Enzymic Availability of Starch in Cooked Black Beans (*Phaseolus vulgaris* L) and Cowpeas (*Vigna* sp.)

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The starch content of black beans and cowpeas was assessed enzymatically in freshly cooked as well as in cooked, stored, and reheated samples. The available starch contents of the variously treated seeds were greater in cowpeas (32−33%, dmb) than in black beans (25−28%, dmb). All samples exhibited relatively high levels of retrograded resistant starch (RS) (8−20%, total starch basis), although beans showed higher contents of this fraction. No major difference in RS formation was observed between conventional and microwave reheating. Total starch was slightly decreased in stored/reheated black beans compared to that in the freshly cooked seeds. In vitro enzymic hydrolysis indices (HI) and corresponding predicted glycemic indices (pGI) of cowpea samples were 2-fold greater than those registered for beans. Reheating, and particularly microwaving, increased the amylolysis course parameters of black beans without altering the enzymic availability of cowpea starch.

Keywords: *Black beans; cooking; cowpeas; glycemic response; resistant starch; starch digestibility; storage*

INTRODUCTION

Numerous studies during the last 15 years have shown that the digestibility of starch in different foods may vary widely (Asp, 1995). Thus, new nutritional concepts such as “slow-release” or “lente” carbohydrates and “indigestible” or “resistant” starch have been introduced (Englyst et al., 1992; Jenkins et al., 1994; Björck and Asp, 1994).

Several reasons explain the heterogeneity in the digestibility behavior of food starches. It is known, for instance, that structural, compositional, and fine chemical characteristics of a particular food influence the enzymatic availability of starch and, hence, the rate of the polysaccharide digestion (Björck et al., 1994). On the other hand, food form, starch supramolecular arrangement and its degree of crystallinity and retrogradation have been identified as major determinants of the extent of starch digestion and absorption in the small intestine (Englyst et al., 1992).

From both the quantitative and qualitative points of view, edible legume seeds are generally regarded as good starch sources (Würsch, 1989; Björck et al., 1994), with beneficial features arising from their remarkably high levels of slow-release and resistant starch fractions (Tovar, 1994). There is, however, ample variability in the bioavailability of legume starches (Würsch, 1989), since intervarietal agronomic heterogeneity and unequal processing protocols may contribute to discrepancies in the digestibility figures reported by different laboratories (Würsch, 1989; Tovar et al., 1990b; Melito and Tovar, 1995).

Although data on the influence of heat treatments on the enzymatic availability of starch in pulses are scarce (Würsch et al., 1988; Lintas and Cappelloni, 1992; Tovar et al., 1992a,b; Tovar and Melito, 1996), it seems feasible to control the nutritional characteristics of the biopolymer by monitoring and manipulating pre- and postcooking seed-handling conditions. This investigation focuses on the impact of postcooking storing and reheating on the in vitro digestibility of starch in black beans and cowpeas, major staple legumes in Venezuela.

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MATERIALS AND METHODS

Preparation of Legume Samples. Black beans (*Phaseolus vulgaris* L cv. Montalban) were provided by The Centro Nacional de Investigaciones Agropecuarias (CENIAP), Maracay, Venezuela. White cowpeas (*Vigna* sp.) were obtained from the local market. The seeds were soaked overnight in water (2:1, water:seed ratio, v:w), drained and boiled (3:1, water:seed ratio) until soft as for eating (Tovar et al, 1990a,b). Cooking times assessed in this way were 90 min for beans and 120 min for cowpeas. Cooked seeds were drained and analyzed immediately for starch content and in vitro digestibility.

In experiments designed to evaluate the postcooking conditions impact on starch content and digestibility, cooked and drained seeds were stored at 4 °C for 24 h and reheated (aproximately 10 g) either following the common domestic procedure, i.e., on a hot cooking pan for 4−5 min (maximum temperature reached within the legume meal was 82 °C), or in an ordinary microwave oven for 90 s (650 W; 2,450 MHz). Reheated seeds were submitted to the above-mentioned tests.

Assessment of Starch Content. Previous to enzymatic analysis of starch, all samples were homogeneized with a SDT Tissumizer (Tekmar, Germany), giving five pulses of 1 min each (Tovar et al., 1990a,b). This treatment releases physically inaccessible starch fractions and thus allows for the assessment of the “potentially available starch content” (Tovar et al., 1990b), which was estimated by the combined Termamyl (Novo A/S, Copenhagen)/amyloglucosidase (Boehringer, Mannheim) method of Holm et al. (1986). Retrograded resistant starch content was measured as starch remnants in dietary fiber residues according to the so-called “Lund method” (Björck and Asp, 1991) as modified by Saura-Calixto et al (1993). Total starch was calculated as the sum of available starch and resistant starch (Tovar et al., 1990b; Tovar and Velasco, 1995).

Starch Hydrolysis Index. The kinetics of enzymic starch hydrolysis was evaluated with the protocol described by Granfeldt et al (1992). In this method, samples (1 g available starch) were chewed 15 times by six trained subjects and expectorated into a beaker containing an acidic pepsin solution.

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Table 1. Starch Content in Heat-Treated Legumes

treatment	available starch[a] %	resistant starch[b] %	total starch[c] %	relative resistant starch content[d] %
		Black Bean		
boiled	27.8 ± 0.3[e]	6.4	34.2	18.7
boiled, stored, reheated	25.2 ± 1.0[f]	7.6	32.8	23.2
boiled, stored, microwaved	24.8 ± 0.6[f]	7.0	31.8	22.1
		Cowpea		
boiled	34.3 ± 1.2[g]	2.9	37.2	7.8
boiled, stored, reheated	32.8 ± 1.5[h]	4.1	36.9	11.2
boiled, stored, microwaved	32.8 ± 1.5[h]	4.5	37.3	12.2

[a] Starch content (g) × 100/seed weight (g), dry matter basis. Values are means of at least four determinations ± SD. Means not sharing the same superscript letter are significantly different ($p < 0.05$). [b] Values are means of at least two determinations. Maximal deviation accepted was 0.2%, i.e., twice the method's s value (Tovar et al., 1990a). [c] Available starch + resistant starch. [d] Resistant starch content (g) × 100/total starch (g).

Table 2. In Vitro Starch Hydrolysis in Heat-Treated Legumes

legume	treatment	hydrolysis index[a] %	predicted glycemic index[b] %
black bean	boiled	10 ± 4.3[c]	17
	boiled, stored, reheated	12 ± 1.6[c]	18
	boiled, stored, microwaved	16 ± 1.4[d]	22
cowpea	boiled	40 ± 3.4[e]	43
	boiled, stored, reheated	42 ± 7.8[e]	44
	boiled, stored, microwaved	41 ±10.3[e]	44
reference white bread		100[f]	94

[a] Hydrolysis index was calculated graphically referred to white bread (Grandfeldt et al., 1992) and expressed as means ± SD. Means not sharing the same superscript letter are significantly different ($p < 0.05$; $n = 6$). [b] (hydrolysis index × 0.862) + 8.198 (Grandfelt, 1994).

The pepsin-treated samples were neutralized and subsequently incubated with pancreatic amylase in a dialysis tubing. Every half hour, the dialysate was analyzed for reducing power with 3,5-dinitrosalicylic acid in order to evaluate the proportion of starch digested to maltose (% maltose equivalents). The hydrolysis index (HI) for each sample was calculated as 100 times the area under the hydrolysis curve (0−180 min) divided by the corresponding area with commercial decorticated white bread chewed by the same person. Predicted glycemic indices (pGI) were calculated from the HI values, using the empirical equation pGI = 0.862HI + 8.198, for which the correlation coefficient r is 0.826, $p < 0.00001$ (Granfeldt, 1994).

Statistics. Means were compared by one-way analysis of variance followed by the Duncan multiple comparison test, using the number cruncher statistical system (NCSS 5.1). For the HI evaluation, the Wilcoxon matched-pair signed-ranks test was used (Granfeldt et al., 1992).

RESULTS

The enzymatically assessed starch content of cooked cowpeas and black beans is shown in Table 1. Expressed on a dry matter basis, the readily available starch contents were greater in cowpea than in bean samples. In both types of legumes, the potentially available starch level in seeds that were cooked, stored, and reheated was significantly lower than in the freshly cooked ones, changes that were accompanied by a rise in resistant starch content due to amylose retrogradation. This indigestible fraction was more important in beans than in cowpeas, as indicated by the resistant starch content expressed on a total starch basis.

Stored and reheated black beans showed a slight decrease in total starch compared to that of the freshly cooked seeds (Table 1). Such a tendency was not recorded for cowpeas. No major difference was observed between conventionally and microwave reheated pulses.

Results from the in vitro hydrolysis assays are summarized in Table 2. The HI and pGI values of cowpeas were 2-fold higher than those registered for black beans. Reheating, and particularly microwave treatment, increased the hydrolysis course parameters of black beans, but it did not change the amylolytic susceptibility of starch in cowpeas.

DISCUSSION

Current nutritional recommendations outline the convenience of increasing slow-release and indigestible carbohydrates in the regular diet (Jenkins et al., 1994; Asp, 1995). This task demands a sound understanding of the bioavailability of starch in foods and especially of its potential alteration by household and industrial processing (Björck and Asp, 1994).

Nutritionally relevant properties of pulses seem to be sensitive to the thermomechanical treatments involved in food processing (Würsch et al., 1988; Tuan and Phillips, 1991; Tovar et al., 1992b; Melito and Tovar, 1995). Such a nutritional impact of food preparation procedures was put in evidence during the present investigation where boiling resulted in a lowered available starch content in black beans and cowpeas (Table 1). This change may be explained, at least partly, by the formation of resistant starch (RS) fractions (Table 1). Furthermore, cold storing and reheating of cooked seeds, a common domestic practice in Latin America, promoted an additional rise in RS levels, stressing the influence of postcooking handling on the bioavailability of food starches.

Expressed on a total starch basis, RS contents in the variously treated seeds were considerable (8−20%). This is in accordance with the greater RS levels found in legumes compared to levels in other traditional Venezuelan starchy foods (Tovar and Velasco, 1995).

It should be emphasized that dietary fiber-based procedures for the analysis of indigestible starch, such as the one employed here, report only the retrograded amylose constituents of resistant starch (RS_3) (Englyst et al., 1992; Saura-Calixto et al., 1993). Hence, it is not surprising that cooked/stored pulses exhibit greater RS levels than the corresponding freshly boiled seeds, since prolonged cooling favors starch gels retrogradation. A similar tendency has been recorded for potatoes (Englyst et al., 1992).

Regarding the second heat treatment (reheating), it is interesting to note that despite any perceived difference in strength between the two heating methods, microwaving and direct pan-heating had a similar effect on RS content. A previous study with cowpeas found equivalent indigestible starch levels after different cooking procedures (Tuan and Phillips, 1991).

Although retrograded RS values provide good estimates of the in vivo indigestible starch ocurring in most wet-heated foods (Björck and Asp, 1991), the actual form in which legumes are usually eaten, i.e., whole cooked seeds, is a source of physically inaccessible fractions that contribute to the physiologically indigestible starch bulk (Tovar et al., 1992a; Botham et al., 1995). In vivo trials for evaluating the impact of processing on the physiologically resistant starch content of these seeds are therefore granted.

We have demonstrated the reduction in total starch content assessed enzymatically in beans submitted to drastic heating conditions (Tovar and Melito, 1996), suggesting that transglycosidation and other carbohydrate side-reactions may play a role in the process-induced limited digestibility of legume starches. According to present results, relatively mild reheating of cooked/stored black beans also promotes a slight deterioration in starch enzymatic availability. Interestingly, no change in total starch content was noted in the corresponding cowpea samples (Table 1), an observation that emphasizes the interspecies physicochemical and nutritional variability of leguminous starches.

The low HI and pGI values recorded for the two legumes studied are noteworthy. Since the in vitro protocol utilized has a good predictive value for the postprandial glycemic responses to a wide range of foods, including beans and lentils (Granfeldt et al., 1992; Granfeldt, 1994), this result is in agreement with the "slow-release" feature of most legume starches (Würsch, 1989). However, there were differences between black beans and cowpeas also in this respect, the latter showing greater in vitro indices (Table 2). Moreover, storing and reheating of black beans, but not of cowpeas, resulted in increased HI and pGI values, regardless of the type of heating applied (Table 2). It is thus sound to expect augmented metabolic responses when consuming reheated beans.

Variable heat resistance of anti-amylase factors occurring in the legume species studied here (Tovar, 1994) and possible unequal fine structural features of their starch constituents (Botham et al., 1995) may be argued to explain the recorded differences in susceptibility to reheating. Nonetheless, the changes in starch digestion rates after further black bean heating may be also related to increased physical accessibility of starch to amylase. In fact, the "lente" properties of starch in legumes can be modified by the deterioration of cotyledon architecture and cell wall integrity produced by thermomechanical treatments (Würsch et al., 1988; Tovar et al., 1992b).

Present observations reiterate the importance of postcooking handling for the overall bioavailability of starch in legumes. In the particular case of black beans, reheating comes forth as an alternative way to manipulate interesting nutritional features such as the starch hydrolysis rate and digestibility.

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