Formation of Resistant Starch by a Twin-Screw Extruder

Emine Unlu[1] and James F. Faller[1,2]

ABSTRACT

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The objective of this study was to evaluate the potential to increase the level of resistant starch (RS) in extruded products by optimizing extruder conditions. Three experiments were conducted as randomized complete block designs with two replicates. In the first experiment, corn starch, wheat starch, and potato starch were added at a level of 30% (w/w) to degerminated yellow corn meal to investigate the influence of starch type. In the second experiment, citric acid (CA) monohydrate was added to corn meal at levels of 0, 2.5, 5, and 7.5% (w/w). The third experiment was a full-factorial arrangement to evaluate the effect of high-amylose corn starch (HACS) level (0, 15, 30%, w/w) and CA level (0, 5, 7.5%, w/w) at two screw speeds (200 and 300 rpm). In the first experiment, the means for RS plus dietary fiber for the different starch formulations ranged from 1.27 to 2.28%. In experiment 2, adding CA increased RS plus dietary fiber content to a maximum of 5.23% at 7.5% CA. In the third experiment, the means for RS plus dietary fiber ranged from a low of 1.75% for 100% corn meal at 300 rpm to 14.38% for 7.5% CA and 30% HACS at 200 rpm. The results indicated a highly significant positive relationship between CA and RS formation and the same for amylose content. The RS formation had a negative relationship with screw speed, but the influence of screw speed was small when compared with that of CA and HACS.

Resistant starch is the portion of starch that is not digested in the small intestine and passes into the colon where it can be fermented by natural microflora to short-chain fatty acids. In this, it has physiological effects in the human body that are similar to that of dietary fiber (Berry 1986), which has been shown to reduce risks for some diseases, including colon cancer, coronary heart disease, and glycemia. Nutritionally, starch can be classified into three basic groups: rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) (Table I). Resistant starch is further classified into three categories: physically inaccessible starch (RS1), RS granules (RS2), and retrograded starch (RS3) (Englyst et al 1992).

Resistant starch can be found in both processed and raw food materials. Lentils are an example of a raw food material that contains 44% RS (Lintas and Cappelloni 1992). Bread and corn flakes are examples of processed foods that contain <1% and 3% RS, respectively (Englyst et al 1987).

Processing raw food materials in most cases destroys RS1 and RS2, but it can produce RS3. Most researchers suggest that RS formed during processing is associated with amylose retrogradation (Berry 1986, Berry et al 1988, Sievert and Pomeranz 1990). Some factors recorded to have an effect on amylose retrogradation (and therefore RS formation) are polymer chain length (Clark et al 1989, Eerlingen et al 1993b), presence of sugars (Eerlingen et al 1994), presence or absence of lipids (Szczodrak and Pomeranz 1992), and incubation time and temperature (Eerlingen et al 1993a). Freeze-drying and autoclaving are some of the processing methods used in the formation of RS. Even simple cooking followed by cooling and storage produces RS. Some factors associated with RS formation during these processes are physical state of the food material (whole or ground), water content, pH, heating temperature and time, feed composition, number of heating or cooling cycles, freezing methods (slow vs. rapid), and drying (Englyst and Cummings 1987).

Resistant starch has also been commercially produced and marketed (Iyengar et al 1991, Chiu et al 1994). The first step of the method developed by Iyengar et al (1991) involves dispersion of starch in an aqueous medium, either in the presence of a buffer or an organic solvent such as dimethyl sulfoxide (DMSO). Then this dispersion is incubated at 60–120°C for 5–10 hr followed by cooling and lower temperature incubation at 4–20°C for 12–96 hr. The second step involves either enzymatic or chemical hydrolysis of retrograded polysaccharide. A water-insoluble product is obtained after washing the resulting residue. This product is then dried by air-drying, freeze-drying, or by using a water-immiscible organic solvent such as acetone. The method developed by Chiu et al (1994) involves gelatinization of a starch with an amylose content of >40%, enzymatic debranching of gelatinized starch, deactivation of the debranching enzyme, and isolation of the resultant product either by drying, extrusion, or crystallization by adding salt.

Many of the conditions enhancing RS formation occur during extrusion. Starch-based extruded snack foods and breakfast cereals are likely candidates for incorporating this potentially valuable food component into the diet. Therefore, a cost-effective approach to increasing RS levels would be through process optimization. The objectives of this study were to: 1) evaluate simple methods for increasing RS content of extrusion-cooked products, specifically, adding types of starch known to have an affinity for forming RS and altering pH; and 2) optimize RS formation in extrusion-cooked product by varying pH, screw speed, and amylose content.

MATERIALS AND METHODS

Materials

Extruded products were prepared using high-amylose corn starch (HACS) (Amaizo, 172N) donated by the American Maize Company (Hammond, IN); corn starch (Buffalo corn starch 3401) obtained from the Corn Products International Company (Englewood Cliffs, NJ); potato starch (Staley unmodified potato starch) donated by the A. E. Staley Manufacturing Company (Decatur, IL); wheat starch (Gem

TABLE I
In Vitro Nutritional Classification of Starch[a]

Starch Type	Source	Digestibility in Small Intestine
Rapidly digestible starch (RDS)	Freshly cooked starchy foods	Rapid
Slowly digestible starch (SDS)	Most raw cereals	Slow but complete
Resistant starch (RS)		
Physically inaccessible starch (RS1)	Partly milled grain and seeds	Resistant
Resistant starch granules (RS2)	Raw potato and banana	Resistant
Retrograded starch (RS3)	Cooled, cooked potato, cornflakes and bread	Resistant

[a] According to Englyst et al (1992).

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[1] Department of Food Science and Human Nutrition, University of Illinois, Urbana-Champaign, Urbana, IL 61801.
[2] Corresponding author. E-mail: j-faller@uiuc.edu Phone: 217/244-2571.

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of the West wheat starch) obtained from the Manildra Milling Corporation (Minneapolis, MN); degerminated yellow corn meal (CCM 260) donated by the Lauhoff Grain Company (Danville, IL); citric acid (CA) (Fisher Scientific); a total dietary fiber kit (TDF 100, (Sigma Chemical Co., St. Louis, MO), including heat stable α-amylase, amyloglucosidase, protease and diatomaceous earth (Celite). Extrudates were analyzed for their TDF content according to the AOAC Official Method 985.29 (AOAC 1995).

Experimental Plan

This study was conducted in three experiments. The first experiment investigated the influence of starch type on RS formation. Four feed mixtures were prepared: 100% corn meal, and mixtures of 30% (w/w) potato starch, corn starch, or wheat starch with 70% corn meal. The second experiment investigated the influence of added CA (pH) on RS formation. Citric acid monohydrate was added directly to the corn meal at levels of 0, 2.5, 5 and 7.5% (w/w). Extrusion moisture content had to be decreased simultaneously with increased CA to maintain product expansion (Table II). Both the first and second experiments used a fixed screw speed of 200 rpm. Both experiments were replicated.

The third experiment was a 3×3×2 factorial experimental design in which combined effects of HACS (0, 15, and 30%, w/w), CA monohydrate (0, 5, and 7.5%, w/w), and screw speed (200 and 300 rpm) were investigated. Extrusion moisture content was again decreased simultaneously with increasing CA to maintain product expansion (Table II). A feed rate of 13.5 kg/hr was maintained throughout the experiments. Two replicates were conducted as randomized complete blocks. Statistical analysis of results was conducted using the general linear models (GLM) procedure (SAS Institute, Cary, NC).

TABLE II
Adjustment in Extrusion Moisture Content Required for Addition of Citric Acid

Feed Composition[a]	MC (%)
100% CM	25.3
2.5% CA + 97.5% CM	19.3
5% CA + 95% CM	17.0
7.5% CA + 92.5% CM	15.6
15% HACS + 85% CM	23.9
15% HACS + 5% CA + 80% CM	15.8
15% HACS + 7.5% CA + 77.5% CM	12.0
30% HACS + 70% CM	26.3
30% HACS + 5% CA + 65% CM	19.0
30% HACS + 7.5% CA + 62.5% CM	18.6

[a] CM = corn meal; CA = citric acid; HACS = high-amylose corn starch.

TABLE III
Extruder Barrel Screw Configuration[a]

Zone	Flight Angle	Type	Screw Elements	Element Length (mm)
I	20°	FTLS undercut	1	10
(Feed)	42°	FTLS undercut	5	21
II	42°	FTLS	3	42
III	28°	FTLS	14	28
IV	20°	FTLS	11	20
	45°	FP	2	28
V	20°	FTLS	5	20
	45°	RP	2	14
	20°	FTLS	5	20
	20°	FTLS	1	10

[a] FTLS = Forwarding twin lead screw; FP = forwarding paddle; RP = reversing paddle. Total screw length is 120 cm. Ratio of barrel length to diameter (L/D) is 40.

TABLE IV
Resistant Starch (RS) plus Dietary Fiber (DF) Levels in Extrudates

Starch Treatment	RS + DF (%)
Control (100% corn meal)	2.13ab[a]
30% Corn starch + 70% corn meal	2.28a
30% Wheat starch + 70% corn meal	1.36ab
30% Potato starch + 70% corn meal	1.27b

[a] Values followed by the same letter are not significantly different ($P < 0.05$).

TABLE V
Analysis of Variance Results for Starch Treatments on Resistant Starch plus Dietary Fiber

Source	Degrees of Freedom	Sum of Squares	*F* Value	Pr > *F*
Model	4	6.67	4.67	0.0190
Starch	3	3.28	3.06	0.0735
Replicate	1	3.39	9.51	0.0104
Error	11	3.92		

TABLE VI
Resistant Starch (RS) plus Dietary Fiber (DF) Levels in Extrudates with Citric Acid (CA)

Starch Treatment	RS + DF (%)
Control (100% corn meal)	2.13c[a]
2.5% CA + 97.5% corn meal	3.06bc
5% CA + 95% corn meal	3.89ab
7.5% CA + 92.5% corn meal	5.23a

[a] Values followed by the same letter are not significantly different ($P < 0.05$).

TABLE VII
Extruder Conditions for Samples Treated with Citric Acid (CA)

Feed Composition	Motor Torque (%)	Die Pressure (kPa)	Moisture (%)
Control (100% corn meal)	57a[a]	2,380a	25.3
2.5% CA + 97.5% corn meal	55ab	1,380b	19.3
5% CA + 95% corn meal	50bc	1,270b	17.0
7.5% CA + 92.5% corn meal	48c	1,200b	15.6

[a] Values followed by the same letter are not significantly different ($P < 0.05$).

TABLE VIII
Analysis of Variance Results for High Amylose Corn Starch (HACS), Citric Acid (CA), and Screw Speed Treatments (RPM) on Resistant Starch plus Dietary Fiber

Source	Degrees of Freedom	Sum of Squares	*F* Value	Pr > *F*
Model	18	637	38.0	0.0001
Replicate	1	0.13	0.14	0.7125
Treatment	17	636	40.2	0.0001
HACS	2	178	95.7	0.0001
CA	2	277	149	0.0001
HACS + CA	4	22.3	5.97	0.0011
RPM	1	28.4	30.4	0.0001
HACS + RPM	2	18.1	9.72	0.0005
CA + RPM	2	9.68	5.20	0.0113
HACS + CA + RPM	4	10.3	2.78	0.0443
Error	31	28.8	· · ·	· · ·

Extruder Operations

Experiments were conducted on a twin-screw corotating and intermeshing extruder (model ZSK-30, Werner and Pfleiderer) equipped with a volumetric feeder (K-Tron F-1). A dual-orifice die (4-mm i.d. for each orifice) was used in all experiments. Distilled water was pumped into the first extruder zone to maintain sufficient water for the extrusion process. Die pressure and temperature were determined by a pressure sensor (Dynisco, model PT 411-3M 9, MA) and a J-type thermocouple mounted at the die exit. The temperature setting profile in the barrel was kept constant in all experiments at 40, 90, 120, 120, and 140°C starting from the feed port to the die exit, respectively. The screw configuration in the extruder barrel (starting from the feed entrance) is shown in Table III.

RS Plus Dietary Fiber Determination

A TDF assay (AOAC 1995) was used to determine the dietary fiber, including RS, in corn meal extrudates. This method involves the enzymatic removal of protein and available starch with the subsequent determination of protein and ash for correction purposes (Fig. 1). Differences in TDF due to processing treatments are assumed to be associated with RS formation as processing is not expected to change fiber content.

RESULTS

Effect of Starch Type and CA Addition

Corn starch addition to the corn meal produced samples that were significantly different than samples with potato starch added. Based on Fisher's least significant difference (LSD) using analysis of variance (ANOVA), the values were 2.28 and 1.27%, respectively (Table IV). However, this analysis also showed no significant difference between the control and the starch-added treatments and showed an insignificant starch effect (Table V). The levels achieved by adding types of starch with an affinity for forming RS are low and do not appear to have commercial significance.

Citric acid addition increased RS formation significantly (Table VI), ranging from a minimum of 2.13% at 0% CA to 5.23% at 7.5% CA. However, not all of the CA levels were separated statistically by Fisher's LSD, probably due to the small intervals between percentages of CA treatment. Extruder conditions for CA-treated samples are given in Table VII.

TABLE IX
Die Pressure (kPa) Response Values in Extrudates Treated with High Amylose Corn Starch (HACS) and Citric Acid (CA) at Different Screw Speeds

HACS (%)	0% CA	5% CA	7.5% CA
200 rpm			
0	2,460a[a]	1,290cd	1,070d
15	2,410a	1,260cd	1,120d
300 rpm			
0	1,880b	1,260cd	1,120d
15	1,760b	1,290cd	1,240cd
30	1,410c	1,120d	1,100d

[a] Values followed by the same letter are not significantly different ($P < 0.05$).

TABLE X
Motor Torque Response Values (% Available) in Extrudates Treated with High Amylose Corn Starch (HACS) and Citric Acid (CA) at Different Screw Speeds

HACS (%)	0% CA	5% CA	7.5% CA
200 rpm			
0	61.0a[a]	51.3d–g	53.3c–f
15	53.3c–f	56.0b–d	58.5ab
30	54.5b–e	44.8h	49.5f–h
300 rpm			
0	56.3a–c	47.8gh	49.5f–h
15	54.3b–f	51.0e–g	47.5gh
30	52.8c–f	50.8e–g	50.5e–g

[a] Values followed by the same letter are not significantly different ($P < 0.05$).

Effects of HACS, CA, and Screw Speed

In this experiment, both the HACS and the CA levels showed significant influence on the RS levels (Table VIII). The means for RS plus dietary fiber ranged from a low of 1.75% for 100% corn meal at 300 rpm to 14.4% for 7.5% CA and 30% HACS at 200 rpm (Figs. 2 and 3). Control samples at both screw speeds differed significantly from the other samples. Overall, increased RS formation was observed with an increase in CA and HACS but with a decrease in screw speed. Statistical analyses of die pressure and motor torque responses for the extrudates containing CA plus HACS are given in Tables IX and X.

DISCUSSION

As shown in Table IV, the percent of RS plus dietary fiber results for samples with added corn starch and potato starch were the only ones significantly different from each other. The higher TDF plus RS content in the sample treated with corn starch may also be related to amylose content. This significant difference is reasonable in that potato starch is easily solubilized during boiling, while cereal starches like corn starch still contain considerable amounts of undispersed granules, even after boiling at 100°C for several hours (Banks and Greenwood 1975, Bornet 1993). Therefore, if gelatinization of cereal starches is incomplete, the rate of digestion will also be incomplete. Table IV also shows samples treated with corn starch (Buffalo corn starch 3401) resulted in the highest RS with dietary fiber. The reason for this may have been the enhancing effect of corn starch addition to the corn meal. The amylose determination for corn meal resulted in an amylose content of 25.3%, while the amylose content of corn

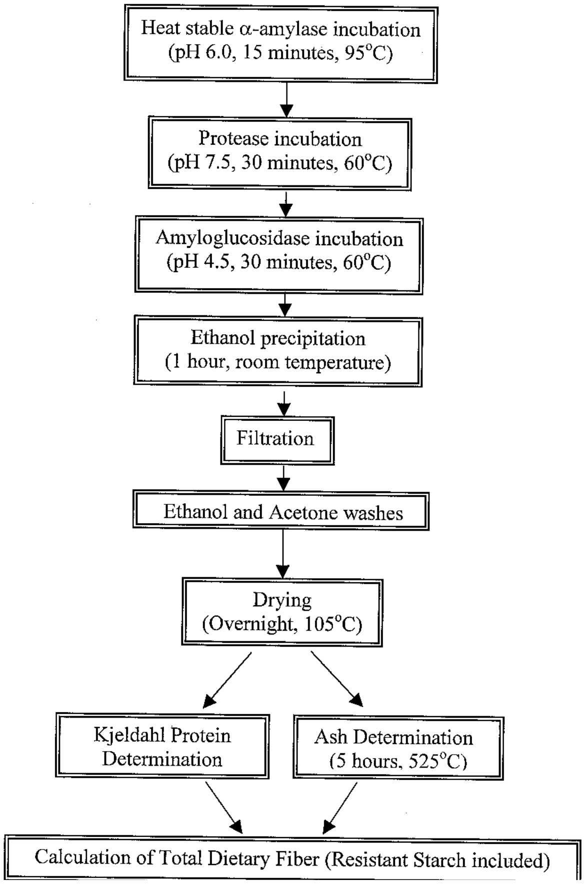

Fig. 1. Analytical procedure for total dietary fiber analysis.

starch, wheat starch, and potato starch was 33.6, 29.7 and 31.2%, respectively. The presence of higher amylose content in the sample treated with corn starch naturally resulted in formation of more retrograded amylose, which is a highly accepted mechanism for RS formation (Berry 1986; Berry et al 1988; Russel et al 1989; Sievert and Pomeranz 1989, 1990; Czuchajowska et al 1991; Sievert et al 1991).

The increase in TDF plus RS with increase in CA level might be the result of acid hydrolysis in which the starch polymers (amylose and amylopectin) are reduced in size. In granular starches, linear parts containing α-D-(1,4) linkages are involved in the formation of crystalline regions where acid attack is difficult. On the other hand, the amorphous regions mostly made up of α-D-(1,6) linkages are more susceptible to acid hydrolysis (Wurzburg 1995). In corn starch, especially, acid hydrolyzes amylopectin more extensively than amylose (Whistler and Daniel 1990). Therefore, CA may have caused size reduction in amylose and amylopectin molecules. As a result of this size reduction, smaller amylose chains might have been produced which may have enabled the formation of amylose-amylose self associations (as opposed to suggestion by Eerlingen et al [1993b]). If this were the case, the samples containing greater amounts of CA would then have a greater opportunity to form these self associations than would the samples containing lower amounts of CA. Acid hydrolysis during extrusion of CA-treated samples is indicated by a decrease in torque and pressure values in spite of a decrease in moisture content (Tables VII, IX, and X) resulting from lower viscosity associated with lower molecular weights.

The increase in TDF and RS with CA addition may also have been caused by polydextrose formation. Polydextrose is an amorphous white powder that is water soluble and mainly tasteless (Anonymous 1989). This glucose polymer is prepared by melt polymerization of a mixture of 89% glucose, 10% sorbitol and 1% CA catalyst (Rennhard 1973, Torres 1986). In addition, a mixture of glucose and CA in the absence of sorbitol produced polydextrose as well at 160°C under vacuum (Rennhard 1973). The glucosidic linkages in polydextrose are random with sorbitol end groups and CA attached to the polymer by mono- and diester bonds, which makes these bonds more resistant to enzyme or acid hydrolysis than the bonds found in starch (Rennhard 1973, Figdor and Rennhard 1981). Also, polydextrose has a minimum number of enzyme-susceptible α-1,4 and α-1,6 linkages. Therefore, polydextrose is poorly digested in the mammalian small intestine by carbohydrases (Anonymous 1989). According to the study by Figdor and Rennhard (1981) in rats, ≈60% of orally ingested polydextrose is excreted in feces and ≈30% of it is fermented by the natural microflora in the lower bowel, giving rise to volatile fatty acid and carbon dioxide production. Poor digestibility in the small intestine and possible fermentability in the large intestine of dietary polydextrose makes it a soluble substance similar to dietary fiber (Yoshioka et al 1994).

Commercial preparation of polydextrose requires purification and decolorization to render it suitable for use in food products (Cherukuri et al 1993). Several methods have been used for purifying and bleaching. One of the methods for this purpose involves contacting a food-approved bleaching agent with 5–60% polydextrose A slurry in methanol, ethanol, or isopropanol, followed by filtration and drying of the final precipitate (Torres 1986). This alcohol precipitation method is also used in the determination of dietary fiber by TDF assay (AOAC 1995), which was the method used for the determination of RS plus dietary fiber in the current study. Therefore, the dietary fiber plus RS value would include polydextrose as well.

Formation of high levels of RS plus TDF for HACS-treated samples can be explained by higher amylose content, as discussed above. The amylose content of raw HACS was 73.8%. This high amylose percentage resulted in considerable formation of RS plus dietary fiber (Figs. 2 and 3). Therefore, HACS has been used in the production of commercial RS such as Novelose (Chiu et al 1994). In the current study, the TDF content of Novelose commercial RS was 29.6%. In addition, extrusion of 30% Novelose plus 70% corn meal and 15% Novelose plus 85% corn meal extrudates resulted in TDF (including RS) contents of 15.7 and 8.3%, respectively, under similar extruder conditions.

The decrease in RS plus dietary fiber with an increase in screw speed may have been associated with shorter residence time, resulting in less opportunity for linear amylose chains to associate at the higher screw speed. In contrast, lower screw speed results in longer residence time (Altomare and Ghossi 1986), which gives rise to more opportunity for amylose chain associations. However, higher screw speed, and thus higher shear development, in the extruder barrel has been shown to increase molecular degradation (Vergnes et al 1987). Della Valle et al (1989) stated that degradation of amylose and amylopectin took place during extrusion cooking of starch, and that this degradation was due to chain splitting which was the result of shear. We have speculated that a rise in RS plus dietary fiber with CA addition may be due to increased associations of smaller linear starch molecules produced by acid hydrolysis of amylose and amylopectin. In the case of chain length reduction due to higher screw speed, the smaller chains formed by the action of mechanical shear may not have had

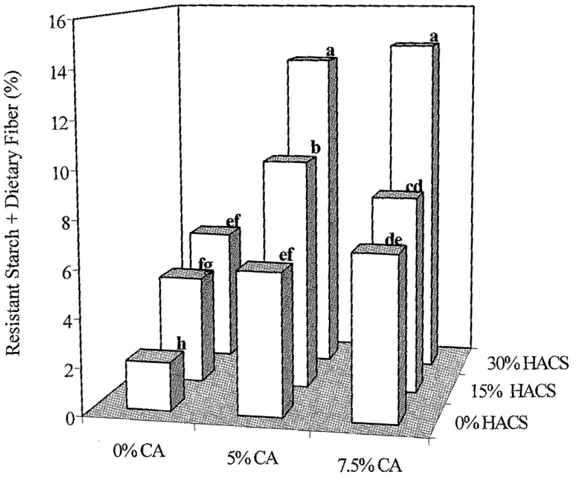

Fig. 2. Combined effects of citric acid (CA) and high-amylose corn starch (HACS) on resistant starch formation at 200 rpm. Means with the same letters are not significantly different ($P < 0.05$).

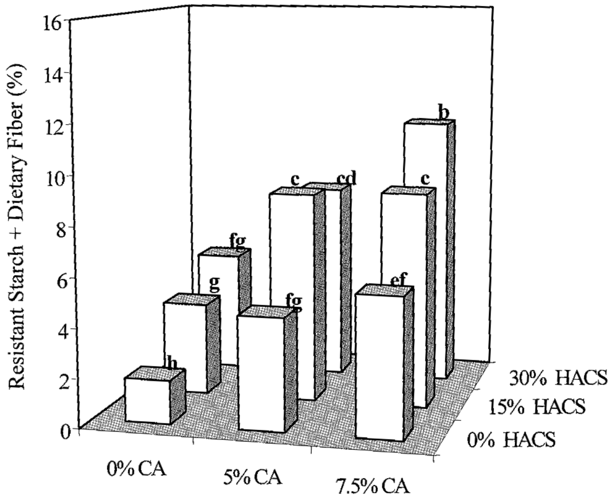

Fig. 3. Combined effects of citric acid (CA) and high-amylose corn starch (HACS) on resistant starch formation at 300 rpm. Means with the same letters are not significantly different ($P < 0.05$).

enough time to associate with each other due to reduced residence time.

CONCLUSION

Addition of HACS is one possible approach for increasing RS content in extruded snacks of acceptable quality. Reasonable levels of RS (as high as 5.62%) were obtained with 30% HACS addition, and products showed good expansion and textural properties. Citric acid addition also shows promise, but products with high CA addition were unpalatable. However, CA addition plus refining could be used to prepare RS for use in other products. Simply adding different types of starch to corn meal does not produce RS levels of practical interest for snack products.

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