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Research Trends of Resistant Starch

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Starch is the most important source of carbohydrate in the human diet, and it is known that its physiological effects depend upon the rate and extent of its breakdown by amylolytic enzymes in the intestine. For nutritional purposes, dietary starch has been classified into three main types rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS). EURESTA (European Flair Concerted Action on Resistant Starch) defined that the resistant starch is the sum of starch and products of starch degradation not absorbed in the small intestine of health individuals. Resistant starches have been assigned to four categories: physically inaccessible to digestive enzyme (RS1); B type crystalline granular starch (RS2); retrograded starch (RS3), and chemically modified starch (RS4). RS is included in the definition of dietary fiber of American Association of Cereal Chemists (AACC), but McCleary argues RS should be reported separately.

Health functional RS in the human diet

Most definitions for dietary fiber incorporate variable amounts of resistant starch as dietary fiber, because they are based on AOAC procedures that do not analyze a portion of starch during fiber analysis (AOAC 991.43, 994.13, 2001.03). But the definitions proposed by LSRD (Life Science Research Office) and COMA (Committee on Medical Aspects of Foods) specifically exclude resistant starch. Total fiber is the sum of dietary fiber and functional fiber (IOM, 2002) and includes non-starch polysaccharide, resistant starch and lignin.

RS has similar beneficial physiological effects as dietary fiber. Resistant starch has been reported to increase stool bulk, decrease fecal pH, alter the colonic microflora, decrease secondary bile acid concentrations and cytotoxicity of fecal water, decrease colonic mucosal proliferation, increase colonic fermentation, and contribute to short-chain fatty acid (SCFA) synthesis, especially butyrate. The physiological benefits of RS include maintained healthy blood sugar levels, altered lipid metabolism (fat absorption and oxidation), improved cholesterol

metabolism, enhancing mineral absorption and sustaining insulin sensitivity. Resistant starch is also a prebiotic effect on colon microflora, which referentially generates butyrate a strong biomarker for maintaining colon health. The addition of a RS to oral rehydration solution reduces fecal fluid loss and shortens the duration of diarrhea in patients with cholera.

Subjects consuming high level of RS supplementation reported higher percent of flatuence or a bloated feeling, diarrhea than that with glucose supplementation.

The energy value of RS calculated from degree of fermentation in large bowel. Each hexose molecule is fermented to release two molecules of acetate, or two molecules of propionate, or one molecule of butyrate and if 95% of net SCFA production is absorbed from the lumen, it is estimated to contribute 2-3 kcal/g of food energy.

Hydrolysis by α-amylase and molecular feature of RS

The specificity of an α-amylase depends on its source, optimal condition (temperature) and its subsite specificity. A structure for retrograded amylose was proposed in which there are crystalline, double helical regions that are 10 nm long, interspersed with amorphous regions. The amorphous regions are hydrolyzed by alpha-amylase or by acid, and do not hydrolyze the crystalline region. Human-salivary and porcine-pancreatic alpha-amylolysis of retrograded amylose gave a resistant fragment of d.p. 43, and *Bacillus subtilis* alpha amylase gave a resistant fragment of d.p. 50, because of the difference of the subsite specificity existed. The combining site of pig pancreatic alpha-amylases has a combining site of 5 contiguous α-1,4-inked glucosyl units versus 9 for the *Bacillus subtilis* enzyme. Thus the porcine α-amylase can "cut" closer to the crystalline region than the bacterial enzyme.

Some native starches are resistant to amylolytic enzymes because it is impossible to fit a double -helical arrangement of amylose chains in the amylasic cleft.

RS3 *in vitro* consisted of semi-crystalline, two main molecular sized subfractions (DPn>100 and DPn 20-30) with a third, minor subfraction (DPn≤5). HPAEC analysis also shows a periodicity in chain length for DP multiples of 6 above DP 18 for all three enzyme-resistant retrograded starches. It means that the consequence of enzyme action was revealed by the periodicity of six glucose units in the aggregated substrate

RS assay methods

The RS level can be determined by direct method like the AOAC Prosky method (enzymatic – gravimetric method) for total dietary fiber or indirect method by Englyst et al using pancreatin. Indirect methods quantify the RS as the difference between total starch and digestible starch, but direct methods consider the RS as the weight of residue after incubation of starch with α-amylase.

The AOAC Prosky procedure (AOAC, 1990) is based on a definition of dietary fiber as the sum of indigestible polysaccharides and lignin. This method does not measure inulin or resistant starch (RS2), and appears to underestimate NSP (non-starch polysaccharide). Prosky values represent an unspecified mixture of NSP and starch, and a range of substances, including Maillard reaction products. The AOAC method is unavailable to measure the RS level of RS1, RS2 and RS4, because starch is gelatinized during heating of RS1 and RS2, and heat stable α-amylase cannot attack the membrane of RS4 granule. Therefore the pancreatin-gravimetric method was suggested to assay RS level of RS4 including RS3, because of its reproducibility, simplicity and ease.

AOAC (Method 2002.02) and AACC (Method 32-40) was developed and accepted as a standard by interlaboratory evaluation (37 labs) in 2002.

RS preparation

Some physical and chemical modifications of starches have been used for improvement of RS level and properties of RS.

In case of RS2, heat moisture treatment is used for high amylose corn starch (Nevelose 240) and novel cultivation to get higher amylose content is attempted to high amylose corn (Novelose 260).

RS3 has formed by autoclaving-cooling cycle (Novelose 330, CrystaLean, C*ActiStar) and RS level is affected with amylose content of starch, heating and cooling temperature during autoclaving-cooling cycle, number of cycles, starch level, lipid in starch, and the botanical origins of the starch. Heat moisture treatment, surfactant addition after retrograded starch, and mild acid

adding to starch slurry before heating are possible to increase RS level.

RS4 is chemically modified starch that is inaccessible to α-amylase digestion, even if dissolved. Cross-linking of starch tends to inhibit the mobility of the starch chains, such that the chains cannot move into the combining site of α-amylase. RS4 shows also granular type starch with low solubility and consistency (from low to high swelling power) and is prepared by using cross-linking agent. Annealing and/or mild acid hydrolytic treatment of starch increases RS level of RS4.

RS in foods

Resistant starch is known to develop in starchy foods after heating and during the cooling period. RS can be easily incorporated in food without altering flavor, texture and appearance and used as stabilizer and/or fat replacer, and increase dietary fiber content in food.

The effects of RS on the physiological aspects in human are not known clearly, RS serves as a source of dietary fiber and has reduced calories. In addition, foods with increased levels of RS3 frequently have high levels of slowly digestible starch with a low glycemic index. Because RS3 has high melting temperature around 150℃, RS3 may be used as a heat stable prebiotic food additive in cooked or baked goods. The new recommendations from the Institute of Medicine recommend 38 g of fiber for adult males and 25 g for females. Daily dietary fiber intakes average 14.5 g in America and range from 8 to 14 g in Europe. Recommended daily intake of dietary fiber (16.3-43.4 g) is composed of total non-starch polysaccharide (11.8-16.4 g), inulin and fructooligosaccharide (2-12 g), lignin (1 g) and resistant starch (1.5-15 g).

References

American Association of Cereal Chemists. 2000. Approved Methods of the AACC, 10th ed. Method 44-15A. The Association: St. Paul, MN.

AOAC. 2000. Official Methods of Analysis of the Association of Official Analytical Chemists. International 17th ed Method 991.43 Total dietary fiber. Enzymatic-gravimetric method. The Association: Gaithersburg, MD. USA.

Asp, N-G. 1992. Resistant starch. Proceedings of the 2nd plenary meeting of EURESTA: European Flair Concerted Action No.11 on physiological implications of the consumption of resistant starch in men. Eur. J. Clin. Nutri. 46: S1

Baghurst, P.A., Baghurst, K.I., Record, S.J. 1996. Dietary fibre, non-starch polysaccharides and resistant starch A review CSIRO Division of Human Nutrition, Adelaide, SA 5000. Supplement to Food Australia 48 S1-S35.

Cho, S.S., Prosky, L., Dreher, M. 1999. Complex Carbohydrates in Foods. Marcel Dekker, Inc.

Englyst, H.N., Kingman, S.M., Cummings, J.H. 1992. Classification and measurement of nutritionally important starch fractions. Eur. J. Clin. Nutri. 46: S33-S50.

Institute of Medicine 2001. Dietary Reference Intakes, Proposed Definition of Dietary Fiber 2001.

Kim, J-H., Choi, I-S., Park, S-A., Shin, M-S., Oh, S-H. 2000. Effects of resistant starch on metabolism of bile acids in college women. Kor. J. Nutri. 33: 802-812.

Kim W.K., Chung, M.K., Kang, N.E., Kim, M.H., Park, O.J. 2003. Effect of resistant starch from corn or rice on glucose control, colonic events, and blood lipid concentrations instreptozotocin-induced diabetic rats. J. Nuti. Biochem. 14: 166-172.

McCleary, B.V. 2001. Two issues in dietary fiber measurement. Cereal Foods World 46: 936-938.

McCleary, B.V., McNally, M., Rossiter, P. 2002. Measurment of resistant starch by enzymatic digestion in starch and selected plant materials: collaborative study. J. AOAC Int. 85: 1103-1111.

Mun, S-H., Bae, C-H., Shin, M-S. 2002. RS levels and characteristics of retrograded maize starches with heat-moisture treatment. Food Sci. Biotechnol. 11: 350-354

Oh, S-H., Shin, M-S., Choi, I-S. 2002. The effect of resistant starch on physio-nutrition availability in human. Kor. J. Nutri. 35: 932-942.

Shin, M., Woo, K., Seib, P.A. 2003. Hot-water solubilities and water sorptions of reisistant starches at 25°C. Cereal Chem. 80: 564-566.

Shin, M., Seib, P.A. 2004. Morphological change and RS level of RS mixture with cross-linked RS4 starch granule during in vitro digestion. Food Sci. Biotechnol. accepted.

Shin, M., Song, J. Seib, P.A. 2004 In vitro digestibility of cross-linked RS4 starches. Starch. submitted.

Woo, K.S., Seib, P.A. Cross-linked resistant starch: Preparation and properties. Cereal Chem. 2002. 79: 819-825.