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Bioavailability of carbohydrates in legumes: Digestible and indigestible fractions

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SUMMARY. Despite their important contribution to seed weight, carbohydrates in pulses have received limited attention. However, experimental evidence acumulated during the last two decades indicate that legumes are rich sources of slowly digestible starch promoting moderate postprandial glycemic and insulinemic responses. Although the reasons for this phenomenon are not completely understood, some intrinsic properties of the starch itself and the microstructure of cotyledon cells appear to determine much of the slow release character. This beneficial feature is rather sensitive to thermal and mechanical processing. A minimum of 10% of the starch occurring in common beans and lentils escapes digestion and absorption in the normal small intestine, and is therefore referred to as «resistant starch». This material consists mainly of retrograded amylose fractions generated upon cooling of wet-heated pulses. Physically inaccessible starch fractions resulting from cotyledon microstructural properties may also contribute to incomplete digestibility, accounting for up to 40% of the indigestible starch. These indigestible starch fractions are fermented in the large intestine generating gases and volatile fatty acids, compounds that have important influence on the physiology of the colonic mucosa and peripheral metabolism.

RESUMEN. Biodisponibilidad de los Carbohidratos de las Leguminosas: Fracciones Digeribles e Indigeribles. En contraste con el amplio conocimiento que se tiene del valor nutritivo del componente proteíco de las leguminosas, y pese a su indiscutible relevancia en términos cuantitativos, aún se sabe poco sobre los carbohidratos presentes en estos granos. El componente mayoritario de las semillas maduras de leguminosas es la fracción compleja. Este renglón, que engloba a la fibra dietética y al almidón, puede representar hasta el 60% del peso de la semilla. Una fracción importante del almidón es hidrolizada a una tasa relativamente baja, lo cual se traduce en una respuesta glicemica moderada. Por ello, las leguminosas constituyen un renglón alimentario atractivo para el manejo dietético de la diabetes mellitus. Los mecanismos que determinan este comportamiento incluyen tanto a las propiedades del almidón en sí, como de la microestructura del cotiledón y sus células constituyentes, lo cual lo hace dependiente del procesamiento al que se someten los granos. Otra porción del almidón presente en los granos tiene carácter indigerible. Esta propiedad ha sido caracterizada in vitro e in vivo y parece resultar tanto de la retrogradación de la amilosa, como de la inaccesibilidad física impuesta por la microestructura del cotiledón. La evidencia mas reciente sugiere que al menos el 10% del almidón de las semillas cocidas de P. vulgaris y L. culinaris escapa a la digestión y absorción en el intestino delgado. Así, una porción importante del componente amiláceo de las leguminosas ingresa al intestino grueso donde, junto con los α-galactósidos, es fermentada por la microflora colónica. Este último proceso da origen a los inconvenientes asociados a la generación de gases, pero tiene influencia beneficiosa sobre la fisiología de la mucosa del colon y, posiblemente, sobre el metabolismo lipídico hepático.

Carbohydrates in most edible pulses represent up to 65% of the seed weight. More than a half of this portion consists of the so-called «complex carbohydrates», i.e. starch and dietary fiber (1), while the sugar fraction (mono, di- and oligosaccharides) is significantly smaller (2).

Although from a quantitative point of view carbohydrates are the main constituent of most dried legumes, nutritional studies on pulses have focussed mainly on their proteins. Two major reasons may be argued to explain such a contradiction: *i)* the early perceived idea of food carbohydrates being easily digested and absorbed in the human small intestine, and *ii)* the apparently low impact that these dietary compounds have on health, specially when compared to fat and proteins. Nevertheless, the outcome of numerous investigations during the last 15 years indicate that the nutritional role of carbohydrates has been largely underestimated, and leguminous foods have provided important evidences for this conceptual change.

In the early 80s, David Jenkins and his research team made an interesting finding when monitoring the evolution of blood glucose in human subjects after the ingestion of experimental breakfasts. Surprisingly, the glycemic responses to legume-based meals were remarkably lower than those registered after other starchy foods or free sugars (3). After these and other experiences, legumes were accepted as sources of «slowly released carbohydrates». The evolution of this pioneer concept has led to a new nutritional classification of carbohydrates (4), which attends to the rate and extent of their digestion in the small intestine, parameters that determine the consequences of carbohydrate ingestion on intestinal and peripheral physiology. Thus, the existence of rapidly digested, slowly digested and indigestible carbohydrates is currently recognized. As it will discussed later, legume seeds contain important quantities of each of these nutritionally relevant carbohydrate types.

The above mentioned «slow release» feature of legume carbohydrates seems to be consequence of the limited susceptibility to enzymatic break down shown by their starches. Compared to cereal and tuber samples, a high proportion of the starch present in lentils and common beans behaves as «slowly digestible starch» *in vitro* (4). This characteristic results in a delayed hydrolysis and absorption of the digestion products *in vivo* promoting, therefore, moderate postprandial hyperglycemia and insulinemia, a fact of potential use in the dietary management of diabetes mellitus (5).

Although the mechanism governing the slow digestion of starch in pulses is not completely understood, it clearly depends on multiple factors. Some of them seem to be intrinsic to the starch itself. This was illustrated for isolated black bean (Phaseolus vulgaris) starch, which is less digestible by pancreatic amylase than wheat, corn or rice starches (Table 1) (6), a fact that might be explained by the relatively high amylose:amylopectin ratio commonly found in leguminous starches (7).

TABLE 1
IN VITRO DIGESTIBILITY OF ISOLATED CEREAL AND BEAN STARCHES[a]

Treatment	Degree of Hydrolysis (%)			
	Black Bean Starch		Corn Starch	
	15 min	60 min	15 min	60 min
None	5	10	3	8
Boiling (30 min)	30	50	50	75

[a]Modified from Socorro, Levy-Benshimol & Tovar (6)

Microstructure is, however, a major determinant of the digestion rate of starch in legumes. The remarkable mechanical resistance of common bean cotyledon cell walls was demonstrated by Würsh et al. (8), who found that gentle mashing of boiled white beans yields a paste rich in rather intact cotyledon cells containing starch granules. Such a unique physical property allowed us to process common beans and lentils into slowly digestible precooked flours (9,10), in which the occurrence of cell wall-surrounded starch was noticeable (11). The influence of cell wall integrity on the *in vitro* starch hydrolysis of the processed powders was made evident by the substantial increase in the amylolysis rate recorded after exhaustive homogenization (Table 2). Recent studies indicate that this phenomenon may also limit protein digestibility in cooked beans (12).

TABLE 2
EFFECT OF CELL WALL DISRUPTION ON *IN VITRO* DIGESTIBILITY OF STARCH IN PRECOOKED LEGUME FLOURS[a]

Treatment	Degree of Starch Hydrolysis (%)			
	Red Beans		Lentils	
	15 min	60 min	15 min	60 min
None (control)	8	19	11	31
Homogenization	50	54	55	73

[a]Modified from Tovar et al. (11)

In vivo trials have confirmed that microstructural alterations induced by processing modify metabolic responses to starch in legumes (13). Table 3 summarizes the glycemic and insulinemic indices of variously treated red kidney beans. The cooking procedure has an evident impact on both glycemic and insulinemic responses to the seeds, since autoclaved samples have greater indices than boiled ones, suggesting that pressure cooking might produce a more extensive damage to cell wall structures. Further deterioration of the seed structural integrity, achieved by milling during the preparation of precooked flours, resulted in increased metabolic responses (Table 3). Therefore, the architecture of the seed tissues appears to constitute a physical barrier that limits digestive enzymes action on their starch substrate. Higher levels of structural damage, such as cell wall disruption by homogenization of the precooked flours, yielded more rapidly digested preparations with greater metabolic indices.

TABLE 3
GLYCEMIC AND INSULINEMIC INDICES OF VARIOUSLY TREATED RED KIDNEY BEANS [a]

Preparation	Glycemic Index	Insulinemic Index
Boiled Beans[b]	44	34
Autoclaved Beans[c]	58	51
Precooked Bean Flour	62	52
Precooked Flour (without cells)	76	51
Wheat Bread (reference)	100	100

[a]Modified from Tovar et al. (13)
[b]70 min
[c]121 °C, 20 min

It should be stressed that even if postprandial blood responses increase, not all the beneficial 'slow' features are lost during processing, as the greatest glycemic/insulinemic indices recorded for the legume-based breakfasts are still smaller than for the reference cereal sample (bread) (Table 3). This is also valid for the long-lasting satiating effect of red bean meals (Table 4), which correlates with the pace of starch processing in the small intestine (2).

TABLE 4
SATIETY SCORE OF RED BEAN AND WHEAT BREAD MEALS[a]

	Red Bean		Wheat Bread
Postprandial Time (min)	Autoclaved	Precooked Flour (Without Cells)	
30	5.6	6.3	3.3
95	3.4	2.5	-0.3
120	2.3	0.7	-1.0
180	0.5	-1.8	-3.9

[a] Modified from Tovar (2)

The rate of starch digestion in freeze-dried samples from cooked beans appears to be different from that of the material leaching out to the cooking water (Tovar J, unpublished). In spite of the presence of cell-enclosed starch in both fractions, the digestibility of the cooking outflow was significantly lower. Although it is tempting to especulate about differential deterioration of cellular structures in each preparation, different starch dispersion properties may also account for unequal susceptibilities to amylolysis. This finding deserves further attention, particularly in view of the traditional latinamerican use of bean cooking water for infant feeding. In this context, slow -and perhaps incomplete- digestion of ingested starch might be undesirable rather than beneficial.

In vitro studies carried out at this laboratory suggest that, on top of the structural influence of cotyledon cell walls on starch bioavailability, polymeric constituents of legume dietary fiber may interfere with α-amylolysis. The phenomenon is observed even when the fiber is present in a less organized physical state, such as in the indigestible residue isolated by enzymatic procedures. For instance, black bean starch degradation is atenuated by the indigestible residue obtained from the same seeds (Table 5) (6). Among possible reasons for this inhibitory effect of bean fiber, enzyme insolubilization and substrate masking (14), as well as anti-amylase activity of fiber-associated tannins (14,15) have been proposed. It is important to mention, however, that the physiological relevance of these observations is yet unclear.

TABLE 5
EFFECT OF BLACK BEAN INDIGESTIBLE RESIDUE ON *IN VITRO* HYDROLYSIS OF ISOLATED BEAN STARCH[a]

	Degree of Hydrolysis[b] (%)		
Treatment	15 min	30 min	60 min
None (control)	28	43	45
+ Indigestible Residue	17	24	32

[a]Modified from Socorro, Levy-Benshimol & Tovar (6)
[b]Porcine pancreatic amylase

Indigestible starch fractions occur in most starchy foods (4,16), but legumes are among the richest sources of such a novel dietary constituent. As a matter of fact, at least 10% of the starch in processed legume seeds escapes digestion and absorption in the small intestine and is, therefore, regarded as «enzyme resistant» or, more simply, «resistant starch» (16,17). Accurate assessment of the resistant starch content in these seeds poses the same technical problems faced with practically any other edible vegetable (17). Thus, we currently rely on estimates obtained with a number of different experimental approaches.

Attempts to measure «true digestibility» of starch in processed common beans gave values ranking from 80 (18) to 90% (19), depending on the *in vivo* model employed. Similar figures have been reported for heat treated lentils (19) and cowpeas (20) (Table 6). These values correspond to a 10-20% resistant starch content, expressed on a total starch basis. In addition to the experimental model, differences in sample origin, agronomical variety and processing conditions may explain the observed discrepant results. Interestingly, and regardless of the digestibility index recorded, undigested starch seems to consist of two major fractions. The predominant one is likely to be retrograded amylose (18-20), a crystalline and enzyme-resistant form that is produced after cooling of heat treated starch or starchy foods (4,16). This observation is consistent with the known tendency of legume starches to generate *in vitro* indigestible retrograded fractions (9,10,16,22).

TABLE 6
IN VIVO DIGESTIBILITY OF STARCH IN PROCESSED LEGUMES

Sample	Method of Assessment[a]	Digestibility Index (%)[b]	Reference
White Beans	HI	80	Schweizer (18)
White Beans	AR	88	Asp et al. (21)
Red Beans	AR	90-92	Tovar et al. (19)
Cowpeas	IC	90	Tuan,Phillips (20)
Lentils	AR	89	Tovar et al. (19)

[a]HI: Human ileostomists; AR: Antibiotic-treated rats; IC: Analysis of ileal content in rats
[b]Undigested starch measured (g) x 100)/starch intake (g)

The remaining of the *in vivo* indigestible starch - approximately 30 to 40% of the resistant starch in beans and lentils- has been poorly characterized, although it is thought to be the sum of part of the physically inaccessible (cell-enclosed) starch mentioned above (19) and the recently acknowledged losses due to physiological inefficiency, an apparently general feature of normal starch assimilation (23).

The occurence of significant amounts of indigestible starch in pulses raises two major questions: should this starch be added to the dietary fiber content of the food? and, is it lost from the physiological point of view? No conclusive answer can be given for the first query which is subject of controversy when the composition of processed foods is to be considered (16). Nonetheless, there is no doubt about the contribution of *in vitro* resistant starch to the dietary fiber residue obtained by enzymatic means (Table 7).

TABLE 7
STARCH CONTRIBUTION TO THE INDIGESTIBLE RESIDUE IN RED KIDNEY BEANS[ab]

Flour	Indigestible Residue[c]	Starch[d]	Dietary Fiber[e]
Raw	18.9	-	18.9
Precooked	22.8	3.5	19.3

[a]Modified from Tovar et al (9)
[b]Values are expressed in g per100 g original flour (dry weight basis)
[c]Obtained after sequential digestion with Termamyl[R], pepsin and pancreatin
[d]Assessed enzymatically in the indigestible residue after alkaline dispersion
[e](indigestible residue - starch)

Regarding the physiological use of legume resistant starch, it has proven to be fermented to a variable extent in the large intestine. Indirect estimates suggest that bean indigestible starch is more easily degraded in the rat caecum than the corresponding lentil fraction. Similarly, *in vitro* studies with rat intestinal microflora indicated important differences between resistant starch isolated from these two legume species (24). Hence, indigestible starch adds to the well known legume indigestible oligosaccharides (25) as substrates for bacterial fermentation, a process that generates gases (hydrogen, carbon dioxide and methane) with concomitant undesirable effects. Fermentation, however, also results in volatile fatty acid production (acetate, propionate and butyrate), compounds that contribute to metabolizable energy (26) and seem to exert beneficial action on the colonic mucosa physiology and hepatic metabolism (27,28).

Further research on chemical and nutritional features of legume carbohydrates may help technological manipulation aiming to retain, or even increase, the amount of slowly and indigestible fractions in pulse-based foods for affluent societies. Research for developing countries should be, in contrast, focussed on the improvement of the overall digestibility.

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REFERENCES

1. BNF (The British Nutrition Foundation's Task Force). Complex Carbohydrates in Foods. London, Chapman and Hill, 1990, pp. 3-21.
2. Tovar J. Bioavailability of starch in processed legumes. Importance of physical inaccessibility and retrogradation. PhD Thesis, University of Lund. 1992.
3. Jenkins DJA, Wolever TMS, Taylor RH, Barker H & H Fielden. Exceptionally low blood glucose response to dried beans. Comparison with other carbohydrate foods. Br. Med. J. 281:578-580, 1980.
4. Englyst HN, Kingman SM & JH Cummings. Classification of nutritionally important starch fractions. Eur J. Clin. Nutr. 46:S33-S50, 1992.
5. Jenkins DJA, Wolever TMS, Taylor RH, Fielden H, Baldwin JM, Bowling AC, Newman HC, Jenkins AL & DV Goff. Glycemic index of foods: a physiological basis for carbohydrate exchange. Am. J. Clin. Nutr. 34:362-266, 1981.
6. Socorro M, Levy-Benshimol A & J Tovar. In Vitro Digestibility of Cereal and Legume (Phaseolus vulgaris) Starches by Bovine, Porcine and Human Pancreatic α-Amylases. Effect of Dietary Fiber. Starch/Stärke 41: 69-71, 1989.
7. Tovar J. Bioavailability of Starch in Processed Legumes: Importance of Physical Inaccessibility and Retrogradation. Scand. J. Nutr. 36:164-172, 1992.
8. Würsch P, Del Vedovo S & B Koellreuter. Cell structure and starch nature as key determinants of the digestion rate of starch in legumes. Am. J. Clin. Nutr. 43:25-29, 1986.
9. Tovar J, Björck I & N-G Asp. Analytical and Nutritional Implications of Limited Enzymic Availability of Starch in Cooked Red Kidney Beans. J. Agric. Food Chem. 38: 488-493, 1990.
10. Tovar J, Björck I & N-G Asp. Starch Content and α-Amylolysis Rate in Precooked Legume Flours. J. Agric. Food Chem. 38:1818-1823, 1990.
11. Tovar J, de Francisco A, Björck I & N-G Asp. Relationship Between Microstructure and In Vitro Digestibility of Starch in Precooked Leguminous Seed Flours. Food Struct. 10: 19-26, 1991.
12. Melito C & J Tovar. Cell walls limit in vitro protein digestibility in processed legume seeds. Food Chemistry. 53: 305-307; 1995.
13. Tovar J, Granfeldt Y & I Björck. Effect of Processing on Blood Glucose and Insulin Responses to Starch in Legumes. J. Agric. Food Chem. 40: 1846-1851, 1992.
14. Effect of indigestible Residue from Foodstuffs on Trypsin and Pancreatic Amylase Activity In Vitro. Morón D, Melito C & J Tovar. J. Sci. Food Agric. 47:171-179, 1989.
15. Carmona A, Seidl DS & WG Jaffé. Comparison of Extraction Methods and Assay Procedures for the Determination of the Apparent Tannin Content of Common Beans. J. Sci. Food Agric. 56:291-301, 1991.
16. Asp N-G & I Björck. Resistant Starch. Trens Food Sci. Technol. 3:111-114, 1992.
17. Tovar J. Natural and Man-Made Resistant Starch. Agro-Food Industry Hi-Tech. 6 (5):23-25, 1994.
18. Schweizer TF, Andersson H, Langkilde AM, Reiman S & Torsdottir I. Nutrients Excreted in Ileostomy Effluents After Consumption of Mixed Diets with Beans and Potatoes. II: Starch, Dietary Fibre and Sugars. Eur. J. Clin. Nutr. 44:567-575, 1990.
19. Tovar J, Björck I & Asp N-G. Incomplete Digestion of Legume Starches in Rats: A Study of Precooked Flours Containing Retrograded and Physically Inaccessible Starch Fractions. J. Nutr. 122:1500-1507, 1992.
20. Tuan YH & D Phillips. Effect of the Hard-to-Cook Deffect and Processing on Protein Digestibility of Cowpeas. Cereal Chem. 68:413-418, 1991.
21. Asp N-G, Tovar J & S Bairoliya. Determination of Resistant Starch in vitro with Three Different Methods, and in vivo with a Rat Model. Eur. J. Clin. Nutr. 46:S117-S119, 1992.
22. Melito C, Contreras L, Levy-Benshimol A & J. Tovar. Enzymic Availability of Starch is Affected by Phytohemagglutinin and the Isolation Procedure. In: Proceedings from EURESTA Summing Up Meeting. N-G Asp (Ed.) in press.
23. Faisant N, Champ M, Colonna P, Buleon A, Molis C, Langkilde A-M, Schweizer T, Flourie B & J-P Galmiche. Structural Features of Resistant Starch at the End of the Human Small Intestine. Eur. J. Clin. Nutr. 47:285-296, 1993.
24. Abia R, Buchanan CJ, Saura-Calixto F & MA Eastwood. Structural Changes During Retrogradation of Legume Starches Modify the in vitro Fermentation. J. Agric. Food Chem. 41:1856-1863, 1993.
25. Liener IE. Factors Affecting the Nutritional Quality of Soya Products. J. Am. Oil Chem. Soc. 58:406-415, 1981.
26. Mathers JC. Energy Value of Resistant Starch. Eur. J. Clin. Nutr. 46:S129-S130, 1992.
27. Todesco T, Rao AV, Bosello O, & DJA Jenkins. Propionate Lowers Blood Glucose and Alters Lipid Metabolism in Healthy Subjects. Am. J. Clin. Nutr. 54:860-865, 1991.
28. Berggren AM, Björck IME, Nyman MGL & BO Eggum. Short-Chain Fatty Acids Content and pH in Caecum of Rats Given Various Sources of Carbohydrates. J. Sci. Food Agric. 63:397-406, 1993.