

Effect of fractionation and processing conditions on the digestibility of plant proteins as food ingredients

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Table S1. Summary of digestion assays, sample treatment and measurement from studies investigating the effect of processing on protein digestion.

| Crop | Digestion assay gastric | small intestinal | Digestion sample treatment/measurement | Effect | Reference |
|------------------------------|----------------------------|------------------------------|--|--|----------------------------------|
| Pre-fractionation treatments | | | | | |
| High temperature soaking | | | | | |
| soybean | 3 h | N/A | TCA-soluble nitrogen | h | Wally-Vallim, <i>et al.</i> [1] |
| cowpea | 2 h | 2 h pancreatin | supernatant, ultrafiltered protein cutoff 3 kDa TCA-soluble nitrogen defined as DH | h | Marques, <i>et al.</i> [2] |
| Germination | | | | | |
| soybean | 2 h | 24 h trypsin | TCA-soluble nitrogen | h | Dikshit and Ghadle [3] |
| black bean | 1 h | 2 h pancreatin | supernatant DH by ninhydrin reaction Samples digested for 24 h considered as 100% DH | h | López-Barrios, <i>et al.</i> [4] |
| soybean | N/A | trypsin (time not specified) | DH by OPA reaction | hi | Aijie, <i>et al.</i> [5] |
| black soybean | 30 min | 30 min pancreatin | supernatant: molecular weight distribution | lower proportion of <0.15 kDa peptides in germinated samples | Sefatie, <i>et al.</i> [6] |
| Fermentation | | | | | |
| lupin | N/A | 10 min trypsin, α - | pH drop | h | Bartkiene, <i>et al.</i> [7] |

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| chymotrypsin and serine-type protease | | | | | |
| Conventional protein fractionation | | | | | |
| soybean | <i>in vivo</i> rat assay | | PDCAAS | = | Hughes, <i>et al.</i> [8] |
| soybean | <i>in vivo</i> pig assay | | Standard ileal digestibility | = | Pedersen, <i>et al.</i> [9] |
| soybean | 3 h | N/A | TCA-soluble nitrogen | = | Da Silva Pinto, <i>et al.</i> [10] |
| Alkaline treatment | | | | | |
| rice | 2 h | 2 h trypsin and <i>in vivo</i> rat assay | TCA-soluble nitrogen and absorption | i | Zhang, <i>et al.</i> [11] |
| lupin | N/A | up to 28 h trypsin and chymotrypsin | TCA-soluble nitrogen | i | Yu, <i>et al.</i> [12] |
| quinoa | 6 h | N/A | DH by OPA reaction Size-exclusion chromatography | i | Ruiz, <i>et al.</i> [13] |
| Alternative protein fractionation | | | | | |
| 'alternative' chemicals | | | | | |
| soybean | 2 h | 24 h pancreatin | TCA-soluble nitrogen | = | Chamba, <i>et al.</i> [14] |
| lupin | N/A | 10 min trypsin, chymotrypsin and peptidase | pH drop | h | Lqari, <i>et al.</i> [15] |
| chickpea | N/A | 10 min trypsin, α -chymotrypsin and peptidase | pH drop | = | Sánchez-Vioque, <i>et al.</i> [16] |
| brown lentil | 1 h | 24 h pancreatin | Free amino groups by TNBS reaction | = | Joehnke, <i>et al.</i> [17] |
| alternative drying methods | | | | | |
| hempseed | N/A | 10 min trypsin, α -chymotrypsin and protease | pH drop | freeze>oven>vacuum oven drying | Lin, <i>et al.</i> [18] |
| buckwheat | 2 h | 2 h trypsin | TCA-soluble nitrogen release and SDS-PAGE | freeze>spray drying | Tang [19] |
| enzyme assisted fractionation | | | | | |
| soybean | (not specified) | N/A | nitrogen solubility | = | De Almeida, <i>et al.</i> [20] |
| white sorghum | 2 h | N/A | nitrogen solubility | = | De Mesa-Stonestreet, <i>et al.</i> [21] |
| air classification | | | | | |

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| pea, fava bean and lentil | | <i>in vivo</i> mouse assay | protein digestibility | i | Bhatty and Christison [22] |
| fava bean | 1 h | 24 h pancreatin | Free amino groups by TNBS reaction | i | Vogelsang-O'Dwyer, <i>et al.</i> [23] |
| quinoa | 6 h | N/A | DH by OPA reaction Size-exclusion chromatography | h | Opazo-Navarrete, <i>et al.</i> [24] |
| Post-fractionation processing | | | | | |
| Fermentation | | | | | |
| yellow pea | N/A | 10 min trypsin, α -chymotrypsin and protease | pH drop in vitro PDCAAS | h (lower IVPDCAAS) | Çabuk, <i>et al.</i> [25] |
| soybean | | soft rat gastrointestinal model with dynamic gastric and duodenal (pancreatin) parts | TCA-soluble free-amino acids | h | Huang, <i>et al.</i> [26] |
| Ultrasound | | | | | |
| fava bean | N/A | 10 min trypsin, α -chymotrypsin and peptidase | pH drop | i | Martínez-Velasco, <i>et al.</i> [27] |
| Proteolysis | | | | | |
| soybean | 30 min | 30 min chymotrypsin (non-sequential) | TCA-soluble nitrogen | h | Ge and Zhang [28] |
| soybean | N/A | 2 h trypsin and α -chymotrypsin | pH drop and free amino groups by ninhydrin reaction | = | Nguyen, <i>et al.</i> [29] |
| soybean and zein | 30 min | 2 h pancreatin | Tricine-SDS PAGE | = soy, h zein | Kaur, <i>et al.</i> [30] |
| soybean, wheat gluten and zein | | <i>in vivo</i> rat assay | true ileal digestibility | = soy, wheat gluten, h zein | Rutherford, <i>et al.</i> [31] |
| soybean | | <i>in vivo</i> rat assay | true gastric total protein digestion | h | Montoya, <i>et al.</i> [32] |
| green lentil | N/A | 10 min trypsin, α -chymotrypsin and peptidase | pH drop and size-exclusion chromatography | i | Aryee and Boye [33] |
| rapeseed | | <i>in vivo</i> rat assay <i>in vivo</i> human assay | true digestibility PDCAAS | h | Fleddermann, <i>et al.</i> [34] |
| rice bran | 2 h | 2 h pancreatin 10 min trypsin, α -chymotrypsin and peptidase | TCA-soluble nitrogen | h | Singh, <i>et al.</i> [35] |
| chickpea | N/A | | pH drop | = | Clemente, <i>et al.</i> [36] |

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| chickpea | N/A | 11 min trypsin, α -chymotrypsin and protease | pH drop | = | Goertzen, <i>et al.</i> [37] |
| Heat treatment | | | | | |
| soybean | 1 h | N/A | DH by OPA reaction | hi | Tian, <i>et al.</i> [38] |
| soybean | 1 h | 3 h pancreatin | TCA-soluble nitrogen and SDS PAGE | h | Chen, <i>et al.</i> [39] |
| soybean | 1 h | 2 h trypsin | DH by OPA reaction | h | Ren, <i>et al.</i> [40] |
| soybean | | Infogest 1.0 | TCA-soluble nitrogen | h | Zhang, <i>et al.</i> [41] |
| soybean | | <i>in vivo</i> rat assay | Apparent digestibility from faeces | i | Kim and Barbeau [42] |
| lupin | N/A | 10 min trypsin, α -chymotrypsin and peptidase | pH drop | h | Sathe, <i>et al.</i> [43] |
| winged bean | N/A | 10 min trypsin, α -chymotrypsin and peptidase | pH drop | h | Sathe, <i>et al.</i> [44] |
| red bean, red kidney bean and mung bean | 2 h | 2 h trypsin | TCA-soluble nitrogen | i red bean, = red kidney bean hi mung bean | Tang, <i>et al.</i> [45] |
| soybean and pea | 2 h | N/A | DH by OPA reaction and size-exclusion chromatography | = | Rivera del Rio, <i>et al.</i> [46] |
| quinoa | 6 h | N/A | DH by OPA reaction and size-exclusion chromatography | i | Opazo-Navarrete, <i>et al.</i> [47] |
| lupin | 30 min | N/A | <3 kDa peptides from size-exclusion chromatography | = i | Pelgrom, <i>et al.</i> [48] |
| navy bean | N/A | 24 h trypsin, α -chymotrypsin and peptidase | DH by TNBS reaction | h | Sathe, <i>et al.</i> [49] |
| sweet potato | | <i>in vivo</i> rat assay | PDCAAS | h autoclaved> microwaved> dry heated>native | Sun, <i>et al.</i> [50] |
| lentil | N/A | 2 h trypsin and chymotrypsin 2 h trypsin and chymotrypsin | DH by TNBS reaction | h | Neves and Lourenço [51] |
| chickpea | 2 h | or 10 min trypsin, chymotrypsin and peptidase | DH by TNBS reaction and pH drop | h | Tavano and Neves [52] |
| white common bean | | <i>in vivo</i> rat assay | absorption or protein intestinal digestibility | i | Carbonaro, <i>et al.</i> [53] |

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| and fava bean | | | | | |
| navy bean | | not specified | " <i>in vitro</i> protein digestibility" | hi | Chang and Satterlee [54] |
| soybean | N/A | 10 min trypsin, α -chymotrypsin and peptidase | pH drop | i | Wu, <i>et al.</i> [55] |
| soybean and rapeseed | 30 min | pancreatin and diffusion through a 1000 MWCO membrane | nitrogen and amino acid release in dialysates | i | Savoie, <i>et al.</i> [56] |
| soybean | | <i>in vivo</i> rat assay | true protein digestibility | i | Sarwar, <i>et al.</i> [57] |
| High pressure processing | | | | | |
| yellow pea | | Infogest 1.0 | SDS PAGE and pH stat | h | Laguna, <i>et al.</i> [58] |
| Modifications | | | | | |
| Transglutaminase-catalysed polymerization | | | | | |
| red kidney bean | N/A | 2 h trypsin | TCA-soluble nitrogen | i | Yin, <i>et al.</i> [59] |
| <i>Phaseolus vulgaris</i> L. bean | 1 h | 1 h trypsin (non-sequential) | SDS PAGE | i | Mariniello, <i>et al.</i> [60] |
| soybean | 2 h | 2 h trypsin | TCA-soluble nitrogen | i | Tang, <i>et al.</i> [61] |
| red kidney bean | N/A | 2 h trypsin | TCA-soluble nitrogen | h | Tang, <i>et al.</i> [62] |
| soybean | 1 h | 3 h pancreatin | SDS PAGE | i | Li and Damodaran [63] |
| soybean | 2 h or 1 h for sequential digestion | 1 h trypsin | TCA-soluble nitrogen | i | Sheng and Zhao [64] |
| soybean | time not specified | trypsin (time not specified) | TCA-soluble nitrogen | h | Fu and Zhao [65] |
| soybean | 2 h or 1 h for sequential digestion | 1 h trypsin | TCA-soluble nitrogen | h | Zhu, <i>et al.</i> [66] |
| soybean | N/A | 10 min trypsin, α -chymotrypsin and peptidase | pH drop | i | Gan, <i>et al.</i> [67] |
| Acylation | | | | | |
| red kidney bean | N/A | 2 h trypsin | TCA-soluble nitrogen | h | Yin, <i>et al.</i> [68] |
| mung bean | N/A | trypsin and pancreatin | pH stat | h | El-Adawy [69] |

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| soybean | N/A | (time not specified) 10 min trypsin, α -chymotrypsin and peptidase | pH drop | h | Achouri and Zhang [70] |
| soybean | <i>in vivo</i> rat assay | | protein efficiency ratio | h = | de Regil and Calderón de la Barca [71] |
| Complexation with phenolic compounds | | | | | |
| soybean | 2 h | 24 h trypsin and chymotrypsin | size exclusion chromatography | = | Budryn, <i>et al.</i> [72] |
| soybean | time not specified | trypsin and chymotrypsin (time not specified) | TCA-soluble nitrogen | i | Rohn, <i>et al.</i> [73] |
| | <i>in vivo</i> rat assay | | nitrogen efficiency true nitrogen digestibility PDCAAS | | |
| yellow pea | 3 h | 4 h pancreatin | Free amino groups by TNBS reaction | i | Strauch and Lila [74] |
| soybean | 1 h | 2 h trypsin | SDS PAGE | i | Yang, <i>et al.</i> [75] |
| Other modifications | | | | | |
| soybean | 1 h | 2 h pancreatin | SDS PAGE and free amino acids | i | Chen, <i>et al.</i> [76] |
| soybean, cottonseed and peanut | N/A | 10 min trypsin, α -chymotrypsin and peptidase | pH drop | i | Rhee and Rhee [77] |
| soybean | 2 h | 2 h pancreatin | TCA-soluble nitrogen | h | Wang, <i>et al.</i> [78] |
| Protein oxidation | | | | | |
| soybean | Infogest 1.0 | N/A | DH by OPA reaction | hi | Zhao, <i>et al.</i> [79] |
| soybean | 1 h | 2 h pancreatin | SDS PAGE | i | Chen, <i>et al.</i> [80] |
| soybean | 3 h | N/A | DH by OPA reaction | = | Duque-Estrada, <i>et al.</i> [81] |
| Structure formation | | | | | |
| Gelling | | | | | |
| soybean and yellow pea | 3 h | N/A | DH by OPA reaction and size-exclusion chromatography | i soybean = yellow pea | Opazo-Navarrete, <i>et al.</i> [82] |
| lentil and fava bean | | Infogest 1.0 | ultrafiltration through 50 kDa, 30 kDa, and 10 kDa Mw cut-offs SDS PAGE | = | Hall and Moraru [83] |
| soybean | 1 h | 2 h pancreatin | pH stat | h | Rui, <i>et al.</i> [84] |
| soybean | 2 h | N/A | TCA-soluble nitrogen | i | Ou, <i>et al.</i> [85] |
| Extrusion and texturization | | | | | |

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| soybean | N/A | 10 min trypsin, α -chymotrypsin and peptidase | pH drop | h | Omosebi, <i>et al.</i> [86] |
| yellow pea | N/A | 10 min trypsin, α -chymotrypsin and peptidase | pH drop | h | Wang, <i>et al.</i> [87] |
| rice | 1 h | 2 h pancreatin | fluorescence spectral analysis | i | Li, <i>et al.</i> [88] |
| Intragastic gelling | | | | | |
| soybean | 2 h | N/A | SDS PAGE of aqueous phase | (| Hu, <i>et al.</i> [89] |
| soybean | Infogest 1.0 | N/A | SDS PAGE | (| Huang, <i>et al.</i> [90] |
| Animal-plant hybrid foods | | | | | |
| soybean, rice and yellow pea | 1 h | 4 h trypsin and chymotrypsin | TCA-soluble nitrogen, amine content by ninhydrin reaction, in vitro PDCAAS | h | Khalesi and Fitzgerald [91] |
| yellow pea, rice and lentil | | Infogest 2.0 | TCA-soluble free amino acids | i | Baugreet, <i>et al.</i> [92] |
| Nutrient interactions | | | | | |
| yellow pea | 2 h | 2 h pancreatin | SDS PAGE | i | Oñate Narciso and Brennan [93] |
| chickpea | N/A | 10 min trypsin, α -chymotrypsin and peptidase | pH drop | i | Sánchez-Vioque, <i>et al.</i> [94] |

DH, degree of hydrolysis; N/A, not applicable; OPA, o-phthalaldehyde; PDCAAS, protein digestibility-corrected amino acid score; SDS PAGE, sodium dodecyl sulphate–polyacrylamide gel electrophoresis; TCA, trichloroacetic acid; TNBS, trinitrobenzenesulfonic acid. The pH drop method normally refers to the method by Hsu, *et al.* [95].

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