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■연구논문■

Resistant Starch

Mal-Shick Shin†

Dept. of Food and Nutrition, Home Economics Research Institute, Chonnam National Univ.

저항전분

신 말 식

전남대학교 식품영양학과, 가정과학연구소

국 문 초 록

저항전분(RS)은 건강한 사람의 소장에서 소화되지 않는 전분이나 전분질 식품의 부분이다. 저항전분은 4가지 형태로 구분하는데 RS 1은 물리적으로 효소와 만나지 않는 부분, RS 2는 생전분으로 감자, 바나나와 고아밀로오스 옥수수전분, RS 3는 노화된 전분 그리고 RS 4는 화학적으로 변성시킨 전분이다. RS 함량은 열에 안정한 α -아밀라아제나 pancreatin, pancreatic α -아밀라아제와 미생물에서 분리된 아밀라아제 등을 이용한 몇 가지 방법에 의해 분석되고 있다. RS는 대장에서 미생물에 의해 발효되어 단쇄지방산을 생성하는데 특히 부티릭산이 생성된다. 아세트산이나 프로피온산은 간의 대사에 영향을 주며 부티릭산은 항 종양(항 대장암) 특성이 있다. RS는 소화가 되지 않아 저열량원이므로 당뇨병 환자나 운동에 의한 혈당 조절이 필요할 때 조절능력을 갖는다. RS가 건강에 중요한 인자임이 알려지면, 건강을 위해 매일 섭취량의 증가를 권장해야 할 것이다.

주요어 : 저항전분, RS 3형, RS 4형, 항 대장암, 저 포도당 계수
KEY WORDS : resistant starch, RS 3 type, RS 4 type, anti-colon cancer, low glycemic index.

I. Introduction

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 in 1990 that resistant starch

†Corresponding author : Dept. of Food and Nutrition, Chonnam National Univ., 300 Yongbong-dong, Buk-ku, Kwangju 500-757, Korea
Tel 82-62-530-1336, Fax 82-62-530-1339
E-mail : msshin@chonnam.ac.kr

is the sum of starch and products of starch degradation not absorbed in the small intestine of healthy individuals(Asp, 1992). Englyst and Cummings (1992) differentiated three main forms of RS : 1) RS 1 type starch is physically inaccessible to digestive enzymes owing to enclosure in food structures such as intact cells or partly milled or whole grains or seeds : 2) RS 2 is resistant B-type starch granules occurring in(uncooked) potatoes and green banana : 3) RS 3 is retrograded amylose occurring in processed foods and manufactured sources. RS 4 is chemically modified starch with reduced susceptibility to digestion, as suggested by Eerlingen et al.(1993).

Ⅱ. Physiological effects of RS

RS is included in the category of dietary fiber because non-digestible starch like fiber may show physiological health benefits in humans. The physiological effects associated with RS include, mainly, reduced levels of plasma glucose and insulin(Carins et al., 1995), increased faecal bulk, and short-chain fatty acid(SCFA) production through fermentation in the large intestine.

Ranganathan(1994) compared resistant starch and dietary fiber on metabolic indexes in seven healthy men(23-26 year) and concluded the effect of resistant starch on energy expenditure, colonic fermentation (breath-hydrogen test) and blood glucose, insulin, and free fatty acid concentrations are similar to those of a known cellulosic fiber. Silvester et al.(1997) studied the fecal excretion of ATNC(apparent N-nitroso compounds) and ammonia from eight healthy men fed low meat diet(40g), high meat diet(600g), and high meat + RS 2 diet(37g RS added to 600g meat diet). Resistant starch significantly increased stool output and decreased fecal pH, but had no significant effect on fecal ATNC and ammonia. Hylla et al.(1998) reported that when 12 healthy volunteers(five women and seven men) ate the diets enriched with high-RS(55.2g HylonVII) and low-RS(7.7g corn starch), fecal concentrations of total neutral sterols and 4-cholesten-3-one in feces decreased by 30% and 36%, respectively, with the high RS diet. So they suggested that RS has potentially important effects on bacterial metabolism in human colon that may be relevant for cancer prevention. But Heijnen et al.(1998) investigated the effect of a daily RS supplement on putative risk factors for colon cancer in > 18 year healthy men and concluded that supplementing the habitual diet for 1wk with 32g/day of RS 2 or RS 3 compared with glucose had no effect on putative risk factors for colon cancer, except for increasing stool weight and colonic fermentative activity.

Asp et al.(1996) reported that some animal experiments indicate RS lowers serum cholesterol level : but such effects have not been confirmed in normolipidaemic man with doses of RS that can be readily incorporated in the diet and tolerated, and hyperlipidaemic subjects have not yet been studied. RS has not shown to affect mineral balance in humans and there is evidence for the production of a comparatively high proportion of butyrate from the fermentation of starch. Butyrate and other short chain fatty acids may have health promoting effects on epithelial cells through the conversion of bile acids, nitrogen metabolism, and feacal bulk.

It would appear that excess RS consumption would seem to be self-limiting, because excess amounts of carbohydrates reaching the large intestine can result in deleterious phenomena like diarrhea and gas. Hylla et al.(1998) observed that diarrhea was not reported in their study period, but other symptoms, including flatulence, abdominal distention, and abdominal cramping were found. Subjects consuming 32g/d RS2 or RS3 supplementation reported higher percentage of flatuence or a bloated feeling than that with glucose supplementation (Heijnen et al., 1998).

Ⅲ. Food energy value of RS

The energetic contribution of unabsorbed carbohydrates in the small intestine depends on the substrate and method of calculation. If 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 9.0~9.2 kJ/g of food energy. RS is thought to produce a high butyrate level in colonic fermentation, so the energy salvaged from RS is to the high side, or 9.2 kJ/g.

When comparing metabolizable energy from two starch sources, normal corn starch and high amylose corn starch, the type of starch consumed in the diet did not affect metabolizable energy. The heat of combustion for corn starch(17.4 kJ/g) was used for the baseline of carbohydrate energy value. All 24 human subjects (av. 37 yr) were able to utilize 67.3% of the resistant starch with food energy of 11.7 kJ/g(2.8 kcal/g), but 14 hyperinsulinemic subjects(av. 41 yr) averaged only 2.2 kcal/g(Behall and Howe, 1996). Ranhotra et al.(1996) determined the energy value of RS using young rats and found that RS provided no energy, although about one-third of RS was fermented.

Ⅳ. Formation of RS 3

Native starch is gelatinized when heated in excess water(> 35% MC) above gelatinization temperature. On cooling and aging, the starch forms gels and eventually a B-type crystallinity is developed in a process called retrogradation. Retrograded non-waxy starches form RS 3 during retrogradation. Thus, RS 3 type starch is produced during processing and storage of starchy foods, and can be increased by storing the wet cooked food at cold temperature, or by freezing.

The yield of RS 3 in the preparation using repeated heating-cooling cycles depends on amylose content of starch, temperature of heating and cooling, starch level, the number of heating-cooling cycles, lipid in starch, and the botanical origins of the starch {Eerlingen et al.(1993a,b, 1994a-d), Sievert and Pomeranz(1989, 1990), Czuchajowska et al.(1991), and Sievert et al.(1991)}. Commercial RS 3 starch products, Novelose and Crystalean, are produced by retrogradation of purified high amylose starches.

Ⅴ. Formation of RS 4

The chemical, physical, and enzymatic modifications of starches are designed to change the physicochemical properties of native starch. Most modified starches are changed in digestibility, depending on the type and degree of modification and starch source.

Hydroxypropylated field pea starch with molar substitutions(MS) ranging 0.04 to 0.12, when digested with α-amylase, decreased in digestibility with increases in MS up to 0.08, but increased with further increases in MS(Hoover et al., 1988). Björck et al.(1989) studied *in vivo* digestibility of chemically modified potato starch derivatives such as distarch phosphate, acetylated di-starch phosphate with acetyl degree of substitution(DS) of 0.08, and hydroxypropyl di-starch phosphate with hydroxypropyl DS of 0.19, and suggested that cross-linking with phosphate at levels used commercially only had minor effect on *in vivo* digestibility. But etherification of potato starch with hydroxypropyl groups to DS 0.19 significantly reduced the extent of digestion and absorption of the gelatinized starch to about 50%, but that was not significantly different from that of raw potato starch.

RS 4 is a chemically modified starch that is inaccessible to α-amylase digestion, even if dissolved. Cross-linking starch tends to inhibit the mobility of the starch chains, such that the chains can not move into the combining site of α-amylase(Casset et al., 1995).

Woo and Seib(1997, 1999) prepared a cross-linked

starch form of RS 4 by reacting starch in aqueous slurry at 40℃ and pH 11.0 with sodium trimetaphosphate (STMP), sodium hydroxide, and sodium sulfate, and it showed a low swelling power and consistency. They also tried to make STMP cross-linked starches with different swelling power. These products of RS 4 appear to be less costly than RS 3. In addition, the procedure is easy to carry out. So it is desirable to develop new cereal products using RS 4 starches.

Ⅵ. Assay methods for RS

EURESTA has developed numerous methods for the quantification of RS fraction from total starch. For determination of this fraction, two types of methodologies have been proposed : Ⅰ) indirect methods, such as Englyst's method(Englyst et al., 1992) which quantify the RS as the difference between total starch and digestible starch, and Ⅱ) direct methods based on the Berry's method(Berry, 1986), which considers the RS as the residue after incubation of starch with α-amylase.

The two main procedures involve determination of the various types of starch using controlled α-amylase/glucoamylase hydrolysis with measurement of the released glucose by glucose oxidase(Englyst et al., 1992), or the Prosky AOAC dietary fiber method(Prosky et al., 1988) involving α-amylase digestion at high temperature (100℃) and gravimetric determination of the residue. The major differences in two procedures that have been developed and published involve enzyme used, the temperature-time conditions of incubation, and the method of measuring the unhydrolyzed residue. The collaborative studies were conducted to compared methods of determining RS in various foods and food products (Prosky et al., 1988, Champ, 1992, Lee et al., 1992, Li, 1995). The AOAC Prosky procedure(AOAC, 1992) is based on a definition of dietary fiber as the sum of indigestible polysaccharides and lignin. Some scientists have used the AOAC method for determination of total dietary fiber in foods(Li and Andrews, 1988, Li and Cardozo, 1993, Sambucetti and Zuleta, 1996). However the method does not measure inulin or resistant starch (RS 2), and appears to underestimate non-starch polysaccharide(NSP) (Englyst et al., 1995). Englyst and Hudson(1996) explained that Prosky values represent an unspecified mixture of NSP and starch, and a range of substances, including Maillard reaction products.

RS content determined *in vitro* depends on the method used, i.e., lower RS content is obtained when more severe conditions are used, such as higher incubation temperature, longer incubation time, higher amount of enzyme, and higher agitation. Würsch and Koellreutter(1992) used two kinds of α-amylase for determining RS in food, both bacterial α-amylase (Termamyl) and pancreatic α-amylase gave higher levels of RS at lower temperature(42℃ > 85℃).

Recently, Saura-Calixto et al.(1993) and Morales et al.(1997) reported the new modified methods for measuring RS in dietary fiber residues, both of which are simple procedures and show a high precision and a good accuracy. Mangala et al.(1999) reported purified RS were analyzed by GPC and SE-HPLC methods and found undigested material recovered from the ileum of rat intestine fed with processed *ragi* flour exhibited a close similarity in some of its properties to that of RS isolated by an in vitro method.

Ⅶ. Limiting factors of hydrolysis by α-amylase

To understand the structural features to release of oligosaccharides from solid starchy substrate in enzymatic hydrolysis, four successive phases have to be considered : the diffusion of the enzyme molecules towards its substrate, the porosity of starchy substrates, the adsorption of enzymes on the substrate, and finally the catalytic event(Colonna et al., 1992). The diffusion is considered as a limiting step of hydrolysis with regard to the macromolecular nature of amylase and porosity of

substrate or associated products in foods. The particle size of substrate is a critical parameter that must be carefully controlled. Upon adsorption, specific forces between the enzyme and binding sites of the substrate are formed leading to an enzyme-substrate complex. The adsorption of α-amylase of *Bacillus* species on highly crystalline amylose spherulites of the B-type is specific and reversible, and occurs following a monolayer mechanism. The hairy structure of spherulites is necessary for α-amylase adsorption to occur. Some different results of degree of hydrolysis of granular starch by α-amylase could be due to the presence of a great number of crystallites at the surface of granules.

The specificity of an α-amylase depends on its source, optimal condition(temperature) and its subsite specificity(Würsch and Koellreutter 1992, Faisant et al., 1993). Jane and Robyt(1984) studied the structure of amylose-V-complexes and retrograded amylose by three kinds of α-amylase from human-salivary, porcine-pancreatic, and *Bacillus subtilis*. 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, but not 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 differences in the subsite specificities. 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.

Molecular modelling studies of interaction between the catalytic site of porcine pancreatic α-amylase (PPA) and amylose fragment have been performed by Casset et al.(1995). The stereochemical refinement confirmed the presence of a maltopentose moiety within the catalytic site, in the absence of water. It was confirmed that the three acidic amino acids of the catalytic site(Asp 197, Asp 300 and Glu 233) are close to their glucosidic target, and there is no steric reason to propose an alteration of the $_4C_1$ conformation of the glucose residue prior to hydrolysis. A minimum of five glucose units are required to completely fill the binding site, where as many as three to four adjacent units can be either tightly bound to the surrounding surface or exit towards the solvent. They explained that 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.

Ⅷ. Molecular features of RS

Faisant et al.(1993) found that ileal RS appeared to consist of three fractions, high molecular weight α-glucan(DPn > 100, amorphous and potentially digestible material), B-type retrograded amylose crystallites (DPn ~35), and oligosaccharides. However *in vitro* RS fractions showed no high molecular weight molecules, due to extensive hydrolysis in the *in vitro* procedure (Champ, 1992, Prosky et al., 1988, Englyst et al., 1992). RS 3, which has a melting temperature near 150°C (Eerlingen and Delcour, 1995) is composed of essentially linear(1-4)-D-glucan chains, DPn ranging from c. 30 to 65, depending on source and preparation conditions, and short linear segments(DPn 30) of α(1-4)-glucans arranged in an A- or B-type crystalline structure (Russell et al., 1989, Leloup et al., 1992). The chain of DPn 30-65 are in reasonable agreement with chain lengths from enzyme-resistant retrograded amylose (Eerlingen et al., 1993, Jane and Robyt, 1984).

Cairns et al.(1995) studied hydrolysis of retrograded amylose gels(RS 3) with porcine pancreatic α-amylase to construct an *in vitro* model of resistant starch. Hydrolysis of these gels produced significant increase in crystallinity during storage, and the amount of

resistant material remaining after hydrolysis increased with increasing gel concentration. Amorphous portions of amylose gels were found to become crystalline during enzymic hydrolysis, and thereby increased level of RS 3. The resistant portion of amylose gels was modelled *in vitro* with a view to explaining the structure of RS *in vivo*. The molecular structure of RS was characterized by X-ray diffraction, size exclusion chromatography and methylation analysis(Carins et al., 1996). RS *in vitro* consisted of semi-crystalline, two main molecular sized subfraction(DPn > 100 and DPn 20~30) with a third, minor subfraction(DPn ≤ 5). Analysis of RS 3 *in vivo*, recovered during an ileostomy study, produced results that were similar to those obtained from RS 3 *in vitro*.

The mobility and conformation of amylose in a gel form and its α-amylase resistant residue were measured by NMR(Colquhoun et al., 1995). Amylose gel contained a double helical, rigid fraction and a more mobile amorphous fraction, but treatment of the gel with α -amylase resulted in the hydrolysis of part of the amorphous fraction, with further ordering of amylose chains. Gildly et al.(1995) suggested that RS 3, such as enzyme-resistant retrograded wheat, amylomaize V and amylomaize Ⅶ starches prepared using porcine pancreatic α-amylase and pullulanase, reflect features both of aggregated/gelled amylose which has high double helix content(60-70%) by ^{13}C CP/MAS NMR spectroscopy, low crystallinity(20-30%) by X-ray diffraction, and DP range from junction zones of DP 10~100 by high performance anion exchange chromatography(HPAEC). 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.

Ⅸ. RS in foods

A high fiber, low fat, and low-caloric food is an important objective in today's food product development. While 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 RS 3 frequently have high levels of slowly digestible starch with a low glycemic index. The amount of RS consumed in the human diet(at time of consumption) is not exactly known, and is thought to be variable. Current European diets in different countries provide an average of about 4 g/d ranging from 3.2 to 5.7 g of RS per day. There is no evidence that this amount is detrimental to health(Asp et al., 1996, Englyst et al., 1996).

Daily dietary fiber intake is average 14.5 g in America and range from 8 to 14 g in Europe. Increased consumption of RS may afford a practical and efficacious way of promoting a healthy environment in the colon leading to less incidence of disease(Gordon et al., 1996). The amount of RS type in a food can be altered by processing, or by adding independently prepared ingredients. RS 3 can impart uniform and small particle size, low bulk density, low water holding capacity, and a light-textured crispness. It can function as a source of fiber without altering flavor and texture, partly due to its partial solubility and non-hydrating properties(Alexander, 1995).

In the daily diet, the main sources of starch are cereals in which only limited amount of RS are produced by usual processing(2~6% RS based on starch) (Würsch, 1995), new products with higher RS content than common cereal products are being investigated. RS 3 from amylomaize VII starch was used to replace up to 50% of the total shortening and up to 15% of the flour in a yellow layer cake. No significant effects were observed when RS replaced 15% replaced of the flour. When 12.5% of the shortening was replaced with RS 3, the quality of cake improved. Replacement

of 25% of the shortening produced a cake with physical characteristics of batter and crumb similar to that of the control(Lin et al., 1994). The RS(RS 2 and 3 type) content of a straight-dough bread was increased by replacing 24% of the wheat flour with 4% vital wheat gluten and 20% high-amylose corn starch or 20% extruded retrograded high amylose corn starch. Increasing the amount of RS in bread to about 10% did not impair bread quality when judged by softness, acceptable volume and sensory analysis(Eerlingen et al., 1996).

Even if every meal has some underestimation of the RS content, RS from daily intake is small amount which we think. Some evidences were proved that RS is beneficial to the consumption of the different amounts and forms of RS, RS containg foods are considered to include every meal. If some desirable properties of RS are verified, cereal products with added RS will be considered to be fiber-fortified, calorie-reduced, or slowly-digestible foods.

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