# Phaseolin diversity as a possible strategy to improve the nutritional value of common beans (*Phaseolus vulgaris*)

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**Running title:** Improving the nutritional value of bean protein

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**Abstract**

This article proposes a new way to improve the protein quality of the common bean (*Phaseolus vulgaris*). It is based on the natural variability found in the different types of phaseolin, its main storage protein (40-50% of the total protein). Despite the fact that it is deficient in methionine content, phaseolin still represents the main source of that amino acid in the seed. More than 40 genetic variants, differing in subunit number (2-6) and molecular weight (40-54 kDa) have been analyzed. The similarity of the amino acid composition among phaseolins, suggests that a nutritional improvement cannot be expected from that side. Conversely, important variation in phaseolin susceptibility to proteolysis (ranging from 57 to 96% after cooking) has been observed, increasing the theoretical availability of methionine by up to 37%. Therefore, breeding programs based on highly digestible phaseolin types could lead to the production of beans with higher protein quality.

**Keywords:** common bean, phaseolin diversity, sulphur amino acid, nutritional value

## 1. Introduction

The common bean (*Phaseolus vulgaris*) represents one third of the total world production of pulses (19.3 Mt/year; FAO, 2007). It is mainly produced in Latin America and Central Africa, where it is a staple food for many people due to its energy, protein, dietary fiber and minerals content (Haytowitz, Marsh & Matthews, 1981; Norton, Bliss & Brezan, 1985). In those regions, the intake per capita ranges from 1 to 40 kg/year (Leterme & Muñoz, 2002; FAO 2007). In developed countries, bean consumption is also encouraged due to its health promoting properties. For example, the daily intake of pulses is known to reduce the risk of coronary heart disease and type-II diabetes (Leterme, 2002; Tharanathan & Mahadevamma, 2003).

However, as a protein source, common beans have several disadvantages: they require long cooking periods (Leterme & Muñoz, 2002), their proteins are poorly digested -even after cooking- and the presence of water-soluble oligosaccharides can cause flatulence. The low apparent protein digestibility of beans can be explained by the low digestibility of its protein fractions (Genovese & Lajolo, 1996), endogenous losses as a result of consuming beans (Oliveira & Sgarbieri, 1986; Marquez & Lajolo, 1991) and the presence of anti-nutritional factors (Genovese & Lajolo, 1996). Additionally, the low methionine content of beans make worse the nutritional value of their proteins.

Attempts have been made to improve the protein quality of the common bean through breeding programs or genetic manipulation (Gepts & Bliss, 1984; Aragao et al., 1999; Taylor, Chapman, Beyaert, Hernandez & Marsolais, 2008). The principal target has usually been phaseolin, since it is the main storage protein in seeds and makes up a high and variable (30-50%) proportion of the total protein and despite the fact that phaseolin is deficient in methionine, cysteine and tryptophan. To our knowledge, the high diversity in phaseolin types and their susceptibility to digestion have barely been considered as parameters to take into account in order to improve bean protein quality.

The present review examines these parameters as possible ways to improve the protein quality of the common bean.

## 2. Protein fractions of common bean

The major components of pulse proteins are globulins and albumins (Table 1). In contrast to other legumes, common bean contain high amounts of glutelin (7-15 vs 20-30%, respectively). Moreover, its main globulin fraction, phaseolin (7S fraction) represents 40 to 50% of the total

seed nitrogen whereas the other globulin fraction (11S) represents only 10% (Derbyshire, Wright & Boulter, 1976; Ma & Bliss, 1978). The other nitrogenous fractions of common bean are prolamin (2 to 4%) and the free AA pool (5 to 9%) (Ma & Bliss, 1978). The AA composition of the seed and its different protein fractions is detailed in Table 2. Differences in AA composition can be observed between the different fractions, even for AA present in low amounts, such as methionine.

## 3. Phaseolin diversity

Phaseolin is a glycoprotein containing neutral sugars, conferring on it a high source of variation in the MW of its subunits (Brown, Ma, Bliss & Hall, 1981). The molecular diversity of phaseolin has been used as an evolution indicator of the common bean domestication in Central America and in the Andes region. It provides solid botanic, archaeological and historical information due to polymorphism, environmental stability and biochemical complexity characteristics (Gepts, 1988).

Each electrophoretic profile of phaseolin subunits is the result of a series of complex events at molecular level, avoiding two identical types of phaseolins. Therefore, it is possible that each type of phaseolin is derived from a unique ancestor (Gepts & Bliss, 1986).

Bean domestication studies have shown that two phaseolins are mainly found (90%) in cultivated beans: the S (Sanilac) and T (Tendergreen) phaseolins (Gepts & Bliss, 1986; Koening, Singh & Gepts, 1990). The S phaseolin is mainly present in the cultivars of Central America, from Mexico to the North of Colombia. The T phaseolin is mainly present in cultivars of the Andes, including south of Peru, Bolivia, Argentina and Chile (Gepts & Bliss, 1986; Beebe, Rengifo, Gaitan, Duque, Tohme, 2001). However, within each centre of domestication, other

93 phaseolin types have been found in wild cultivar. For example, the I phaseolin (Inca) was found
94 between the two geographical centres defined above (i.e. between Ecuador and Peru) (Koening
95 et al., 1990).
96 The electrophoretic profile in one dimension shows that phaseolins are composed of 2 to 6
97 polypeptides differing according to their molecular weight (MW) (ranging from 40 to 54 kDa)
98 (Figure 1; Salmanowicz, 2001; Montoya et al., 2008c). These polypeptides also differ in their
99 isoelectric point (Brown et al., 1981). Phaseolin is thus a family of proteins varying in isoelectric
100 point, polypeptide composition and MW, due to the proportion of each polypeptide present in the
101 whole molecule (Bollini & Vitale, 1981). The differences in MW and isoelectric point observed
102 among polypeptides reflect differences in DNA sequences, coding for two different polypeptide
103 sub-families, α-phaseolin polypeptides (435 to 444 AA residues) and β-phaseolin polypeptides
104 (421 AA residues) (Slightom, Drong, Klassy & Hoffman, 1985), both derived from the same
105 ancestor. Recently, different subunit precursor profiles for S, T and I phaseolin (S, αβ; T, αββ; I
106 ββ) have been evidenced by mass spectrometry (Montoya, Leterme, Beebe, Souffrant, Molle &
107 Lallès, 2008b). It could be explained by differences in the sequences of the α- and β-gene
108 precursors for each phaseolin type (Kami & Gepts, 1994; Kami, Becerra, Debouck & Gepts,
109 1995). Differences in MW could also be due to pre- and post-translational modifications that
110 lead to the differentiation of polypeptides of phaseolin, or small insertions-deletions, limited
111 duplications and nucleotide substitutions (Brown et al., 1981). Moreover, the carbohydrate
112 composition and the number of phosphate binding sites of phaseolin (Paaren, Slightom, Hall,
113 Inglis & Blagrove, 1987; Lawrence, Izard, Beuchat, Blagrove & Colman, 1994) also contribute
114 to the MW diversity observed for the same protein precursor (Montoya et al., 2008b). Similarly
115 with wild soybean lines, Fukuda et al. (2005) found variations in AA sequences of the subunits

of soybean storage globulins β-conglycinin (7S) and glycinin (11S) that affected their electrophoretic mobility.

## 4. Digestibility of the protein fractions

In general, legume proteins are usually regarded as highly resistant to proteolysis in the digestive tract of monogastric animals and humans. The resistance has been confirmed in vitro (Nielsen, Deshpande, Hermodson & Scott, 1988; Shutov, Kakhovskaya, Bastrygina, Bulmaga, Horstmann & Müntz, 1996), although there are contradictory results (Aubry & Boucrot 1986; Rubio, Grant, Caballe, Martinez-Aragon & Pusztai, 1994; Clemente, Vioque, Sanchez-Vioque, Pedroche, Bautista & Millan, 1999). The resistance or susceptibility to digestion depends on the structural characteristics of each protein. For example, a high percentage of β-sheet structures, typical for 11S and 7S fractions, may limit the access of proteolytic enzymes (Deshpande & Damodaran, 1989; Yu, 2005). Similarly, other constituents in the protein, including carbohydrates (glycoprotein), can also increase protein resistance to hydrolysis (Deshpande & Nielsen, 1987b; Genovese & Lajolo, 1996).

### *4.1 Phaseolin is resistant to digestion*

Raw phaseolin is highly resistant to in vitro hydrolysis (digestion from 10 to 27%) and in vivo digestion (digestibility values ranging from 28 to 36%) (Table 3; Levy-Benshimol & Garcia, 1986; Genovese & Lajolo, 1998; Montoya, Lallès, Beebe, Montagne, Souffrant & Leterme, 2006). The low degree of hydrolysis could be explained by: a compact and rigid structure (Desphande & Damodaran, 1989); a secondary structure rich in β-sheets (10% of α-helix, 50% β-sheet, 9% β-turns and 31% of random conformation) (Deshpande & Damodaran,

1989); glycosylation (Paaren et al., 1987) and the fact that phaseolin is not very hydrophilic, which limits the accessibility of proteases (Nielsen et al., 1988). The central region of raw phaseolin subunits (MW of 45, 48 and 52 kDa) is the most sensitive to protease attacks, thus generating large indigestible fragments with MW ranging from 22 to 33 kDa (Deshpande & Nielsen, 1987a; Jivotovskaya, Senyuk, Rotari, Horstmann & Vaintraub, 1996).

*4.2 Thermal treatment improves digestibility*

The structure of phaseolin changes during thermal treatment, resulting in an increase in the rate of hydrolysis in vitro (82%; Nielsen et al., 1988; Nielsen, 1991) and digestion in vivo (90%; Phillips, Eyre, Thompson & Boulter, 1981; Marquez & Lajolo, 1991; Montoya et al., 2006). Heat treatment does not cause a major change in the secondary structure of phaseolin but alters its tertiary and quaternary structures. The result is a 7- to 9-fold increase in hydrophilic surfaces (Deshpande & Damodaran, 1989), indicating a breakdown of the phaseolin subunit interactions, leading to a higher degree of hydrolysis (Nielsen, 1991). Denatured β-phaseolin subunits are more susceptible to trypsin hydrolysis than α-phaseolin subunits (+20% in the predicted cleavage sites). Some differences between β-phaseolin subunits have also been observed (Montoya, Lallès, Beebe, Souffrant, Molle & Leterme, 2009).

For comparison, in soybean β-conglycinin, only α-subunit polypeptides were recognized in ileal digesta of pigs consuming soybean (Fisher et al., 2007). In contrast, for the 11S fraction of various legume seeds, α-polypeptides were shown to be more susceptible to in vitro hydrolysis than β-polypeptides (Plumb & Lambert, 1990; Perrot, Quillien & Guéguen, 1999). Differences in thermal stability, surface hydrophobicity, solubility and heat-induced association of individual α, α' and β subunits of β-conglycinin were observed (Maruyama et al., 1999; 2002). Also, the same

research group, screening wild soybean lines, found variations in AA sequences on the same subunit that affected electrophoretic migration and thermal stability in β-conglycinin and glycinin (Fukuda et al., 2005). In spite of high sequence homologies between β-conglycinin subunits, differences in antibody immune-reactivity were observed due to differences in the AA sequence of the recognised epitopes (Fu, Jez, Kerley, Allee & Krishnan, 2007). Slight differences in the structure of a monomer can cause changes in quaternary structure (Banerjee, Das, Ravishankar, Suguna, Surolia, Vijayan, 1996) and thus susceptibility to hydrolysis.

Heating seems to have variable effects on the different phaseolin types. Comparing the degree of hydrolysis (DH) of 43 different phaseolin types by means of an in vitro technique, Montoya et al. (2008c) found that DH range from 57 to 96%, depending on the phaseolin type (Figure 2). Such variations in DH values can be ascribed, as mentioned previously, to differences in subunit composition (Figure 1), subunit precursor origin (α or β) and trypsin susceptibility between phaseolin subunits (Montoya et al., 2008b; 2009). Montoya et al. (2009) hypothesize that the lowest DH values of S phaseolin, compared with the T and I phaseolins, could be explained by the high α-phaseolin content in the whole molecule (DH values of 50, 33 and 0% in S, T and I phaseolins, respectively).

*4.3 Other protein fractions*

Raw albumin and glutelin have also low DH values (26-32 and 42%, respectively) (Genovese & Lajolo, 1998). For albumin, it is due to a high number (e.g. n = 7 for the Bowman-Birk trypsin inhibitor) of disulphide bridges and the presence of carbohydrates (12% by weight; Genovese & Lajolo, 1996). After heat treatment, the resistance of albumin to proteolysis is maintained or slightly increased (DH 13-18%, Table 3) (Marquez & Lajolo, 1981; Moreno, Maldonado, Wellne

& Mills, 2005). For bean glutelin, heat treatment has virtually no effect (Genovese & Lajolo, 1998). For common bean legumin (11S fraction), only α-polypeptides may be partially degraded, while β polypeptides remain intact, even after heat treatment (Momma, 2006).

## 5. Improvement of the nutritional value of common bean

### *5.1 Treatments*

In general, common beans are consumed by humans after soaking and thermal treatment, reducing the concentration of tannins, phytic acid and soluble- and heat-labile anti-nutritional factors such as phytohemagglutinin, protease inhibitors and oligosaccharides. This improves palatability and the digestibility and availability of some nutrients (Barampama & Simard, 1994; Wu et al., 1996), although protein digestibility generally remains low (Oliveira & Sgarbieri, 1986; Marquez & Lajolo, 1991).

### *5.2 Transgenic plants*

In pulses, improving the sulphur-containing AA content has been a challenge for many research groups. Several studies have been conducted with soybeans to improve the lysine and tryptophan contents (Falco et al., 1995; Galili, Galili, Lewinsohn & Tadmor, 2002). In common beans, attempts have been made to improve methionine deficiency by introducing a transgene coding for a methionine-rich proteins (e.g. 2S albumin) from the Brazil nut (*Bertholletia excelsa* H.B.K., *Lecythidaceae*) (Aragao et al., 1999). The methionine content increased from 10 to 23% in the bean lines expressing this albumin. However, during seed maduration, the albumin was either not stored correctly in the cotyledon tissue and degraded prematurely or that the 2S mRNA is less stable in beans than in Brazil nut in some transgenic bean lines. Additionally, the 2S

albumin is characterized by high resistance to proteolytic hydrolysis, both in raw and heat-treated forms, as explained above. Therefore, an increase in the methionine content using the 2S albumin would not increase the methionine availability of common bean. Other attempts have been made to increase the methionine and tryptophan content of common beans by modifying the sequence of β-phaseolin subunits. However, such modified subunits were poorly expressed (only 0.2% present in beans) due to either degradation in the Golgi vesicles or in the formation of protein bodies (Hoffman, Donaldson & Herman, 1988; Nutall, Vitale & Frigerio, 2003).

*5.3 Changing the percentages of protein fractions*

Another strategy to increase the methionine content of common beans consists of modifying its protein fractions by decreasing the percentage of those with low contents of limiting AA (Gepts & Bliss, 1984). Various attempts have been made to change the amount of phaseolin (Gepts & Bliss, 1984), phytohemagglutinin (Osborn & Bliss, 1985) or both (Burow, Ludden & Bliss, 1993). However, seeds containing phaseolin still have higher available methionine levels, compared to seeds devoid of phaseolin. Despite a low methionine content (Table 1), phaseolin is still the major source of that AA. This is due to its high proportion (40 to 50%) in common bean, the little differences in methionine content (Gepts & Bliss, 1984) and to a higher DH value after thermal treatment, compared to other protein fractions.

Recently, Taylor et al. (2008) evaluated the overall AA composition of genetically related lines of common beans deficient in selected seed storage proteins (phaseolin, phytohemagglutinin and/or arcelin). They found several changes in the free AA content in bean lines deficient in storage proteins, including a reduction of S-methyl-cysteine and γ-glutamyl-S-methyl-cysteine (a non-protein AA that cannot substitute the requirements of methionine or

cysteine in the diet). In contrast, the sulphur-containing AA (especially cysteine) content increased by 40% in beans devoid of store protein (phaseolin, phytohemagglutinin and arcelin) as compared to beans with only high phytohemagglutinin and arcelin contents. However, further work is required to evaluate the potential nutrional value of bean lines deficient in storage proteins.

*5.4 Amino acid composition of phaseolin*

Differences in methionine content (7.5 to 10 mg/g protein) have been reported between phaseolin of different beans lines (Ma & Bliss, 1978; Chagas & Santoro, 1997). Montoya et al. (2008c) compared 18 purified phaseolins and found only slight differences in AA composition among phaseolins. Also, differences in methionine content have been evidenced between some of the phaseolin subunits (Kami & Gepts, 1994). However, those differences are not sufficient to increase the nutritional value of phaseolins, according to the AA score (Montoya et al., 2008c).

*5.5 Diversity in phaseolin digestibility*

Montoya et al. (2008c) explored the possibility of taking advantage of the wide diversity in phaseolin types by investigating their DH, since the DH value may reflect AA availability. Therefore, we compared the sequential hydrolysis (pepsin for 2h followed by pancreatin for 4h) of 43 phaseolin types (Montoya et al., 2008c). We found DH values ranging from 11 to 27% for uncooked phaseolins and from 57 to 96% for heat-treated phaseolins (Figure 2).

The protein digestibility-corrected AA score (PDCAAS) of these isolated phaseolin types was calculated. PDCAAS is the reference method for measuring protein quality in humans and is based on the comparison of the digestible content of each essential AA in a test protein with that

254 of the essential AA requirements of preschool-age children (2 to 5 years-old). The AA score is
255 corrected for the digestibility, determined by in vivo or in vitro methods (Nielsen, 1998;
256 Schaafsma, 2000). Based on PDCAAS, the S-containing AAs are the limiting AAs in phaseolins,
257 followed by threonine (Montoya et al. 2008c). The DH value of the heat-treated phaseolins
258 combined with PDCAAS values were then used to estimate the potential nutritional quality of
259 each phaseolin. The phaseolins with the highest DH value could provide 37% more of sulphur-
260 containing AA requirement than those with the lowest DH value (Figure 2). Moreover, only the
261 phaseolins with the highest DH values could provide the whole requirement of leucine, lysine,
262 aromatic AAs and threonine. In other words, the estimated nutritional value of heated phaseolins
263 was influenced more by their DH value than their AA composition (Montoya et al., 2008c).
264 The effect of the DH of phaseolin on its nutritional value cannot be extrapolated to the total
265 protein fraction of the whole seed, as the seed contains different protein fractions, anti-nutritional
266 factors and structural components (e.g. fibre) that could affect protein digestibility. Therefore,
267 one common bean line was selected to express either S, T or I phaseolins in the same genetic
268 background and the protein DH values of these selected beans were determined after thermal
269 treatment. The DH value of the total bean protein containing the I phaseolin was found to be
270 higher than the one for the bean containing the S phaseolin (Montoya, Gomez, Lallès, Souffrant,
271 Beebe & Leterme, 2008a). Interestingly, a similar ranking was observed for heat-treated S, T,
272 and I purified phaseolins (Figure 2). This result suggests that differences in the DH between bean
273 lines could be essentially explained by the susceptibility of different phaseolins to hydrolysis
274 (Montoya et al., 2008a).
275 The pattern of DH values of heated phaseolins (Figure 2) clearly showed that the S and T
276 phaseolins, present in more than 90% of cultivated beans, were among the ten phaseolins with

277 the lowest DH and lowest estimated nutritional value. Thus, if the phaseolin type influences the
278 DH value of total bean protein as previously presented, we could hypothesize that phaseolins
279 with the highest DH values would increase the nutritional value of common bean protein. In
280 order to demonstrate this, we calculated the possible effect of phaseolin type (with different DH
281 values) on the potential nutritional value of the total bean protein. The PDCAAS of each protein
282 fraction was combined with its percentage in total protein (Table 4). As an example, we observed
283 that the beans containing the To1 and J1 phaseolins (DH = 96%) provided 28% more sulphur-
284 containing AA than the bean containing the S phaseolin (DH = 58%) and 16% more than the
285 bean with the T phaseolin (DH = 71%). The requirements of histidine, isoleucine and aromatic
286 AAs for preschool children could only be met with the beans containing the To1 and J1
287 phaseolins, as compared to the beans with S phaseolin. Additionally, the phaseolins with the
288 highest DH values provided amounts of leucine, lysine, threonine and valine in excess of the
289 corresponding requirements for this child population.
290 Given this, estimates such as those presented above must be confirmed in vitro on beans with
291 similar composition characteristics but differing in their phaseolin type. Transferring a phaseolin
292 type from one cultivar to another can be made by plant breeders using backcrossing and it is
293 possible to obtain genetically-selected beans after only 2 or 3 generations (Montoya et al.,
294 2008a). This does not affect the balance in the different protein fractions or the viability of the
295 seed. Thus, it is likely that the use of highly-digestible phaseolin will generate highly-digestible
296 beans. Finally, the true nutritional value of those beans should always be assessed in vivo, since
297 high digestibility values of legume proteins do not necessarily guarantee a high nutritional
298 utilization of the proteins (Rubio & Seiquer, 2002).
299

In conclusion, exploiting the natural variability of phaseolin types with respect to their protein digestibility seems to be a promising strategy to improve the nutritional quality of bean protein. The phaseolins with the highest DH values could increase the bio-availability of sulphur-containing AAs and other essential AAs. Therefore, DH values of heat-treated phaseolins could be used as a criterion in breeding programs for improving the nutritional value of common bean.

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**Table 1.** Crude protein content and protein fractions in various pulses.

| Pulse | Crude protein (g/kg DM) | Protein fractions, % of total protein | | | | Reference |
|---|---|---|---|---|---|---|
| | | Albumin | Globulin | Prolamin | Glutelin | |
| *Canavalia ensiformis* | 240-280 | 30-36 | 52-60 | 2-4 | 7-8 | Seena et al. (2005); Gomez et al. (1993) |
| *Glycine max* | 300-500 | 10 | 85-95 | - | - | Adsule & Kadam (1989) |
| *Lupinus albus* | 310-350 | 10-20 | 80-90 | - | - | Babar et al. (1989) |
| *Phaseolus vulgaris* | 213-313 | 12-30 | 54-79 | 2-4 | 20-30 | Ma & Bliss (1978); Sathe et al. (1984) |
| *Pisum sativum* | 212-329 | 21 | 66 | - | 12 | Adsule & Kadam (1989); Kadam et al. (1989) |
| *Vicia faba* | 229-385 | 20 | 65 | - | 15 | Adsule & Kadam (1989); Kadam et al. (1989) |
| *Vigna unguiculata* | 209-346 | 45 | 51 | 1 | 3 | Kadam et al. (1989); Freitas et al. (2004) |

**Table 2**. Amino acid composition (mg/g protein) of the total and different protein fractions of the common bean.

| | | | Protein fraction | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Requirements[a] | Total | Phaseolin | 11S | Albumin | Glutelin | Prolamin | NNP[b] |
| Essential | | | | | | | | |
| Arginine | | 63 | 53 | 48 | 65 | 61 | 71 | 65 |
| Histidine | 18 | 30 | 32 | 30 | 35 | 35 | 24 | 31 |
| Isoleucine | 31 | 48 | 49 | 49 | 43 | 62 | 57 | 64 |
| Leucine | 63 | 95 | 83 | 87 | 66 | 114 | 101 | 109 |
| Lysine | 52 | 76 | 64 | 78 | 109 | 81 | 59 | 70 |
| Methionine | 26[c] | 12 | 9 | 15 | 10 | 20 | 16 | 12 |
| Phenylalanine | 46[d] | 65 | 31 | 36 | 40 | 54 | 74 | 97 |
| Threonine | 27 | 47 | 30 | 49 | 74 | 46 | 39 | 44 |
| Valine | 42 | 57 | 59 | 70 | 49 | 66 | 79 | 71 |
| Non-essential | | | | | | | | |
| Alanine | | 51 | 36 | 69 | 49 | 49 | 47 | 55 |
| Aspartic acid | | 120 | 152 | 95 | 142 | 112 | 95 | 102 |
| Cysteine | | 1 | 3 | 6 | 2 | | 1 | 1 |
| Glutamic acid | | 140 | 160 | 131 | 128 | 145 | 124 | 124 |
| Glycine | | 56 | 40 | 80 | 47 | 44 | 52 | 53 |
| Proline | | 38 | 35 | 51 | 50 | 40 | 91 | 38 |
| Serine | | 68 | 102 | 73 | 55 | 43 | 48 | 43 |
| Tyrosine | | 40 | 47 | 29 | 35 | 28 | 23 | 22 |
| References | | 1,2 | 3, 4 | 5, 6 | 6 | 6 | 6 | 6 |

[a] Suggested pattern of AA requirement for preschool children (aged 2 to 5 years) (FAO/WHO/UNU, 2007)
[b] NNP, N non protein
[c] Value includes Met + Cys
[d] Value includes Phe + Tyr

1 Marzo et al. (2002)
2 Montoya et al. (2008a)
3 Bhushan & Pant (1986)
4 Montoya et al. (2008c)
5 Derbyshire et al. (1976)
6 Ma & Bliss (1978)

**Table 3.** In vivo and in vitro protein digestibility values of raw and cooked phaseolins in rats.

| | Treatment | | |
|---|---|---|---|
| Site | Raw | Cooked | Reference |
| *In vivo* | | | |
| Fecal | | 96 | Phillips et al. (1981) |
| Fecal | 28 | 91 | Levy-Benshimol & Garcia (1986) |
| Fecal | | 90 | Marquez & Lajolo (1990) |
| Fecal | 33 | 91 | Montoya et al. (2006) |
| *In vitro* | | | |
| Pepsin-pancreatin | 10 | 82 | Marquez & Lajolo (1981) |
| Pepsin-pancreatin | 23 | 88 | Genovese & Lajolo (1998) |

**Table 4.** In vitro protein digestibility corrected amino acid score (PDCAAS) of common bean protein fractions and estimated PDCAAS for the total protein of beans, assuming that each seed has a different phaseolin type and according to its degree of hydrolysis (DH).

| | Total seed | Phaseolin | | | | 11S | Albumin | Glutelin | N-fraction | Total estimated | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **S** | **T** | **J1** | **To1** | | | | | **S** | **T** | **J1** | **To1** |
| DH (%) | 64 | **58** | **71** | **96** | **96** | 60 | 21 | 42 | 100 | | | | |
| Total protein (%) | | **45** | **45** | **45** | **45** | 8 | 20 | 20 | 5 | | | | |
| PDCAAS[a] (%) | | | | | | | | | | | | | |
| Histidine | 106 | **105** | **127** | **172** | **172** | 100 | 41 | 82 | 172 | **90** | **100** | **120** | **120** |
| Isoleucine | 98 | **93** | **113** | **153** | **153** | 95 | 29 | 8 | 206 | **85** | **94** | **112** | **112** |
| Leucine | 96 | **78** | **94** | **128** | **128** | 83 | 22 | 76 | 173 | **72** | **80** | **95** | **95** |
| Lysine | 93 | **73** | **88** | **119** | **119** | 90 | 44 | 65 | 135 | **70** | **77** | **91** | **91** |
| Met + Cys | 33 | **27** | **33** | **45** | **45** | 49 | 10 | 32 | 50 | **28** | **31** | **36** | **36** |
| Phe + Tyr | 145 | **100** | **122** | **164** | **164** | 85 | 34 | 75 | 258 | **89** | **99** | **118** | **118** |
| Threonine | 111 | **65** | **80** | **108** | **108** | 109 | 58 | 72 | 163 | **74** | **81** | **93** | **93** |
| Valine | 86 | **83** | **101** | **136** | **136** | 100 | 25 | 66 | 169 | **74** | **82** | **98** | **98** |

[a] PDCAAS = (AAx/ReqAAx) * DH. Where AAx the level of a X AA in the protein; Req AAx the requirements for children of 2 to 5 years-old- in X AA; and DH the degree of hydrolysis of the protein

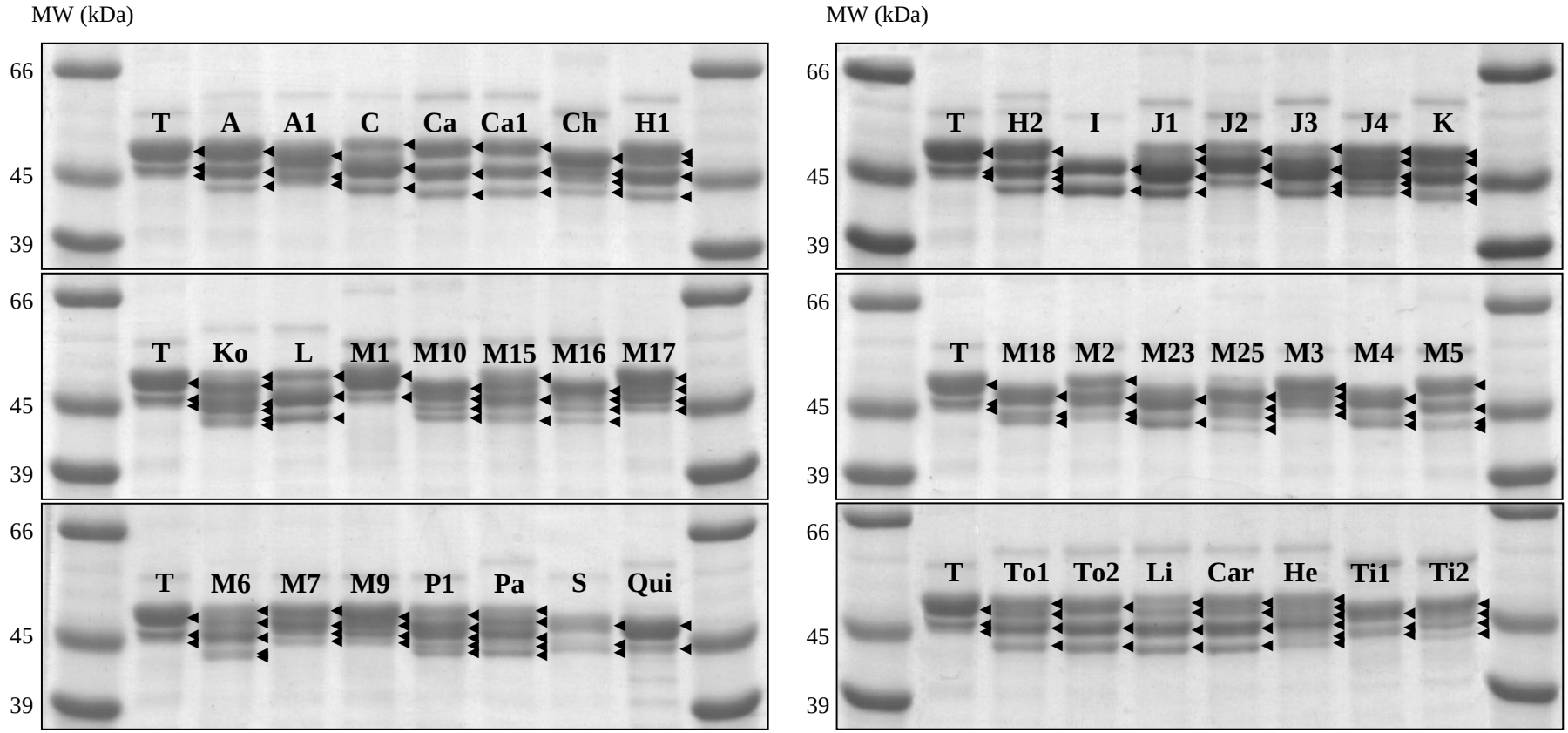


**Figure 1.** Electrophoretic subunit pattern of different phaseolin types determined using 1-D SDS-PAGE. Arrow heads indicate each subunit of a phaseolin type. Molecular weight markers (MW) are indicated on the left of the figure. Reproduced from Montoya et al., 2008c with the kind permission of JAFC (license number; 2175541338291).

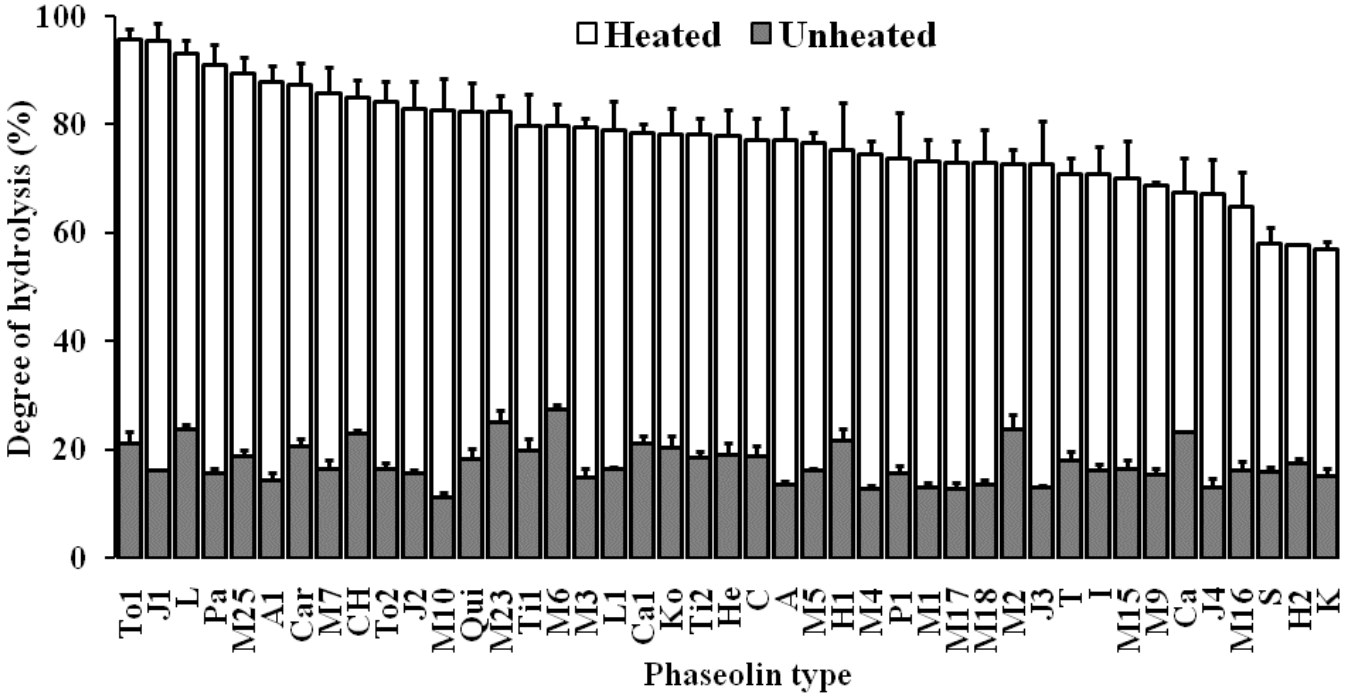


**Figure 2.** Degree of hydrolysis of different unheated and heated phaseolins after in vitro hydrolysis (120 min pepsin + 240 min pancreatin). Values are means of 3 measurements for each phaseolin. Reproduced from Montoya et al., 2008c with the kind permission of JAFC (license number; 2175541338291).