94.28ef	0.81n	93.47d
F5	92.22kl	1.19g	91.03ij
F6	92.11kl	0.95l	91.16i
F7	94.34e	1.70c	92.64g
F8	92.03l	1.11i	90.92j
F9	91.63m	2.21a	89.42k
F10	90.25n	1.03k	89.22kl
F11	89.75o	1.70c	88.05l
F12	92.16kl	1.16h	91.00j
F13	93.97fg	0.73	93.24e
F14	93.89gh	0.94l	92.95g
F15	90.30n	1.13i	89.17kl
F16	89.39p	0.20t	89.19kl
F17	88.27q	1.15h	87.12n
F18	87.55r	0.38s	87.17n
F19	93.60h	0.19t	93.41d
F20	88.46q	0.76o	87.7m
F21	86.62s	0.72p	85.9p
F22	86.97s	0.84m	86.13o
F23	88.53q	0.84m	87.69m
F24	93.07i	1.19g	91.88h
F25	90.17n	0.73p	89.44k
SF1	85.95t	1.32f	84.63r
SF2	75.59v	0.19t	75.44t
SF3	79.60u	0.67q	78.93s
T1	98.57b	0.67q	97.9a
T2	95.36d	0.56r	94.8c
S	91.93lm	0.85m	91.08ij
S1	98.14c	1.06j	97.08b
S2	99.14a	1.11i	98.03a

Means followed by different letters in a column are different (Tukey test $p < 0.05$).

from Acre state (F19) and dry/toast flour from São Paulo state (F16). Significant differences were also observed for the flours with the same classifications, which may be due to differences in the processing methods for the same type of flour in Brazil.

Resistant starch is a natural component which is present in many foods. Certain types of processing, such as sterilizing, drying in ovens, or drying at high temperatures, increases the level of resistant starch (Pereira, 2007). During the processing of cassava root to obtain flour, an important step is the drying in an oven. In this stage, the moisture of the mass of grated cassava is strongly influenced by the type of flour produced, the type of oven used, the load of material in the oven, and the temperature of drying.

Drying of cassava mass can be carried out in ovens or roasters; the most commonly used types of oven are: "baiano", semi-spherical pot with a central paddle, and "Sao Paulo" or rotary over, consisting of a rotating circular plate seated on a masonry stove with a mechanical distributor bottom sieve for the distribution of mass on the plate and a brush to remove the flour. Flat ovens are commonly found in the North and Northeast regions of Brazil, which are made from a flat sheet of clay or iron and the tilling of cassava mass is done manually with using squeegees or mechanically by a system of blades. These ovens operate at different temperatures and loads. Cold ovens with low load produce the finest white flour. Hot ovens with high loads produce flours with particle size characteristic of water group flour. Rotary ovens with low load on the surface produce the typical flocculation of flocked flour (Cereda & Vilpoux, 2003; Matsuura et al., 2003). These differences in cassava flour production can influence the starch gelatinization and retrogradation, affecting the content of resistant starch.

The differences in the process of drying the grated cassava mass to obtain the different types of flour, besides affecting the particle size, affect the integrity of starch granules, interfering in digestibility. The digestion resistance can or cannot be related to the integrity of the physical state of starch granules, which in turn is related to crystalline organization or packing of the glucose chains of amylose and amylopectin (Thompson, 2000; Liu, 2005).

In the analyses performed in the commercial flour, the content of resistant starch found for the raw cassava flour was 0.55 g/100g, below the values found for wheat bran (0.73 g/100 g), yellow corn flour (2:46 g / 100 g), and oat flour (1.78 g/100 g) (Universidade de São Paulo, 1998). Comparing these data with the results obtained for products derived from cassava, it can be observed that the seasoned and cassava flours can be considered interesting sources of resistant starch.

As for the seasoned cassava flours, the highest content of resistant starch was observed in the flour from Paraná (SF1). The influence of lipids on starch digestibility has been reported. Amylose-lipid complexes are enzyme-degradable, and an increase in complexed amylose reduces yields of resistant starch. Amylose recrystallization in RS formation is competitively affected by complexation of amylase with lipids (Czuchajowska et al., 1991).

Since crystallisation of amylose takes place above the glass transition temperature, all components (e.g. other polymers such as proteins but also sugars and salts) which have an impact on the glass transition temperature can theoretically have an influence on the formation of RS type III (Eerlingen & Delcour, 1995).

Tapioca, product obtained from cassava starch under a granular form, showed low content of resistant starch. This result is probably due to the type of processing in which the mass of starch lumps with moisture content of approximately 55% are broken up when heated in a "*baiano*" oven at temperatures of 70 to 80 °C leading to the starch gelation. The starch is mixed and dried in the rotary drying device allowing the aggregation of irregular particles (Cereda & Vilpoux, 2003).

Formation of RS is also affected by the water content. A maximum in RS yield is found when a starch:water ratio of 1:3,5 (w/w) is applied. Indeed, as the amylase concentration increases, RS yield increases. A minimum of water, however, is necessary for plasticization of the environment and for incorporation into the crystal structure (Eerlingen & Delcour, 1995).

The sago of cassava showed significantly high RS content. Sago is a product of partially gelatinized starch with spherical shape and eaten as dessert or as porridge. It is produced in a continuous process with three rotating drums. The starch with 55% of moisture is placed into the upper part of the drum, where it is granulated. The beads of sago selected are placed into another double surfaced drum (120 to 200 °C) to gelatinize the starch. Sago passes through this rotating drum resulting in surface gelatinization, while remaining white inside. After this step, the product is dried in the third drum. Since the starch cannot be fully gelatinized in this process, it can contribute to the presence of resistant starch in this product.

The content of digestible (DS) and resistant starch (RS) in commercial cassava starches were higher than those found by Walter et al. (2005a) for corn starch. These authors evaluated the available digestible (DS) and resistant (RS) starch using different protocols to determine these fractions of starch (adjusted to the AOAC 996.11 method) and obtained (dry mass):0.10 ± 0.01 to 0.24 ± 0.04% RS and 97.6 ± 0.98 to 99.30 ± 0.82% for DS.

In the processing of cassava starch, the "starch milk" concentrated in centrifuges is subjected to pre-drying, which in most companies is performed in a vacuum filter. After this step of pre-drying, the starch with about 45% moisture is dried in a flash dryer at temperature ranging 120 to 150 °C. This type of process often promotes gelatinization and retrogradation of starch and can interfere in the resistant starch content. In the production of cassava starch using the peeler centrifuges, it is possible to obtain a product in the pre-drying stage at lower moisture contents (30-40%) by reducing the gelatinization and retrogradation.

The amylose content, temperature, physical form, the degree of gelatinization, cooling, and storage affect the contents of RS III. These indications serve as a basis to explain why, unlike dietary fiber, the amounts of resistant starch in foods can be modified by relatively simple processing techniques influencing the rate and expected extent of starch digestion in the human intestine (Walter et al., 2005b).

4 Conclusions

Cassava products contain varying amounts of resistant starch, and the processes for obtaining these products can interfere in RS content. The consumption of cassava products can contribute to the intake of resistant starch in the Brazilian diet.

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