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Evaluation of resistant starch content of cooked black beans, pinto beans, and chickpeas

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ABSTRACT

Resistant starch (RS) is associated with many of the health benefits attributed to dietary fiber. In this study, RS content was determined in black beans, pinto beans, and chickpeas freshly cooked and sampled at 15-min intervals for 90 min. A second set of black bean samples, cooked identically, was held at room temperature (25 °C) for a 24-h period prior to being assayed. The analysis showed that resistant starch levels fall sharply between 15 and 30 min of cooking before achieving a steady resistant starch concentration of approximately 4 g/100 g of sample dry weight. Beans allowed to sit at 25 °C showed similar behavior, but had increased levels of resistant starch after leveling off (approximately 5 g/100 g dry weight). A texture analysis of black beans was also completed along with resistant starch analyses for pinto beans and chickpeas to provide additional results that contributed to the overall conclusions. Pinto beans had slightly higher levels of resistant starch at each time interval, but followed a pattern similar to black beans. Chickpeas had low levels of resistant starch initially and expressed little change as cooking time increased. After 60 min of cooking, all bean samples had between 3 and 5 g of resistant starch per 100 g. In order to maximize dietary consumption of RS, a cooling period for cooked legumes is advisable.

1. Introduction

Legumes are an important source of proteins and complex carbohydrates. Starch is a polymeric carbohydrate that serves as a major source of energy in the human diet [1]. The two components of starch, amylose and amylopectin, both exist as polysaccharides of D-glucose residues [2,3].

RS is defined as the portion of starch and starch products that resist digestion, passing directly through the small intestine. The RS can be divided into four types: category one (RS1) is starch physically protected from digestive enzymes in grains that haven't been fully milled. Category two (RS2) refers to starch in less stabile, tightly packed crystalline granules that are partially resistant to hydrolysis. Category three (RS3) is starch (amylose) that has been retrograded into more highly stabile crystalline structures, and category four (RS4) refers to starch that has been modified using chemical reagents. RS3 is considered the most stable of the natural resistant starches to heat (over 100 °C) and further processing. Upon entering the colon, RS undergoes a high degree of anaerobic fermentation by local microbiota into a wide variety of products. These products include gases (hydrogen, methane, and carbon dioxide) and short-chain fatty acids (acetate, propionate, and butyrate). Butyrate is the predominate short-chain fatty acid produced from RS [4–8]. According to studies published over 30 years, there is no doubt of the important role that butyrate plays in maintaining intestinal homeostasis [9].

Resistant starch is associated with many of the health benefits attributed to dietary fiber, such as the reduction of type II diabetes risk, the production of short-chain fatty acid in the colon, the increase of calcium absorption and the reduction of inflammatory bowel disease [10–17].

It must be pointed out that resistant starch is not a precise physical entity but a concept developed to explain why some starch is not readily digested [18]. As a result, RS is characterized by analytical methods rather than a specific chemical structure. The search for an accurate analytical method for determining the RS content of food has gone on for more than 30 years. Although methods vary widely, nearly all involve mimicking the in vivo process of digestion [19]. The RS content of food therefore depends largely on the analytical method. As an example, Hughes et al. [20] found the raw starch of chickpeas contained 24–41% RS using the Englyst method [21], while the more recent AACC approved

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method 32–40 [22] found 8.4–18.4% RS. Whole chickpea flour contained just 3.1–6.3% RS using the AACC method [23,24].

Given the interest in RS from a nutritional standpoint and availability of the approved AACC 32-40 method of analysis, a study of resistant starch levels in black beans, pinto beans, and chickpeas after cooking for increasing periods of time was performed.

2. Materials and methods

2.1. Materials and instrumentation

Dry Beans: Goya Dry Black Beans, Goya Dry Pinto Beans, Goya Dry Chickpeas.

Processed Beans: Goya Canned Chickpeas, Ortega No Fat Refried Beans.

Chemicals: Potassium hydroxide 2 M solution prepared from potassium hydroxide (Fisher Scientific Company); sodium maleate buffer pH 6, prepared from maleic acid (Sigma–Aldrich Chemical Company) and sodium hydroxide (Fisher Scientific Company); Sodium acetate buffers pH 3.8 and pH 4.5, prepared from glacial acetic acid and sodium hydroxide (both from Fisher Scientific Company); Aqueous ethanol (50% v/v) prepared from 95% ethanol (Fisher Scientific Company), Spectrophotometric Grade, and Megazyme Resistant Starch Assay Kit.

Instruments: Bausch and Lomb Spectronic 20 Spectrophotometer; Brookfield Engineering LFRA Texture Analyzer; Vertis Freezemobile 12ES Lyopholizer; Fisher Scientific Vortex Mixer; Precision Circulating Water Bath Model 260; Adams Analytical Centrifuge manufactured by Becton, Dickenson and Company.

2.2. Sample preparation

All beans were cooked in 2 L of boiling distilled water – with prior soaking overnight (12 h) at room temperature using standard procedures – and removed every 15 min for 60 or 90 min. Sample sizes for dry and wet beans were 100 and 500 mg respectively. After preparation, fresh bean samples were kept at room temperature for less than 30 min prior to being processed in the shaking water bath. Samples were tested in duplicate with each glucose solution tested twice spectrophotometrically for a total of four data points for each time interval. Black beans were allowed to remain for 24 h at room temperature, and then were analyzed for RS.

2.3. Methods

2.3.1. Resistant starch

The samples were incubated in a shaking water bath with pancreatic α-amylase and amyloglucosidase (AMG) for 16 h at 37 °C, during which time non-resistant starch was solubilized and hydrolyzed to D-glucose, by the combined action of the two enzymes. The reaction was completed by the addition of an equal volume of ethanol, and the RS was recovered as a pellet on centrifugation. Then, it was washed twice by suspension in ethanol (50% v/v), followed by centrifugation (with free liquid removed by decantation). RS in the pellet was dissolved in 2 M KOH by stirring in an ice water bath over a magnetic stirrer. This solution was neutralized with acetate buffer and the starch was quantitatively hydrolyzed to glucose with AMG. D-Glucose was measured with glucose oxidase/peroxidase reagent (GOPOD), and this was a measure of the RS content of the sample. Non-resistant starch was determined by combining the original supernatant and the washings, adjusting the volume to 100 mL and measuring D-glucose content with GOPOD. The methodology for Resistant Starch determination was supplied by the Megazyme Starch Assay Procedure [25], essentially the same as AACC Approved Method 32–40. A collaborative study verifying the reproducibility of this procedure was conducted [26]. One difference of note was the use of a coffee grinder and strainer instead of a grinding mill and meat mincer in sample preparation.

2.3.2. Moisture and texture analysis

Moisture content was determined by lyophilization [27], followed by oven drying (18 h). Texture analysis was performed using the needle probe with measurements taken over a five 5 mm penetration of the bean sample.

3. Results and discussion

Raw black beans (Fig. 1) had an average RS concentration of 31 g per 100 g of dry sample weight. After 15 min of cooking in boiling (100 °C) water, RS concentration dropped by 15.3% in freshly cooked beans. By 30 min, RS levels had fallen a total of 86.8% on average to approximately 4 g. Over the next hour RS levels stabilized and fell on average 5.3% every 15 min until the end of the experiment. After 90 min of exposure to excess heat and water, RS levels were slightly over 10% of what was originally found in raw black beans.

Variations in individual RS measurements were higher (up to 8.2% from the mean) in raw and 15-min beans, but were reduced considerably for 30–90-min samples. By 60 min of cooking, the black beans were judged suitable for consumption when load weight was less than 100 g.

Black beans allowed to stand at 25 °C for 24-h (Fig. 1) had a similar behavior to the freshly cooked black beans. Fifteen and thirty minutes of cooking led to a 22.5% and 84.5% reduction respectively in RS. Levels of RS stabilized again after 30 min and the concentration of RS at the experiment's termination was 14.1% of that present in raw black beans. The major difference between fresh and 24 h beans was that after 30 min of cooking, beans allowed to cool had an average of 29.4% more RS.

A texture analysis was performed on both sets of black beans (Fig. 2). The average load weight needed to penetrate a sample bean 5 mm dropped exponentially for both fresh and 24-h beans as cooking time increased. Regression lines had correlation coefficients of 0.9669 and 0.9481, respectively. Load weights for 20-h cooled beans were higher than freshly cooked beans at each cooking interval, but decreased in

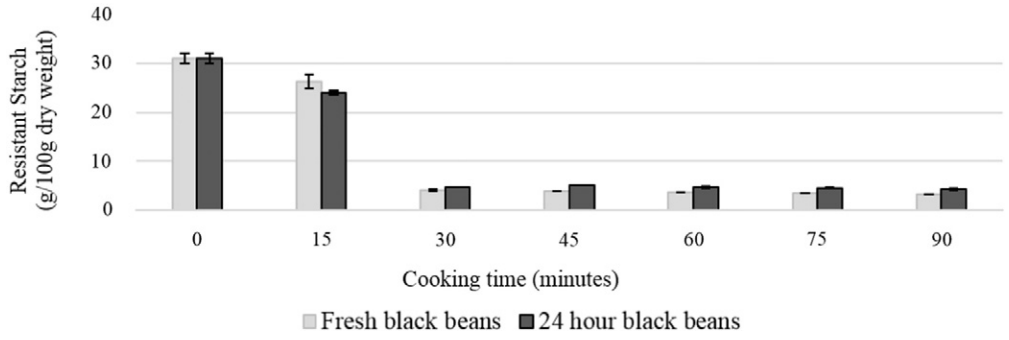

Fig. 1. Resistant starch in Black beans versus cooking time.