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Review Article

In vitro and in vivo hydrolysis of legume starches by α-amylase and resistant starch formation in legumes—a review

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Abstract

Starch represents the major source of available carbohydrate in the human diet. Starch is the most abundant (22–45%) carbohydrate in the legume seed. The rate of starch digestion in legumes is lower both in vitro and in vivo, than that of cereals. In vivo, starch is hydrolyzed by salivary and pancreatic α-amylase. However, a proportion of starch in starchy foods generally escapes complete digestion. This fraction is called 'resistant starch'. Resistance starch has properties similar to fermentable fibers. This review summarizes the current knowledge on: (1) the extent of in vitro hydrolysis of native legume starches by α-amylases of different origin; (2) the structural and morphological changes that occur in legume starches as a result of α-amylolysis; (3) the reactivity of amylases towards gelatinized, retrograded and modified legume starches; (4) effect of food processing on in vitro hydrolysis; (5) in vivo digestibility; and (6) resistant starch formation in legumes.

Keywords: Legume starches; Digestibility; Resistant starch; α-Amylolysis

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1. Introduction

Legumes are dicotyledonous seeds of plants that belong to the family Leguminosae (16,000–19,000 species ~750 genera; Allen & Allen, 1981). The grain legumes, collectively, are ranked fifth in terms of annual world grain production (171 million metric tons). Total carbohydrates of food legumes vary from 24 to 68%. Starch is the most abundant carbohydrate in the seed (22–45%; Hoover & Sosulski, 1991). Native legume starches have been found to be more digestible than native potato or high amylose maize starch, but less digestible than native cereal or cassava starch (Dreher, Berry, & Dreher, 1984; Frins, Fornal, Ring, & Vidal-Valverde, 1998; Hoover & Sosulski, 1985; Ring, Gee, Whittam, Orford, & Johnson, 1988; Socorro, Levy-Benshimol, & Tovar, 1989; Tovar, de Francisco, Björck, & Asp, 1991). Hoover and Sosulski (1985) have shown that during a 6 h digestion with porcine pancreatic α-amylase, maize starch was hydrolyzed to the extent of 75%. Whereas, at the same enzyme concentration, the corresponding value for legume starches belonging to the biotype *Phaseolus vulgaris* ranged from 25 to 35%.

Differences in the in vitro digestibility of native starches, among and within species have been attributed to the interplay of many factors, such as starch source (Ring et al., 1988), granule size (Snow & O'Dea, 1981), extent of molecular association between starch components (Dreher et al., 1984), amylose/amylopectin ratio (Hoover & Sosulski, 1985), degree of crystallinity (Hoover & Sosulski, 1985), type of crystalline polymorphic (A, B or C) form (Jane, Wong, & McPherson, 1997), distribution of B type crystallites in the granule (Gerard, Colonna, Buleon, & Planchot, 2001), amylose–lipid complexes (Guraya, Kadan, & Champagne, 1997; Holm et al., 1983; Hoover & Manuel, 1995; Nebensy, Rosicka, & Tkaczyk, 2002; Seneviratne & Biliaderis, 1991; Tufvesson, Skarabanja, Björck, Elmstahl, & Eliasson, 2001), physical distribution of starch in relation to dietary fiber components (Dreher et al., 1984; Rao, 1969; Snow & O'Dea, 1981), antinutrients (Thompson & Gabon, 1987), α-amylase inhibitors (Lajolo, Finardi-Filho, & Menezes, 1991; Puls & Keup, 1973), physical insulation of starch by thick walled cells (Wursch, Dal Vedovo, & Koellreuter, 1986), porosity (Colonna, Buleon, & Lemane, 1988) and the influence of drying and storage conditions (Kayisu & Hood, 1979). Gallant, Bouchet, and Baldwin (1997) have shown that the crystalline and amorphous lamellae of amylopectin are organized into larger, more or

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less spherical structures called 'blocklets'. This model of starch structure assumes that the crystalline lamellae are made up of double helical amylopectin side chain clusters. The clusters are inter-leaved with amorphous lamellae of the amylopectin branching regions. The susceptibility towards α-amylase has been shown to decrease with increase in blocklet size (Gallant et al., 1997).

The reduced bioavailability of legume starches has been attributed to the presence of intact tissue/cell structures enclosing starch granules, high levels of amylose (30–65%), high content of viscous soluble dietary fiber components, presence of a large number of antinutrients, 'B' type crystallites and strong interactions between amylose chains (Deshpande & Cheryan, 1984; Hoover & Sosulski, 1985; Siddhuraju & Becker, 2001; Tovar et al., 1991; Wursch et al., 1986).

α-Amylase (1,4 α-D glucan glucanohydrolase, EC 3.2.1.1) catalyzes the hydrolysis (endo attack) of the α (1 → 4) glycosidic bond in amylose, amylopectin and related oligosaccharides. α-Amylase hydrolyzes starch by a multiple attack mechanism (Robyt & French, 1967, 1970). The direction of multiple attack is from the reducing end towards the nonreducing end: that is, after the first cleavage, the fragment with the nonreducing end dissociates from the active site while the fragment with the newly formed hemiacetal reducing end remains associated with the active site and repositions itself to give another cleavage and the formation of maltose and maltotriose (Robyt, 1984; Robyt & French, 1967).

The active site of porcine pancreatic α-amylase has been shown to contain five subsites (Robyt & French, 1970; Seigner, Prodanov, & Marchis-Mouren, 1987) with catalytic groups (glutamic acid as acid/base catalyst and an aspartate as the nucleophile) between the second and third subsite from the reducing end subsite (Robyt & French, 1970). A discussion of the catalytic mechanism of α-amylase action (which proceeds by a double displacement (Tao, Reilly, & Robyt, 1989) covalent S_N2 mechanism) is outside the scope of this review, but is discussed in detail in a review by Kuriki and Imanaka (1999).

Starch hydrolysis by α-amylase involves, an enzyme in solution acting on a solid substrate. Thus, the surface area accessible to the enzyme and the efficiency of the adsorption of the enzyme onto this surface are critical kinetic parameters (Bertoft & Manelius, 1992). Robyt (1984) has shown that the products of hydrolysis of porcine pancreatic α-amylase are mainly maltose, maltotriose and maltotetraose. However, α-amylase from *Bacillus amyloliquefaciens* produces primarily maltotriose, maltohexose and maltoheptose (Yook & Robyt, 2002).Planchot, Colonna, Gallant, and Bouchet (1994) have shown that α-amylase from *Aspergillus fumigatus* produces only glucose as the sole end degradation product. All products resulting from the action of α-amylase on starch have been shown to possess the α-anomeric configuration of the substrate (Kuriki & Imanaka, 1999).

Several researchers have shown that α-amylases can simultaneously solubilize both amorphous and crystalline regions of starch granules (Colonna et al., 1988; Lauro, Forssell, Suortti, Hulleman, & Poutanen, 1999; Leach & Schoch, 1961). This was based on the observation that α-amylolysis did not produce an increase in crystallinity. However, crystallinity and gelatinization enthalpy have been shown to decrease during the later stages of α-amylolysis (Lauro et al., 1999). This suggests that extensive hydrolysis effectively destroys and solubilizes the crystalline areas of the granule. The ability of α-amylase to hydrolyze the crystalline region has been explained (Colonna et al., 1988) as follows: each of the subsites of α-amylase is able to bind one glucose unit of the starch chain. When the substrate binds to the active site, it is distorted by the enzyme in the direction of the transition rate for catalysis by a strain at the glycosidic bond to be cleaved. This enables α-amylase to grasp onto portions of molecule, involved in the crystalline region, leading to an active disentanglement of the crystalline chains. Colonna et al. (1988) have shown by iodine binding studies, that there is no preferential hydrolysis of either amylose or amylopectin by α-amylase.

Legume starches make better substrates than cereal or tuber starches for gaining a deeper insight into the structural factors that influence α-amylolysis due to the following reasons: (1) absence of pores on the granule surface (Hoover & Sosulski, 1985), (2) presence of only trace quantities of bound lipids (Hoover & Sosulski, 1991), (3) wide variations in the amount of 'B' type crystallites (Cairns, Bogracheva, Ring, Hedley, & Morris, 1997, Davydova, Leontev, Genin, Sasov, & Bogracheva, 1995; Gernat, Radosta, Damaschun, & Schierbaum, 1990; Ratnayake, Hoover, Shahidi, Perera, & Jane, 2001), (4) uniformity in granule size (Hoover & Sosulski, 1991) and (5) variations in starch chain interactions within the amorphous and crystalline domains (Hoover & Sosulski, 1985). Thus, a comparative study of the susceptibility of legume starches (belonging to the same and to different biotypes) towards α-amylase may lead to the identification of the structural factors that limit α-amylolysis. This in turn may help us to understand as to why legume starches exhibit a lower glycemic index than cereal or tuber starches (Annison & Topping, 1994; Foster-Powell & Miller, 1995; Jenkins et al., 1982; Jenkins et al., 1987; Jenkins, Wolever, Taylor, Barker, & Fielder, 1980; Tovar, Björck, & Asp, 1992a; Tovar, Granfeldt, & Björck, 1992b; Truswell, 1992; Urooj & Puttaraj, 1994).

This review summarizes the current knowledge on the susceptibility of legume starches towards α-amylolysis, and resistant starch formation during legume processing, with a view to providing suggestions for needed research to obtain a deeper understanding of the influence of starch granule structural organization on α-amylolysis.

2. In vitro amylolysis of legume starches

The in vitro amylolysis of legume starches by α-amylases from different origins are presented in Table 1. It is difficult to rank the legume starches with regard to their susceptibility towards α-amylase, due to differences in enzyme concentration, time of hydrolysis, and source of α-amylase. Furthermore, in most cases, the data represented in Table 1 have been on a single cultivar. Therefore, it is difficult to ascertain whether the reported extent of starch hydrolysis is truly representative of the particular species.

3. Analysis of starch residue left after in vitro α-amylolysis

In legume starches the residue left after α-amylase hydrolysis has been examined by scanning electron microscopy (SEM) and differential scanning calorimetry (DSC).

Table 1
In vitro digestibility of native legume starches

Starch source	Source of α-amylase	Reaction temp (h)	Degree of hydrolysis (%)
Smooth pea (*Pisum sativum* L.)	Pancreatic porcine	24	18.2–22.2[a]
	Pancreatic porcine	29	91[b]
	Bacillus sp.	29	78[b]
	Aspergillus fumigatus	29	100[b]
Wrinkled pea (*Pisum sativum* L.)	Pancreatic porcine	29	72[b]
	Bacillus species	29	66[b]
	Aspergillus fumigatus	29	77[b]
Grass pea (*Pisum sativum* L.)	Pancreatic porcine	24	22[c]
Green pea (*Pisum sativum* L.)	Pancreatic porcine	24	16[c]
Beach pea (*Lathyrus maritimus* L.)	Pancreatic porcine	24	35[c]
Lentil (*Lens culinares* L.)	Pancreatic porcine	24	14.5–35.5[d]
Mung bean (*Phaseolus aureus*)	Pancreatic porcine	24	71.1[d]
Lima bean (*Phaseolus lunatus*)	*Bacillus subtilis*	6	25[e]
Lablab bean (*Lablab purpureus*)	Pancreatic porcine	2	30[f]
Pinto bean (*Phaseolus vulgarisi*)	Pancreatic porcine	1	62[g]
		6	25.2[hi]
Navy bean (*Phaseolus vulgaris*)	Pancreatic porcine	6	32[i]
Northern bean (*Phaseolus vulgaris*)	Pancreatic porcine	6	29[i]
Black bean (*Phaseolus vulgaris*)	Pancreatic porcine	6	34.8[i]
Kidney bean (*Phaseolus vulgaris*)	Pancreatic porcine	3	49.5[j]
Moth bean (*Phaseolus acontifolius*)	Pancreatic porcine	6	31.4[i]
	Pancreatic porcine	Not given	25.4–28.2[j]
	Human salivary	1.6	30.2[k]
Tepary bean (*Phaseolus acutifolius*)	Pancreatic porcine	2	8[l]
Yam bean (*Sphenostylis stenocarpa*)	Saliva	1	18[m]
Chick pea (*Cicer arientum*)	Pancreatic porcine	24	65[n]
	Human salivary	2	15[n]
Horse gram (*Dolichos biflorus*)	Saliva	2	10.2[n]
Cow pea (*Vigna sinensis*)	Saliva	2	10.8[n]

[a] Ratnayake et al. (2001).
[b] Planchot et al. (1994).
[c] Chavan, Shahidi, Hoover, and Perera (1999).
[d] Hoover and Manuel (1995).
[e] Hoover et al. (1997).
[f] Hoover et al. (1991).
[g] Rosenthal and Nakamura (1972).
[h] Grelda, Farias, Moreona-Valencia, del Refugio Falcon-Villa, and Barron-Hoyes (1997).
[i] Hoover and Sosulski (1985).
[j] Socorro et al. (1989).
[k] Wankhede and Ramteke (1982).
[l] Abbas, Scheerens, and Berry (1987).
[m] Agunbiade (1998).
[n] El-Faki et al. (1983).

3.1. SEM

SEM of smooth pea starches has shown that small granules are more resistant than larger granules to hydrolysis by α-amylase from *Bacillus amyloliquefaciens*. This was attributed to the higher apparent amylose content in smaller granules (Bertoft, Manelius, & Qin, 1993a). SEM showed no evidence of enzyme hydrolysis on many of the smaller pea starch (75% amylose) granules (solubilized up to 3%), whereas some small granules and large granules were extensively degraded with surface erosion being restricted to a few granules (Bertoft et al., 1993a). Hoover and Yang (unpublished results) have shown that the action of porcine pancreatic α-amylase on smooth pea starch (at 60% solubilization) results in extensive surface degradation on all granules. Many of the degraded granules were deformed and some of the granules had enlarged pores and cracks on their surface (Fig. 1(b)).

SEM of wrinkled pea starch hydrolyzed by α-amylase from *Bacillus amyloliquefaciens* (Bertoft, Manelius, & Qin, 1993b) have revealed that several of the larger granules (17% solubilized) were fragmented by α-amylase, but some were intact. Small granules and granule fragments that had been solubilized (20%) showed no evidence of enzyme attack. Hoover and Yang (unpublished results) have shown that during isolation of wrinkled pea starch (75% amylose) some granules suffer extensive damage resulting in splitting and exposure of the internal layering (Fig. 2(a)–(c)). Jenkins and Donald (1995) investigated the effect that varying amylose content has on the structure of pea, barley and maize starches. They indicated that amylose disrupts amylopectin crystallites. This could then explain granule disruption during extraction, and hence, the high susceptibility of wrinkled pea starch towards α-amylase (Table 1). The pattern of hydrolysis of porcine pancreatic α-amylase on wrinkled pea starch (Fig. 2(d) and (e)) is similar to that reported for *Bacillus amyloliquefaciens* (Bertoft et al., 1993b).

Hoover and Manuel (1995) showed that the action of porcine pancreatic α-amylase on laird lentil starch (72 h, 22.3% solubilized) resulted in highly roughened surfaces which were covered with numerous fissures (Fig. 3(c)). However, starch from the cultivar cc gold, was more susceptible to attack by α-amylase being hydrolyzed to the extent of 50.3% in 72 h (Fig. 3(d)). At the end of this time period, the surface of cc gold was covered not only with fissures but with numerous craters of varying size and depth as if the α-amylase had entered the granule and preferentially hydrolyzed the inner portion (Fig. 3(d)).

Pigeon pea starch has been shown to be very resistant to attack by porcine pancreatic α-amylase (Hoover, Swamidas, & Vasanthan, 1993). After 72 h, many of the granules were found to exhibit only superficial surface erosion. In, mung bean starch (Hoover, Li, Hynes, & Senanayake, 1997), porcine pancreatic α-amylase initially makes a depression on the peripheral region of the granule and these penetrate deep into the granule interior (Fig. 4(b)–(d)) during subsequent attack. The outer surface becomes spongy as a result of surface erosion over the entire surface.

Hoover and Sosulski (1985) have shown by comparative studies on maize and kidney bean starch granules, that after 1 h hydrolysis by porcine pancreatic α-amylase, maize starch granules showed surface indentations on the peripheral regions of the granule. During the same time period, the granule surfaces of kidney bean starch appeared smooth and showed no evidence of enzymic attack. After 6 h of hydrolysis, many of the large maize starch granules were extensively eroded over the entire surface. The erosion areas were circular ranging from 1 to 3 μm in diameter, and they penetrated through several layers of the granule wall

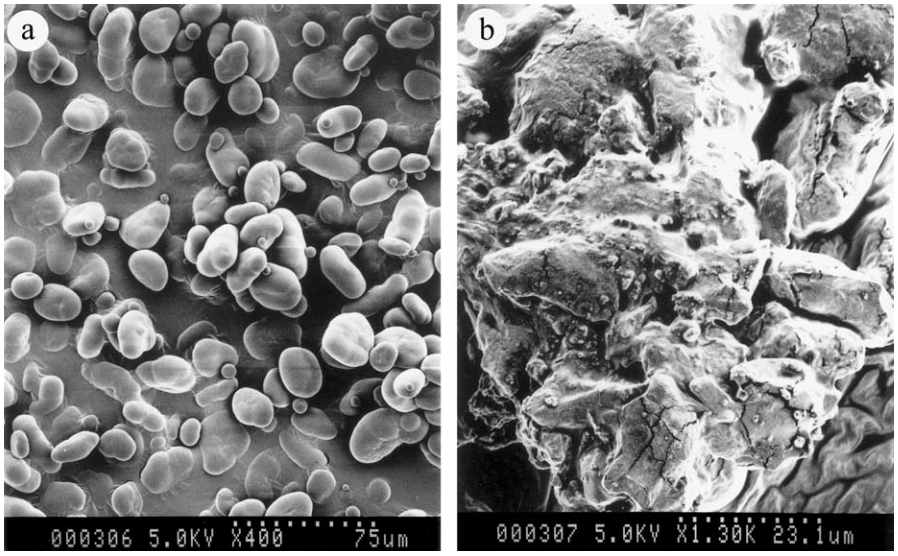

Fig. 1. Scanning electron micrographs of: (a) native smooth pea starch granules; (b) smooth pea starch hydrolyzed (60%) by porcine pancreatic α-amylase. (Hoover & Zhang, unpublished results).

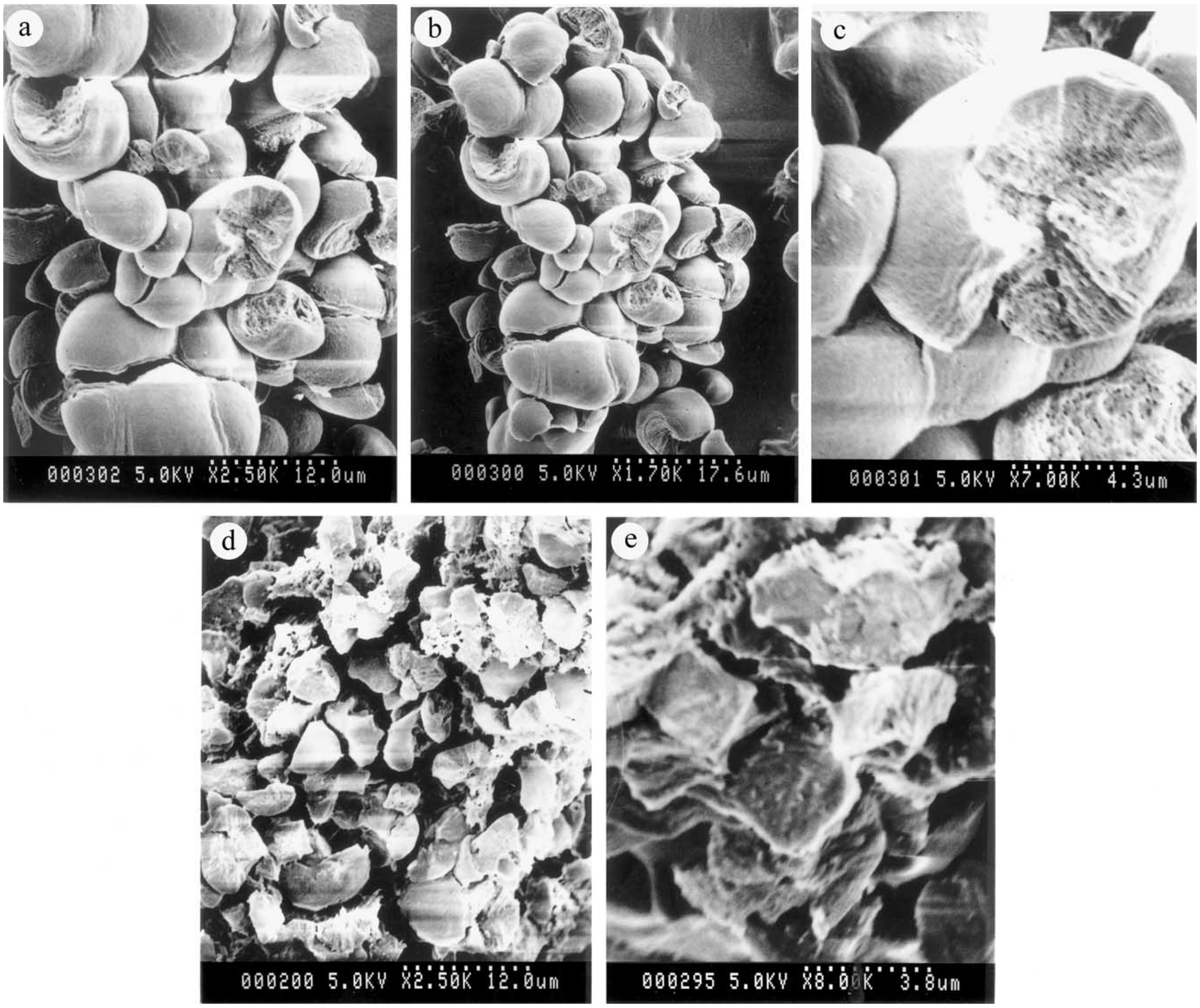

Fig. 2. Scanning electron micrographs of: (a)–(c) native wrinkled pea starch; (d) and (e) wrinkled pea starch hydrolyzed (50%) by porcine pancreatic α-amylase. (Hoover & Yang, unpublished results).

into its interior. However, some small and intermediate size granules were less eroded and had numerous small pin holes on the surface. During the same time period, kidney bean starch was less heavily eroded. Most of the kidney bean granules exhibited roughened surfaces indicating surface erosion. As in maize starch, small and intermediate size granule, were less heavily eroded than larger granules. After 24 h hydrolysis, some of the larger kidney bean starch granules were fragmented. However, fragmentation was not seen in maize starch granules.

El-Faki, Desikachar, Tareen, and Tharanathan (1983) have shown that salivary α-amylase causes pitting and surface erosion in both cow pea and horse gram starches. The extent of these morphological changes was more pronounced in horse gram starch. However, in the case of chick pea starch granules, except for some pitting, morphological changes were not discernible.

3.2. DSC

Hoover and Manuel (1995) showed that the thermal transition temperatures of the residues resulting from hydrolysis (24 and 72 h) of lentil starches (cc gold and laird) by porcine pancreatic α-amylase were higher than that of the control (no enzyme) starches. After 72 h hydrolysis, the increase in T_o, T_p and T_c were 11.8, 5.8 and 12 °C, respectively, in cc gold, whereas the corresponding values for laird were 3.2, 3.5 and 4.2 °C. In both starches, ΔH was only marginally affected by enzyme hydrolysis. Slade and Levine (1988) have ascribed the gelatinization endotherm to the melting of microcrystallites (hydrated clusters of amylopectin branches) in the presence of plasticizing water, in which crystalline melting is indirectly controlled by the kinetically constrained continuous amorphous region. Cooke and Gidley (1992) have shown that ΔH

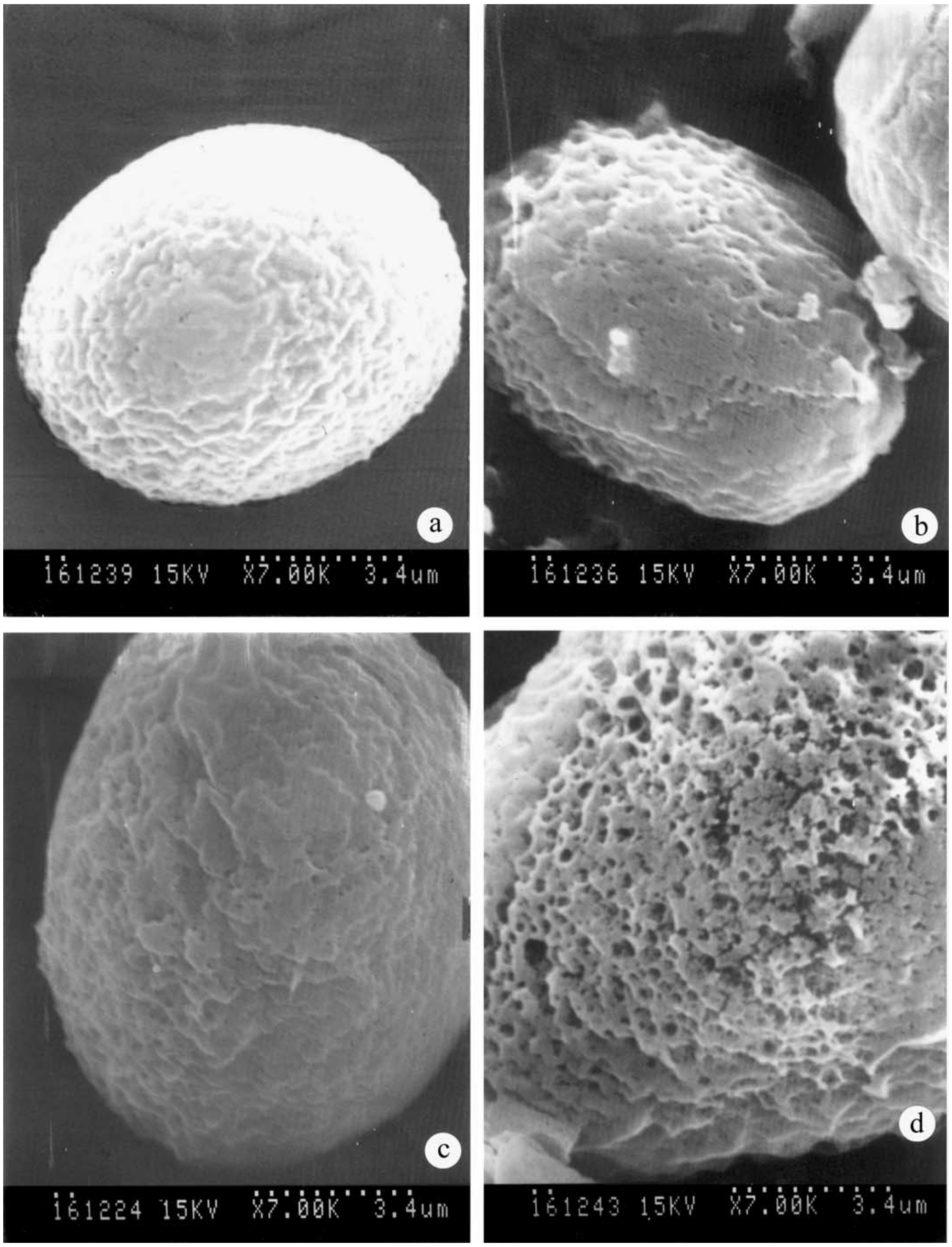

Fig. 3. Scanning electron micrographs of porcine pancreatic α-amylase hydrolyzed native lentil starch granules. (a) Laird after 24 h hydrolysis; (b) CC Gold after 24 h hydrolysis; (c) Laird after 72 h hydrolysis; (d) CC gold after 72 h hydrolysis (Hoover & Manuel, 1995, reproduced with permission from Elsevier Science).

primarily represents the loss of double helical order. Hoover and Manuel (1995) have explained the increase in T_o, T_p and T_c on α-amylase hydrolysis (72 h) as follows: hydrolysis of the bulk and intercrystalline amorphous areas would decouple the crystallites from the amorphous region. Consequently, a higher temperature would be required for crystallite melting. The unchanged ΔH (even after 72 h hydrolysis) was attributed to the resistance of double helices in the amorphous and crystalline domains towards attack by α-amylase (Hoover & Manuel, 1995).

4. In vitro hydrolysis of gelatinized and retrograded legume starches by porcine pancreatic α-amylase

Faulks and Bailey (1990) showed that the extent of hydrolysis of gelatinized legume starches ranged from

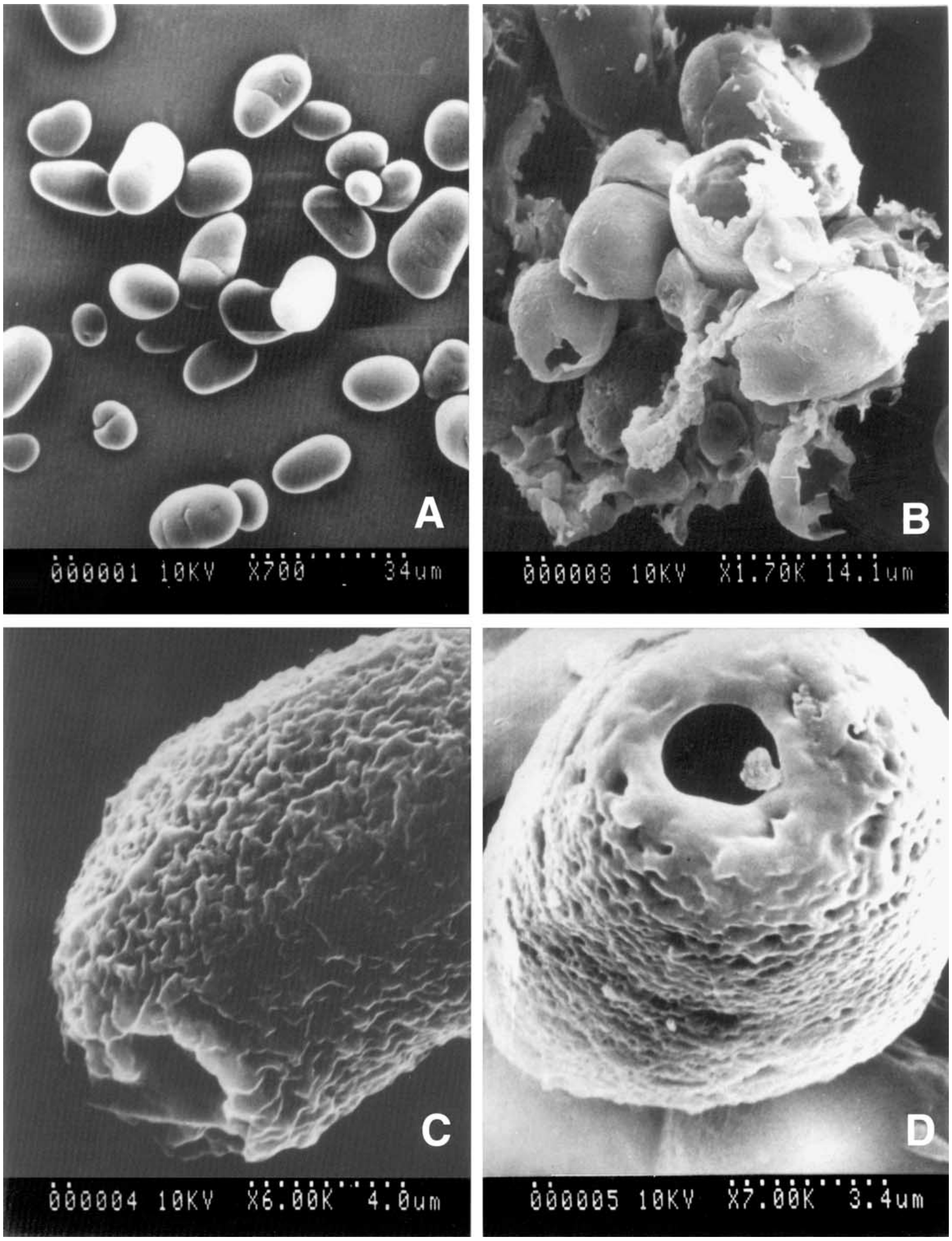

Fig. 4. Scanning electron micrographs of: (a) native mung bean starch granules; (b)–(d) mung bean starch hydrolyzed (71.1%) by porcine pancreatic α-amylase (Hoover et al., 1997, reproduced with permission from Oxford University Press).

70.5% for wrinkled pea to 90.4% for red lentil (Table 2). However, the extent of hydrolysis of retrograded starch gels was lower than that of their freshly gelatinized counterparts (Table 2). The authors have postulated that in gelled starches, there is a hierarchy of structures of differing susceptibility to amylolysis, and that retrogradation leads to an increase in degree of ordering, resulting in a decrease in the extent of hydrolysis.

5. Effect of annealing on in vitro digestibility

Annealing is a process whereby a material is held at a temperature somewhat lower than its melting temperature, which permits molecular reorganization to occur and a more organized structure of lower free energy to form (Blanshard, 1987; Tester & Debon, 2000). Annealing of starches has been studied at various starch to water ratios

Table 2
Sum of the hydrolysis products of gelatinized and retrograded starches after treatment with porcine pancreatic α-amylase for 4 h

Starch source	Gel age[a] (h)	Hydrolysis[b] (%)
Smooth pea	0[c]	89.8
Wrinkled pea	0[c]	70.5
	24[a]	58.9
	48[a]	53.4
Red kidney bean	0[c]	84.1
	24[a]	70.5
Mung bean	0[c]	80.0
Red lentil	0[c]	90.4
Broad bean	0[c]	80.0

Sum of the oligosaccharides up to maltopentose. Adapted from Faulks and Bailey (1990).

[a] The gelatinized starch prepared as above, was aged for 24 and 48 h at 1 °C, prior to treatment with α-amylase.

[b] Results expressed as a percentage of the total, starch.

[c] The starches were heated in a boiling water bath for 2 h, quickly cooled to 37 °C and then treated immediately with α-amylase.

(1:1, 1:3, 1:5) and at temperatures ranging from 50 to 75 °C (Andreev, Kalistratova, Wasserman, & Yuryev, 1999; Chung, Moon, & Chun, 2000; Hoover & Manuel, 1996; Hoover & Vasanthan, 1994a; Jacobs, Eerlingen, Spaepen, Grobet, & Delcour, 1998; Knutson, 1990; Krueger, Walker, Knutson, & Inglett, 1987; Larsson & Eliasson, 1991; Seow & Teo, 1993; Stute, 1992; Tester, Debon, & Karkalas, 1998; Yost & Hoseney, 1986). The physical aim of annealing is to approach the glass transition temperature (T_g), which thereby enhances molecular mobility without triggering gelatinization at the same time (Blanshard, 1987; Tester & Debon, 2000). A great deal is known about the effect of annealing on crystallinity, granular swelling, Brabender viscosities and gelatinization parameters. However, there is a dearth of information on the influence of annealing at different time/temperature/moisture combinations on the susceptibility of starches towards enzyme hydrolysis. Hoover and Vasanthan (1994a) have shown that the extent of in vitro hydrolysis of lentil starch by porcine pancreatic α-amylase decreases by 5% on annealing (starch:water ratio 1:3). This was attributed to an increase in the degree of interaction between starch components in the amorphous regions and/or closer packing of double helices within starch crystallites on annealing. Jacobs et al. (1998) showed that annealing (starch:water ratio 1:2) decreases the susceptibility of pea and wheat starches to hydrolysis by porcine pancreatic α-amylase, during the first (rapid) phase of hydrolysis. However, annealing increased the extent of degradation in the second (slower) phase of hydrolysis. After 120 h of hydrolysis, DSC thermograms of annealed pea and wheat starches showed broader peaks with lower enthalpies (ΔH) than those of the corresponding hydrolyzed native starch. The ΔH decrease for the annealed starches after prolonged hydrolysis was attributed to an increased susceptibility of double helical structures in the granule towards enzyme action as a result of annealing. However, annealed (starch:water ratio 1:2) potato starch showed increased resistance during both the slow and fast stages of α-amylolysis. Jacobs et al. (1998) postulated that the influence of annealing on the slow and fast phases of α-amylolysis is a function of botanical origin and/or crystal type.

6. Effect of chemical modification on in vitro hydrolysis

Modification of legume starches by acetylation (Hoover & Sosulski, 1986a) and hydroxypropylation (Hoover, Hannouz, & Sosulski, 1988) has been shown to decrease their in vitro digestibility by porcine pancreatic α-amylase. Decreases of 5.2, 5.8 and 23.8% were observed in acetylated (DS = 0.05) starches of pinto bean, navy bean and black bean, respectively (Hoover & Sosulski, 1986a). This decrease was explained by the above authors as follows: the presence of a bulky acetyl group on C2 of the glucose unit would sterically hinder the proper positioning of the substrate into the active site of α-amylase. This would make it difficult for the glutamic acid residue in the catalytic site to donate a proton to the glycosidic bond oxygen. In addition, the electron withdrawing effect of the carbonyl oxygen of the acetyl group would result in the development of a positive charge on C2. Thus, there would be two electron deficient centers (positively charged C1 and C2) on the glucose unit, which could be attacked by the nucleophilic carboxylate anion. Consequently, the α-retaining double displacement mechanism of hydrolysis that begins by the donation of a proton by glutamic acid to the glycosidic oxygen, and by the nucleophilic attack of the carboxylate anion of aspartate on C1, would occur more slowly when acetyl groups are esterified at C2.

Hydroxypropylation of field pea starch was also found to influence susceptibility towards α-amylase (Hoover et al., 1988). Increasing the level of molar substitution (MS) caused an initial decrease in hydrolysis followed by increases at MS levels beyond 0.08. The above authors postulated, that the initial decrease in hydrolysis at MS levels below 0.08 was due to the steric hindrance imposed by the large hydroxypropyl groups towards the action of the catalytic residues on the active site of α-amylase. The subsequent increase in hydrolysis at MS levels beyond 0.08 was due to an increase in granular swelling. At high MS levels (>0.08), the extent of swelling of the amorphous regions negated the effect of steric factors in influencing α-amylase hydrolysis. Cross-linked legume starches were found to exhibit only marginal decreases in the extent of hydrolysis (Hoover & Sosulski, 1986b).

7. Influence of processing on in vitro hydrolysis

The in vitro legume starch hydrolysis has been shown to increase on cooking (Aparna, Khatoon, & Prakash, 2000; Bishnoi & Khetarpaul, 1993; Bravo, Siddhuraju, & Saura-Calixto, 1998; Chau and Cheung, 1997; Fleming, 1982; Jood, Chauhan, & Kapoor, 1988; Kumar & Venkataraman, 1976; Kutos, Golob, Kač, & Plestenjak, 2003; Rao, 1969; Rehman, Salariya, & Zafar, 2001; Rosin et al., 2002; Siddhuraju & Becker, 2001), Soaking (Bravo et al., 1998; Chau & Cheung, 1997; Jood et al., 1988; Kataria & Chauhan, 1988; Kutos et al., 2003; Negi, Boora, & Khetarpaul, 2001), Sprouting (Jaya & Venkataraman, 1980; Jyothi & Reddy, 1981; Negi et al., 2001), autoclaving (Bishnoi & Khetarpaul, 1993; Chau & Cheung, 1997; Jood et al., 1988; Siddhuraju & Becker, 2001) and extrusion cooking (Alonso, Aguirre, & Marzo, 2000).

Several researchers (Bishnoi & Khetarpaul, 1993; Chau & Cheung, 1997; Jood et al., 1988; Urooj & Puttaraj, 1994) have shown that pressure cooking was more effective than other processing methods in increasing in vitro starch hydrolysis by porcine pancreatic α-amylase (Table 3). Soaking, cooking, sprouting and autoclaving of legumes reduce the level of tannins, phytate and amylase inhibitors (Bishnoi & Khetarpaul, 1993; Jood et al., 1988; Khokhar & Chauhan, 1986; Rao & Deosthale, 1982) which may to some extent increase starch hydrolysis of processed and cooked legume grains. The increase in starch hydrolysis during different heat treatments has also been ascribed to granular swelling, gelatinization and granule rupture (Bishnoi & Khetarpaul, 1993; Bravo et al., 1998; Tovar et al., 1991).

Table 3
Effect of various processing methods on the in vitro hydrolysis of legume starches by porcine pancreatic α-amylase

Starch source	Extent of hydrolysis
Black gram[a]	Autoclaving > sprouting > cooking of soaked seeds > cooking of unsoaked seeds > cooking of sprouted seeds > soaking
Chick pea[a]	
Field pea[b]	Soaked, dehulled and pressure cooked > soaked and pressure cooked > unsoaked and pressure cooked
Phaseolus angularis[c]	Autoclaving > cooking > germination > soaking
Phaseolus calcaratus[c]	
Bengal gram[d]	Pressure cooking > germination > fermentation > roasting
Cow pea[d]	
Green gram[d]	
Vicia faba L.[e]	Extrusion > germination > dehulling > soaking
Phaseolus vulgaris L.[e]	
Moth bean[f]	Sprouting and cooking > water soaking and cooking > direct cooking
Horse gram[f]	

[a] Jood et al. (1988).
[b] Bishnoi and Khetarpaul (1993).
[c] Chau and Cheung, 1997.
[d] Urooj and Puttaraj (1994).
[e] Alonso et al. (2000).
[f] Bravo et al. (1998).

8. In vivo digestibility

Fleming and Vose (1979) investigated the in vivo digestibility of several legume starches by estimating the starch content of the rat caecum. They reported that, with the exception of high amylose wrinkled pea starch, all other legume starches were 100% digestible. However, the above authors did not take into account fermentation of possible undigested starch that may have passed into the large bowel (Tovar et al., 1992b).

El-Faki et al. (1983) compared the in vivo starch digestibility of chick pea, cowpea and horse gram with that of maize starch by determining the increase in weights and contents of caecum and small intestine, apparent digestibility and fecal weights of rats. They found that the in vivo starch digestibility of legume starches was much lower than that of maize starch. The apparent starch digestibility and weight gain followed the order: maize > chickpea > horse gram, while the fecal weight and weights of organs and their contents followed the order: horse gram > cowpea > chick pea > maize. The caecum pH of rats fed legume starches was acidic (5.5–5.8) in comparison to that of rats fed maize starch. The acidic pH of the caecum was attributed to the formation of organic acids and carbon dioxide resulting from the fermentation of indigestible carbohydrates. SEM showed that chickpea starch granules appeared to be more digestible, both by in vivo and in vitro by salivary α-amylase (El-Faki et al., 1983). These authors have postulated, that in addition to enzyme specificity, the actual composition of starch per se may decide the nature, type and degree of amylolysis in in vitro systems, while in in vivo systems, the combined action of different enzymes, including those from microorganisms and changes in pH may bring about significant hydrolysis of native starch granules.

Schweizer, Anderson, Langkilde, Reimann, & Torsdottir (1990) used seven ileostomy patients to evaluate the extent of digestibility of bean flakes. Between 9.0 and 10.9% of dietary bean starch remained unabsorbed. Dietary fiber and resistant starch were completely recovered in ileostomy effluents.

Hildebrandt and Marlett (1991) used colectomized rats to compare the availability of starch in canned peas, kidney beans, lima beans, maize and cooked rice. The ileal digestion contained 11–15% of starch from the legumes, and 0.2–0.3% starch from rice and maize, respectively.

Tovar et al. (1992a) investigated the digestibility of starch in processed flours from green coat lentils and red kidney beans by balance experiments with rats treated with antibiotics (Nebacitin) to suppress hindgut fermentation. The authors found that between 8% (bean) and 12% (lentils) of the total starch ingested appeared in the feces, indicating a relatively low starch digestibility. Sixty percent of the fecal starch in the bean-fed animals and 70% in the lentil group was found to be retrograded amylose.

Abia, Fry, and Eastwood (1996) investigated the fate of [^{14}C] retrograded and gelatinized broad bean starch in the rat by using an in vivo radiolabel technique. They showed that the rate and extent of degradation of [^{14}C] starch in the small intestine were lower for ^{14}C retrograded starch, as indicated by the higher amounts of α glucans of degree of polymerization >70 in the distal fraction of the small intestine. These authors showed that the higher resistance of retrograded starch to amylolytic degradation provides the body with a supply of starch degradation products available for microbial fermentation, contributing to variations in the metabolism of the liver, carcass, felt, and gut tissues in the rat.

Bornet et al. (1989) have shown that the resistance of retrograded starches to α-amylolysis controls the rate of appearance of glucose in the small intestine and has been found to be a determining factor in glycemia and insulinemia in humans.

9. Resistant starch

Most foods are subjected to a heat processing step prior to consumption. Although heat treatment increases the accessibility of starch to enzymes, a fraction of the starch remains resistant to α-amylase hydrolysis in the small intestine; this fraction is called resistant starch (RS). RS has been classified (Englyst & Cummings, 1987) into three groups: (1) RS 1: starch that is physically inaccessible to digestive enzymes due to enclosure in structures such as intact cells in legumes; (2) RS 2: crystalline regions of native starch granules and retrograded amylopectin; (3) RS 3: retrograded amylose. An additional type of RS, type RS 4 is considered to result from chemical modification (Asp, Van Amelsvoort, & Hautvast, 1996; Brown, 1996; Eerlingen & Delcour, 1995). RS 1 and RS 2 are slowly but completely digested with appropriate pre-processing of foods, but RS 3 resists digestion. In the formation of RS 3, the starch granule is completely hydrated and amylose is leached from the granules into solution as a random coil polymer. Upon cooling the amylose chains begin to reassociate as double helices stabilized by hydrogen bonds. The individual strands in the helix contain six glucose units per turn in a 20.8 Å repeat. Upon further retrogradation the double helices pack in a hexagonal unit cell. The intimate packing of amylose double helices leads to crystal formation (Haralampu, 2000; Wu & Sarko, 1978a,b), which hinders the accessibility of α-amylase to the glycosidic bonds.

Several in vitro procedures have been used for the quantification of RS (Berry, 1986; Björck et al., 1986; Champ, 1992; Englyst, Kingman, & Cummings, 1992; Goni, Gracia-Diz, Manas, & Saura-Calixto, 1996; McCleary & Monaghan, 2002). These procedures differ with respect to enzymes used, pH and time/temperature conditions. Consequently, the RS content will vary according to the procedure used. The methodology proposed by Englyst et al. (1992) and McCleary and Monaghan (2002) are more indicative of the in vivo process.

Some factors that have been reported to influence resistant starch formation and yield are presence of sugars and lipids, amylose chain length, amylose/amylopectin ratio in the starch, physical state of the food material (whole or ground), moisture content, pH, heating temperature and time, freezing methods (slow versus rapid), drying, incubation time and temperature (Araya et al, 2003, Berry, Anson, Miles, Morris, & Russel, 1988; Eerlingen, Crombez, & Delcour, 1993; Eerlingen, Deceuninck, & Delcour, 1993; Englyst & Cummings, 1987; Szczodrak & Pomeranz, 1992; Unlu & Faller, 1998).

Raw and processed legumes have been shown to contain significant amounts of RS in comparison with cereal and potatoes (Bednar et al., 2001; Björck, Granfeldt, Liljeberg, Tovar, & Asp, 1994; Bravo et al., 1998; Elmstahl, 2002; Garcia-Alonso, Goni, & Saura-Calixto, 1998; Lehmann, Rössler, Schmiedl, & Jacobasch, 2003; Lintas & Cappelloni, 1992; Marlett & Longacre, 1996; Osorio-Diaz et al., 2002; Periago, Ros, & Casas, 1997; Rosin et al., 2002; Tovar & Melito, 1996; Tovar et al., 1992a,b; Truswell, 1992; Velasco, Rascon, & Tovar, 1997). For this reason the ingestion of legumes results in reduced glycemic and insulinemic prosprandial responses in comparison with cereals or potatoes (Jenkins et al., 1982, 1987; Tovar et al., 1992b).

RS 3 has been shown to escape digestion in the small intestine, and is slowly fermented in the large intestine (Björck et al., 1987; Englyst & Cummings, 1987; Englyst & MacFarlane, 1986; Gordon, Topp, Shi, Zallie, & Jeffcoat, 1997; Younes, Levrat, Demigné, & Rémésy, 1995). Fermentation by the intestinal microflora produces short chain fatty acids, such as acetate, propionate and butyrate (Kritchevsky, 1995; Muir, Young, & O'Dea, 1994; Phillips et al., 1995). Short chain fatty acids have been shown to have a positive impact on bowel health, including increased absorption of magnesium and calcium, epithelial proliferation, balance of bacterial species, and bacterial metabolism of bile salts. (Asp et al., 1996; Haralampu, 2000).

Cairns, Morris, Botham, and Ring (1996) characterized (X-ray diffraction, size exclusion chromatography and methylation) the RS produced in vitro by hydrolysis of retrograded pea starch gels and pea amylose gels by porcine pancreatic α-amylase. They showed that RS 3 in vitro consists of semicrystalline, mostly linear material that was present in two main molecular size sub-fractions: (1) DP >100 (arising from semicrystalline material present

Table 4
Total and available content in purified starch from raw and steam-heated beans

Legumes	Preisolation treatment	Yield (%)[a]	Total starch (%)[b]	Available starch (%)[b]	Resistant starch (%)[c]
Black bean	Raw	35 ± 0.2	89.8 ± 1.2^{e}	87.6 ± 1.4^{e}	2.2
	Steam-heated[d]	25 ± 0.6	91.2 ± 0.2^{e}	72.3 ± 1.5^{f}	18.9
Red bean	Raw	30 ± 2.5	90.2 ± 1.1^{e}	88.5 ± 1.5^{e}	1.7
	Steam-heated[d]	23 ± 1.7	89.2 ± 0.4^{e}	68.0 ± 1.0^{g}	21.2
Lima bean	Raw	29 ± 2.3	98.5 ± 2.7^{f}	96.5 ± 1.5^{h}	2.0
	Steam-heated[d]	26 ± 0.7	97.4 ± 0.8^{f}	66.7 ± 1.9^{g}	30.7

Tovar and Melito (1996). Reproduced with permission from American Chemical Society.

[a] Starch obtained (g) × 100/seed weight (g); dry matter basis.

[b] Starch content (g) × 100/isolated starch weight (g); dry matter basis. Values are means of at least six determinations ± SEM. Means in columns not sharing the same superscript letter (e–h) are significantly different ($p < 0.05$).

[c] Total starch–available starch.

[d] Steam-heated for 90 min.

in the retrograded part of the gel) and (2) DP 20–30 (composed of recrystallized amylose fragments released during degradation by α-amylase). A third minor subfraction consisting of oligosaccharides (DP ≤ 5) was also present. This model of RS 3 showed that the resistant residue from hydrolyzed amylose gels closely resembled the resistant residue recovered from hydrolyzed starch gels, indicating the primary importance of retrograded amylose in determining the RS content of starch. Analysis of in vivo RS recovered during an ileostomy study produced results that were similar to those obtained from RS 3 in vitro (Cairns et al., 1996).

Botham et al. (1995) assessed the digestibility of retrograded chick pea starch gel using an ileostomist model. The resistant starch contained molecules with a wide range of molecular weights. A major fraction consisted of fragments of amylose with an average degree of polymerization of 70–80. The resistant product was partially crystalline, giving a B-type X-ray pattern. The resistant product obtained in vivo was found to be comparable with the product obtained from the amylolysis of starch and amylose gels in vitro (Cairns, Leloup, Miles, Ring, & Morris, 1990; Ring et al., 1988).

Tovar and Melito (1996) isolated starch from either raw or steam-heated black, red and lima beans. The RS content of the isolates (Table 4) from steam-heated legumes ranged from 19 to 31% dmb, a fact not observed when raw seeds were used (Table 4). Similarly, RS measured directly in conventionally and high pressure steamed beans was 3–5 times higher than in the raw legumes (Table 5), suggesting retrogradation as the major mechanism behind the reduction in digestibility. Prolonged steaming as well as short dry pressure heating decreased the enzymatically assessed total starch content of whole beans by 2–3% (dmb) indicating that these treatments may induce formation of other types of indigestible starch.

Bravo et al. (1998) studied the effect of different processing treatments on the in vitro digestibility and the resistant starch formation in moth bean, horse gram and black gram (Table 6). Samples were cooked without previous treatment, after overnight soaking in water or in a 0.02% $NaHCO_3$ solution, and after sprouting. The RS content of the raw legumes varied between 12.2 and 26.4 g kg^{-1} of dry matter. Whereas, the corresponding range for freshly cooked and cooked and stored samples ranged from 2.7 to 5.2 g kg^{-1} of dry matter and

Table 5
Total and available starch content in variously treated beans

Legumes	Treatment	Total starch (%)[a]	Available starch (%)[a]	Resistant starch (%)[b]
Black bean	Raw	39.2 ± 0.2^{e}	37.4 ± 0.6^{e}	1.9
	Steam-heated[c]	38.7 ± 2.9^{e}	32.7 ± 1.6^{f}	6.0
	High-pressure steaming[d]	41.3 ± 4.0^{e}	35.7 ± 4.5^{g}	5.6
Lima bean	Raw	39.9 ± 0.6	39.1 ± 0.5^{e}	0.8
	Steam-heated[c]	38.9 ± 0.4^{e}	34.9 ± 0.3^{g}	4.0
	High-pressure steaming[d]	40.9 ± 0.7	36.5 ± 0.7^{g}	4.4

Tovar and Melito (1996). Reproduced with permission from American Chemical Society.

[a] Starch content (g) × 100/seed weight (g): dry matter basis. Values are means of at least six determinations ± SEM. Means in columns not sharing the same superscript letter (e–g) are significantly different ($p < 0.05$).

[b] Total starch–available starch.

[c] Steam-heated for 90 min.

[d] High-pressure steaming for 15 min.

Table 6
Total starch (TS), resistant starch (RS), and digestible starch (DS) of raw and processed Indian legumes (grams per kilogram of dry matter)

	Moth bean			Horse gram			Black gram		
	TS	DS	RS	TS	DS	RS	TS	DS	RS
Raw	$39.54 \pm 1.7^{a\alpha}$	27.34	$12.20 \pm 0.77^{a\alpha}$	$36.03 \pm 1.17^{a\alpha}$	9.61	26.42 ± 0.20^{ba}	37.87 ± 1.05^{ac}	18.21	$19.66 \pm 1.67^{e\alpha}$
Freshly cooked									
Cooked	$46.24 \pm 0.66^{a\beta}$	42.34	$3.90 \pm 0.06^{a\beta}$	$47.32 \pm 2.58^{a\beta}$	42.12	$5.21 \pm 0.64^{b\beta\gamma}$	$40.73 \pm 0.23^{b\beta\gamma}$	37.33	$3.40 \pm 0.06^{a\beta\gamma}$
WSC	$47.88 \pm 0.40^{a\gamma}$	44.15	$3.72 \pm 0.41^{a\beta\gamma}$	$46.29 \pm 0.45^{a\beta\gamma}$	40.60	$5.69 \pm 0.24^{b\beta}$	$41.02 \pm 0.14^{c\beta}$	37.35	$3.68 \pm 0.25^{a\gamma}$
SBSC	$44.49 \pm 0.89^{a\delta}$	41.26	$3.23 \pm 0.28^{a\gamma\delta}$	$43.15 \pm 1.30^{a\gamma}$	38.07	$5.08 \pm 0.06^{b\beta\gamma}$	$39.74 \pm 0.22^{b\alpha\gamma}$	36.09	$36.4 \pm 0.24^{a\gamma}$
SpC	$44.75 \pm 0.37^{a\delta}$	42.08	$2.67 \pm 0.22^{a\delta}$	$44.66 \pm 0.30^{a\beta\gamma}$	40.22	$4.44 \pm 0.71^{b\gamma}$	$38.99 \pm 0.83^{c\alpha}$	35.96	$3.03 \pm 0.30^{a\beta}$
Cooked, stored at 4 °C for 24 h									
Cooked		41.45	4.79 ± 0.70^{abc}		41.57	$5.75 \pm 0.17^{b\beta\delta}$		36.74	$3.99 \pm 0.01^{a\delta}$
WSC		41.91	5.97 ± 0.46^{ac}		38.64	7.65 ± 0.42^{bc}		35.73	5.29 ± 0.56^{ac}
SBSC		39.22	$5.27 \pm 0.44^{a\alpha}$		36.83	$6.32 \pm 0.79^{b\delta}$		34.90	$4.84 \pm 0.39^{a\delta c}$
SpC		40.23	4.52 ± 0.34^{ac}		36.77	7.89 ± 0.68^{bc}		33.50	5.49 ± 0.80^{ac}

WSC, water soaked and cooked; SBSC, sodium bicarbonate soaked and cooked; SpC, sprouted and cooked. Different superscript Latin characters denote statistically significant differences among legume species; different superscript Greek characters denote statistically significant differences among processing treatments ($p \leq 0.05$). From Bravo et al. (1998). Reproduced with permission from American Chemical Society.

4.0–7.9 g kg^{-1} of dry matter, respectively (Table 6). Sprouting and direct cooking resulted in the lowest RS content (Table 6).

Skrabanja, Liljeberg, Hedley, Kreft, and Björck (1999) evaluated the formation of resistant starch and the rate of starch hydrolysis in a wild type of green-seeded pea genotype RRRbRbBC$_3$ (32.7% amylose) and in two mutants RRrbrbBC3 (23.3% amylose) and rrRbRb-BC3 (65.1% amylose). Pea samples were intact or homogenized and subjected either to autoclaving or to boiling at atmospheric pressure. The amount of resistant starch (total starch basis) varied from 6.2 to 12.9% in the mutant samples with 23.3% amylose and from 31.2 to 33.4% in the mutant samples with 65.1% amylase (Table 7). The resistant starch level of the wild type (RRRbRbBC3) green-seeded pea (32.7% amylose) was 11.0%. Both mutant products (23.1 and 65.1% amylose) were abundant sources of dietary fiber (39 and 34% dry matter basis, respectively) versus 23.1% in the regular pea product. The hydrolysis indices (HI) and predicted glycemic indices (GI) (Table 8) were lowest in the 65.1% amylose mutant [HI 42-59, GI, 44-59) as compared to the 23.1% amylose mutant (HI, 53–84, GI 54–80). The authors have postulated that both the mutant (23.1 and 65.1% amylose) products represent an abundant source of dietary fiber, which, together with resistant starch should be expected to provide substrate for the colonic microflora.

Vasanthan and Bhatty (1998) have used annealing as a means of enhancing the resistant starch content of legume starches. Annealing (100–140 °C, 30% moisture, 1 h) of retrograded field pea and lentil starch gels increased the resistant starch content of field pea and lentil starches from 8.4 to 14.1% and 6.5 to 9.5%, respectively. Acid hydrolysis (2.2N HCl, 0.5 h) of

Table 7
Potentially available starch and RS contents in pea products, expressed as relative (total starch basis) or absolute (dmb) values

Sample	Form	Potentially available starch ± SD (g/100 g of total starch)	RS ± SD (g/100 of total starch)	Potentially available starch ± SD (g/100 g of dm)	RS ± SD (g/100 g of dm)
23-Am	Autoclaved purée (7%)[a]	101.02 ± 1.9^{a}	6.17 ± 0.24^{b}	27.08 ± 0.32	1.65 ± 0.06
23-Am	Autoclaved purée (10%)	104.23 ± 0.75^{f}	7.23 ± 0.60^{b}	27.93 ± 0.20	1.94 ± 0.16
23-AM	Autoclaved seeds	90.91 ± 2.68^{cd}	7.64 ± 0.24^{b}	24.36 ± 0.72	2.05 ± 0.07
23-AM	Boiled seeds	76.13 ± 5.32^{b}	12.85 ± 5.28^{c}	20.40 ± 1.43	3.44 ± 1.42
33-Am	Autoclaved puréé (7%)[a]	89.36 ± 0.41^{c}	11.03 ± 0.14^{bc}	41.26 ± 0.15	5.10 ± 0.06
65-Am	Autoclaved puréé (7%)[a]	75.84 ± 0.57^{b}	31.79 ± 0.25^{d}	21.84 ± 0.16	9.16 ± 0.07
65-Am	Autoclaved puréé (7%)[b]	76.31 ± 0.71^{b}	31.15 ± 1.57^{d}	21.98 ± 0.20	8.97 ± 0.45
65-Am	Autoclaved seeds	71.75 ± 3.26^{ab}	32.63 ± 3.35^{d}	20.67 ± 0.94	9.40 ± 0.97
65-Am	Boiled seeds	67.42 ± 5.53^{a}	33.40 ± 4.22^{d}	19.42 ± 1.59	9.62 ± 1.21
Wheat bread	Crumb	96.71 ± 2.46^{de}	0.76 ± 0.05^{a}	75.42 ± 1.92	0.59 ± 0.04

Values with different superscript letters (a–f) in the same column are significantly different ($p < 0.001$). Skrabanja et al. (1999). Reproduced with permission from American Chemical Society.

[a] The final concentration of starch in water reached 7% (w/w).

[b] The final concentration of starch in water reached 10% (w/w).

Table 8
HI and predicted GI for different pea products

Sample	HI ± SD	Predicted GI
White bread	100^{e}	100
23-Am autoclaved purée (7%)[a]	84.3 ± 8.34^{d}	81
23-Am autoclaved seeds	83.7 ± 13.32^{d}	80
23-Am boiled seeds	53.0 ± 10.02^{b}	54
33-Am autoclaved purée (7%)[a]	70.8 ± 6.46^{c}	69
33-Am autoclaved purée (7%)[a]	58.6 ± 9.15^{b}	59
65-Am autoclaved seeds	50.9 ± 5.79^{ab}	52
65-Am boiled seeds	41.7 ± 5.05^{a}	44

Values with different superscript letters (a–d) in the same column are significantly different ($p < 0.05$). [a] The final concentration of starch in water reached 7% (w/w). Skranbanja et al. (1999), reproduced with permission from American Chemical Society.

retrograded field pea and lentil starch gels prior to annealing increased their RS 3 content to 19.6 and 16.5%, respectively. This increase was explained as follows: acid hydrolysis of retrograded gel amylopectins at or closer to the branch points could cause the double helices that form the starch crystallites to unravel. This would then increase the amount of free linear chains, which could then associate together during annealing forming more perfect crystallites. The increase in RS 3 content was attributed to the increase in the number of crystallites of greater perfection and stability.

10. Directions for future research

1. Legume starches have been shown to have varying proportions of the A and B crystalline polymorphic forms. Furthermore, starch resistance to α-amylase has been shown to be proportional to the increasing amount of B-crystallites in starch (Gerard et al., 2001; Ratnayake et al., 2001). Hoover and Vasanthan (1994b) have shown that heat-moisture-treatment changes the B-X-ray pattern of potato and yam starches to the A + B type. Thus, a comparative study of the kinetics of α-amylase action on native and heat-moisture treated (at different time/temperature combinations) legume starches may provide a deeper insight into the influence of crystalline arrangement on legume starch susceptibility to α-amylase. Legume starches are ideal for such a study, since they are devoid of phosphate groups and contain only trace quantities of lipids.
2. The residue left after α-amylase hydrolysis has been examined mainly by SEM. There is a need to investigate the thermal characteristics, degree of polymerization, amylose content, crystallinity, B-polymorphic content and ultrastructure (using TEM) of the residue at various time intervals of hydrolysis. Such a study will help to explain the action of α-amylase on the amorphous and crystalline domains of the granule.
3. Research should be directed towards understanding the structure of the enzyme resistant material from retrograded legume starches (of widely varying amylose content). This may provide an insight into the molecular origin of enzyme resistance.

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