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Production of High-resistant Starch (RS3) Ingredient from Pinto Bean Starch

Courtney Simons[1]

[1] Wright State University - Lake Campus, Celina Ohio, USA

Correspondence: Courtney Simons, Wright State University - Lake Campus, 7600 Lake Campus Drive, Ohio 45822, USA. Tel: 419-586-0313

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Abstract

Commercial retrograded resistant starch (RS3) has been successfully produced from cereals and tubers such as corn and tapioca. Pulse starches have the potential to be utilized as a raw material to make RS3 products due to their high amylose content. However, this opportunity has not yet been explored. In this study, RS3 was produced using pinto bean starch, corn starch, tapioca flour, and rice flour. This was accomplished by gelatinizing the starches, followed by refrigeration for 4 days to allow for retrogradation. Afterwards, the retrograded starch was digested with α-amylase to remove soluble starch, and the remaining non-soluble (high-RS) residue collected, dried, and milled to a flour. Pinto bean starch produced a high-RS product containing 52.88% RS. This amount was not significantly different from what was produced from corn starch (49.54%) and tapioca flour (46.08) %. Rice flour produced the lowest percentage RS (10.33%). The findings demonstrated that pinto bean starch can be utilized as a raw material to produce a high-RS product with comparable resistant starch content to corn and tapioca RS3 ingredients.

Keywords: starch, resistant starch, pinto bean starch, pulse starch, ingredients, carbohydrates, digestion, food quality

Abbreviations

RS – Resistant starch

RS3 – Retrograded resistant starch

1. Introduction

Most of the starch we eat are completely digested in the small intestine. A small portion may escape digestion and ferment in the large intestine. This type of starch is known as resistant starch (RS). There are 5 forms of RS, i.e., RS1, RS2, RS3, RS4, and RS5. These are defined as (1) RS1 – physically inaccessible starch in partially milled grain, seeds, and legumes; (2) RS2 – ungelatinized starch that resists digestion due to its highly compact and crystalline structure; RS3 – retrograded starch; RS4 – chemically modified starch; and (5) RS5 – starch that is chemically bound to lipids (Sajilata et al., 2006; Walsh et al., 2021). RS3 is of particular commercial interest because of its high thermostability during most cooking operations (Haralampu (2000). Examples of RS3 products reported in literature include Novelose 330 from corn (47% RS), CrystaLean® from corn (46% RS), and Actistar 11700 from tapioca (50-58% RS). Health properties of RS3 ingredients include: (1) prebiotic effects e.g., increasing short chain fatty acid (SCFA) and SCFA-producing bacteria; (2) cancer prevention, e.g., preventing tumor development and colon carcinogenesis; (3) weight management; (4) cholesterol control; and (5) lowering glycemic index (Bauer-Marinovic et al., 2006; Chen et al., 2023; Jacobasch et al., 2006; Kahraman et al., 2019; Leu et al., 2009; Liu et al., 2020; Sanz et al., 2010; Silvi et al., 1999; Zampa et al., 2003).

Pulse starches may be a good source material for the production of RS3 due to their relatively high amylose content compared to most starches (Guillon & Champ (2002). High amylose content is associated with high retrogradation and RS3 formation (Ren et al., 2021; Haralampu, 2000). Nevertheless, despite its potential, there are currently no commercially available pulse-based RS3 products on the market. Hence the goal of this study was to make RS3 from pinto bean starch, and to demonstrate that it can yield a product with comparable or more RS as can be generated from corn starch, tapioca flour, and rice flour under the same processing conditions. This would encourage food manufacturers to begin to explore pulse starch as an alternative to conventional starches.

2. Materials and Methods

Dry pinto beans, corn starch, tapioca flour, and white rice were purchased from a local grocery. Amylex-3T alpha amylase was donated by DuPont Nutrition and Health (Kansas, USA). Resistant starch kit was purchased from Megazyme International (Bray, Ireland).

2.1 Milling of White Rice

White rice was milled at 10,000 rpm using a Retsch ZM200 centrifugal mill (Retsch GmbH, Haan, Germany) fitted with 0.5 mm sieve.

2.2 Starch Extraction

Dry pinto beans (6 kg) were rinsed and then soaked in excess distilled water at room temperature for 12 hr. Excess water was then drained, and the beans were rinsed. The soaked beans were blended in an Oster (Sunbeam Products, Boca Raton, FL) 14-speed kitchen blender at high speed for 2 min. The ratio of water to beans in the blender was 1:2. The puree was strained using a stack of sieves with decreasing Tyler mesh openings (20, 60, and 80 mesh). The liquid that passed the 80-mesh sieve (i.e., liquid portion) was placed in a refrigerator for 12 hr to cool. This resulted in the precipitation of starch. The supernatant was discarded, and the precipitate was resuspended using excess 0.1N NaOH to solubilize proteins. The mixture was returned to the refrigerator for 12 hr to allow the settling of starch. Afterward, the supernatant was discarded, followed by another resuspension using distilled water. The mixture was adjusted to a neutral pH using 2N of HCl and then returned to the refrigerator for another 12 hr to allow the starch to precipitate. The supernatant was then discarded. The starch was dried in a convection oven (VWR International, Radnor, PA) at 50^{o}C for 12 hr and then milled in a Retsch ZM200 centrifugal mill (Retsch GmbH, Haan, Germany) fitted with 0.5 mm sieve and rotating at 10,000 rpm to distribute starch aggregates uniformly.

2.3 Production of Retrograded Starch

Exactly 80 g of flour (pinto bean starch, corn starch, tapioca flour, or white rice flour), was combined with 750 ml water in an aluminum pot, and the starch cooked to gelatinization on a stove for 6 minutes with continuous mixing. The gels were allowed to cool to room temperature and then transferred to a refrigerator (4^{o}C) for storage for 4 days. The retrograded gels were then removed from the refrigerator and homogenized in 500 ml phosphate buffer (100mM; pH = 6) using a Polytron PT 2500 E rotor stator homogenizer set at 10000 rpm (Kinematica Inc., Bohemia, NY). Two milliliters of Amylex 3T alpha amylase were then added, and the mixture digested at 60^{o}C for 3 hours with continuous mixing in a water bath (SWBR17 Shel Lab, Sheldon Manufacturing, Inc., Cornelius, OR). The digested material was immediately centrifuged and the supernatant discarded to remove the soluble starch. Ethanol (150 ml; 95%) was then added followed by agitation, and then centrifugation to inactivate the enzyme. The previous step was repeated three times using distilled water to remove ethanol residue and any remaining soluble starch. The resulting material was dried in a convection oven (VWR International, Radnor, PA) at 40^{o}C overnight. The dried products were milled at 10000 rpm using a Retsch ZM200 centrifugal mill (Retsch GmbH, Haan, Germany) fitted with a 0.5 mm sieve to produce a flour.

2.4 Determination of Total and Resistant Starch

Resistant starch and soluble starch were determined using a Megazyme RS kit procedure (Megazyme, Ireland). The total starch was calculated as the sum of resistant and soluble starch.

2.5 Statistics

SPSS version 23 statistics software (IBM Corp., 2015) was used to subject total and resistant starch to analysis of variance (ANOVA) and means comparison using Tukey's test ($p< .05$)

3. Results and Discussion

Retrogradation produced the hardest gel in the pinto bean starch, followed by corn starch. The retrograded tapioca and rice gels remained semi-solid. Pinto bean starch demonstrated the highest water leakage during cooling, suggesting higher amylose-amylose association. There was no significant difference in the total starch in the flours (Table 1). However, the RS component of pinto bean starch was significantly higher (41.09%) than the amount found in the other starches (10.7 – 13.40%). The difference may be due to a complex of factors including amylose/amylopectin ratio, starch type, and granule size. Pinto bean starch contains C-type starch which is a combination of A and B-type, and is known to be more resistant to digestion than the A-type found in corn, white rice, and tapioca (Tharanathan and Mahadevamma, 2003; Zhang et al.; 2022).

Table 1. Percentage total starch (TS) and resistant starch (RS) in ungelatinized flour treatments, and the percentage RS3 in their retrograded end products.

Flour	% TS (db)	% RS (db)	% RS3 (db)
Pinto Bean Starch	88.79 ± 6.32	41.09 ± 5.30[a]	52.88 ± 3.93[a]
Corn Starch	84.35 ± 5.02	10.7 ± 0.09[b]	49.54 ± 0.28[a]
Tapioca Flour	90.16 ± 4.37	13.40 ± 1.21[b]	46.08 ± 0.73[a]
White Rice Flour	81.47 ± 0.66	10.90 ± 1.33[b]	10.33 ± 0.21[b]

Letters that are different in the same column indicate significant difference ($p< 0.05$)

The percentage of RS in the RS3 ingredient made from pinto beans (52.88%) was not significantly different from the RS content of RS3 made from corn (49.54%) and tapioca (46.08%). However, white rice produced an RS3 product with a significantly lower RS (10.33%) than the others. This could be attributed to its reportedly smaller granule size. Of the cereals, rice has the smallest granule size, ranging between 2 to 6 μm (Vandeputte and Delcour, 2004). In contrast, Hoover and Ratnayake (2002) reported a mean granule size of approximately 22 μm for pinto bean starch. Yamaguchi et al., 2019 reported corn starch granules to range between 10 and 15 μm; and Molenda et al. (2006) found that tapioca was similar to corn (13.8 μm). Smaller granules size may result in higher solvation rates, lowering gel viscosity, and hence negatively impacting amylose-amylose association during retrogradation and RS formation.

4. Conclusion

This study provides evidence that pinto bean starch can be used as a plant source to make a high-RS RS3 ingredient. It also provides an outline of a procedure that can be used as a guide in developing commercial protocols for manufacturing RS3 from pulse starches. The inclusion of pulse-based RS3 ingredients in the diet, may provide novel functional health benefits worthy of future exploration.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Informed consent

Obtained.

Ethics approval

The Publication Ethics Committee of the Canadian Center of Science and Education.

The journal's policies adhere to the Core Practices established by the Committee on Publication Ethics (COPE).

Provenance and peer review

Not commissioned; externally double-blind peer reviewed.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Data sharing statement

No additional data are available.

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