Food Chemistry 194 (2016) 377–382

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

The impact of heating and soaking on the *in vitro* enzymatic hydrolysis of protein varies in different species of tropical legumes

Julieta Torres [a], Shane M. Rutherfurd [b], Luz S. Muñoz [a], Michael Peters [c], Carlos A. Montoya [a,b,*]

[a] Universidad Nacional de Colombia, Dept. de Produccion Animal, Carrera 32 Chapinero, Palmira, Colombia
[b] Riddet Institute, Massey Institute of Food Science and Technology (Massey University), Private Bag 11, 222, Palmerston North 4442, New Zealand
[c] Centro Internacional de Agricultura Tropical, AA 6713 Cali, Colombia

ARTICLE INFO

Article history:
Received 2 June 2015
Received in revised form 6 August 2015
Accepted 7 August 2015
Available online 8 August 2015

Keywords:
Tropical legumes
In vitro hydrolysis
Protein, Soaking treatment
Heating treatment

ABSTRACT

The effects of different thermal (raw, autoclaving or boiling for 5 and 20 min) and soaking (with or without) treatments on the degree of hydrolysis (DH) of protein were investigated for selected legumes (*Canavalia brasiliensis*; *Lablab purpureus*; pink, red and white colour hulls *Vigna unguiculata*). Each legume preparation underwent *in vitro* simulated gastrointestinal tract digestion comprising either pepsin (120 min) or pepsin/pancreatin (120/240 min) digestion. The DH was determined based on the amount of free amino groups released. Autoclaving for 5 min increased the pepsin/pancreatin DH for all the unsoaked and soaked legumes (+20% to 46% units) except *Canavalia*, while boiling for 5 min only increased DH for two soaked legumes (+12% to 28% units). Extending boiling from 5 to 20 min increased the DH for three soaked legumes (+5% to 29% units). In conclusion, autoclaving, in general, extensively increased the sequential pepsin/pancreatin DH, while boiling only increased it for selected legumes.

1. Introduction

Legumes are important dietary sources of protein, energy, dietary fibre and minerals for both humans and animals, particularly in the tropics (Sandberg, 2002; Tharanathan & Mahadevamma, 2003). Legumes generally have a high protein content (21–29%) but tend to contain lower amounts of tryptophan and sulphur-containing amino acids. Moreover, legumes tend to be poorly digested in their raw form (Genovese & Lajolo, 1996; Torres, Muñoz, Peters, & Montoya, 2013), largely due to the presence of anti-nutritional factors (Duranti & Gius, 1997; Mekbungwan, 2007). Processing, such as heating, soaking, dehulling or germination, can reduce the presence of, or inactivate, some anti-nutritional factors such as trypsin inhibitors or polyphenols (Laurena Van Den & Mendoza, 1984; Rehman & Shah, 2004) and are therefore commonly used to improve the nutritional value and palatability of legumes (Hardy, Parmentier, & Fanni, 1999; Tharanathan & Mahadevamma, 2003). Several *in vitro* and *in vivo* studies, focussing on different species and varieties of legumes (e.g., common bean (*Phaseolus vulgaris*), lentils (*Lens culinaris*), chickpeas (*Cicer arietinum*), cowpea (*Vigna unguiculata*)) have shown that the digestibility of protein can be improved using a range of processing methods, including boiling, autoclaving, microwave cooking and germination (El-Adawy, 2002; Rehman & Shah, 2005). Processing can also modify the structure of proteins (Deshpande & Nielsen, 1987; Tang, Chen, & Ma, 2009), and/or modify the matrix of the legumes (Enwere & Ngoddy, 1986; Guillon & Champ, 2002) which can also affect protein digestibility. However, the relationship between processing methods and the digestibility of legumes is not easily described and the impact of processing on digestibility can vary not only across different legume species but also within the same legume species. For example, autoclaving at 121 °C for 30 min increased *in vitro* protein digestibility of one variety of the common bean (IAC-Aruã; digestibility = 73%), but not another variety (IAPAR; digestibility = 61%) when compared to their raw counterparts (60% and 55%, respectively) (Paiva, Carvalho, & Pizauro, 2011). Moreover, there are a wide variety of parameters (e.g., temperature, exposure time, particle size, moisture content) that can impact the processing effect on digestibility, but predicting how combinations of processing parameters affect the digestibility of legumes is not straightforward.

The aim of this study was to extend the work of Torres et al. (2013) by exploring the effects of different combinations of heating

Abbreviations: CB, *Canavalia brasiliensis*; DH, degree of hydrolysis; DM, dry matter; LP, *Lablab purpureus*; PVU, RVU and WVU, pink, red and white *Vigna unguiculata*.

* Corresponding author at: Riddet Institute, Massey Institute of Food Science and Technology (Massey University), Palmerston North, New Zealand.
E-mail address: c.montoya@massey.ac.nz (C.A. Montoya).

http://dx.doi.org/10.1016/j.foodchem.2015.08.022
0308-8146/

(raw, autoclaving and boiling for 5 and 20 min) and soaking (unsoaked or soaked) treatments on the *in vitro* pepsin (120 min) and sequential pepsin/pancreatin (120/240 min) digestion of selected species and varieties of legumes. The legumes examined in this study were canavalia (*Canavalia brasiliensis*, CB), lablab (*Lablab purpureus*, LP) and 3 varieties of cowpea (*V. unguiculata*, VU; pink PVU, red RVU and white WVU colour hulls). These varieties were selected based on their adaptability to soils with low pH and fertility (Cook et al., 2005). Both the heating and soaking treatments examined in the present report were chosen as they have been shown to improve the degree of protein hydrolysis (DH) for some (e.g., common bean, lentils and chickpeas) types of legumes (Alajaji & El-Adawy, 2006; Rehman & Shah, 2005; Shimelis & Rakshit, 2007).

2. Material and methods

2.1. Sample preparation

Each legume type examined in this study was produced exclusively for the present study at the International Centre for Tropical Agriculture (Cali, Colombia). To determine the effect of heat treatment, a set of unsoaked dry legumes was either unheated (raw), boiled at 96 °C for 5 min (unsoaked B5) or autoclaved at 121 °C under a pressure of 15 psi for 5 min (unsoaked A5). Both boiled and autoclaved legumes were prepared in distilled water (1:3, w:v). To determine the effects of soaking and heating treatments, each legume type was also soaked overnight in distilled water (1:3, w:v) at room temperature, water was then removed and legumes were washed three times with distilled water. The soaked legumes underwent either no further treatment (soaked raw) or were cooked as described above (soaked B5 and soaked A5). To determine the effect of extended heating time, soaked legumes were either boiled or autoclaved as described above for 20 min (soaked B20; soaked A20).

Excess water was removed from the legumes after cooking and the soaked and/or cooked legumes were then dried in a forced-air oven at 60 °C until they reached a constant weight. Finally, all legume preparations were ground through a 0.5 mm-mesh (model 2, Arthur H. Thomas Co., PA, USA) and then analysed in duplicate for dry matter (DM) (105 °C for 24 h) and nitrogen (Kjeldahl method) as described elsewhere (Torres et al., 2013).

2.2. In vitro enzymatic hydrolysis of protein

Porcine pepsin (Merck No. 107197) and pancreatin (a mixture of pancreatic enzymes, Sigma No. P1750) were used for the *in vitro* protein hydrolysis of the legumes as described previously (Montoya et al., 2008; Torres et al., 2013). Briefly, legume samples (containing 24 mg of nitrogen) were incubated at 39 °C in a water-bath with continuous stirring in 0.1 M HCl (pH 2.0) with pepsin (1:67, pepsin:protein) for 120 min. Phosphate buffer saline (0.2 M, pH 6.8) containing pancreatin (1:30, pancreatin:protein) was then added to the incubation medium and the mixture incubated for a further 240 min for a total incubation time for pepsin plus pancreatic of 360 min. Aliquots (1 ml) were taken at 0, 120 and 360 min of digestion, added to Eppendorf tubes containing trichloroacetic acid (TCA) 7.5% (w:v, final concentration) and centrifuged at 20,800*g* for 10 min. The concentration of free amino groups (NH_2) in the resulting supernatants and the total content of NH_2 groups in the legumes themselves (after acid hydrolysis in 6 M HCl for 24 h at 100 °C) were then determined using the *o*-phthaldialdehyde method (Church, Swaisgood, Porter, & Catignani, 1983; Montoya et al., 2008; Torres et al., 2013). Four replicate digests were conducted for each legume preparation and each treatment.

2.3. Calculations and statistical analysis

The degree of protein hydrolysis (DH) was calculated according to the following equation:

$$DH\ (\%) = \left(\left[NH_{2(Tt)}\right] - \left[NH_{2(T0)}\right]\right)/\left(\left[NH_{2(Total)}\right] - \left[NH_{2(T0)}\right]\right) \times 100$$

where $NH_{2(Tt)}$ was the free NH_2 at time *t*, $NH_{2(T0)}$ was the free NH_2 at time 0 and $NH_{2(Total)}$ was the total free NH_2 in the sample.

The statistical analyses were performed using the Mixed Model procedure of SAS (SAS/STAT Version 9.3, SAS Institute Inc., Cary, NC, USA). The response variables examined were the free NH_2 before digestion, after simulated gastric digestion (i.e., pepsin hydrolysis for 120 min) and after simulated gastric plus small intestinal digestion (i.e., 120 min pepsin + 240 min pancreatin digestion). A completely randomised factorial treatment arrangement ($5 \times 3 \times 2$) (Steel & Torrie, 1980) was performed to examine the effect of legume type (CB, LP, PVU, RVU and WVU), heat treatment (raw, B5 and A5), soaking treatment (with or without), and their interactions on the response variables described above. In addition, to examine the effect of extended cooking time (i.e., soaked B5 vs. soaked B20 and soaked A5 vs. soaked A20) on the response variables within each legume type, a completely randomised design was conducted to compare all the treatments (i.e., raw, B5, B20, A5, A20 for soaked and raw, B5 and A5 for unsoaked legumes).

The model diagnostics (e.g., normal distribution, equal variance across treatments) of each response variable were tested combining the PROC UNIVARIATE and the ODS GRAPHICS options of SAS. When the *F*-value of the analysis of variance was significant ($P < 0.05$), the means were compared using the adjusted Tukey test. Finally, correlation analysis was carried out between gastric hydrolysis and both small intestinal and gastrointestinal hydrolyses ($n = 40$) using the PROC CORR of SAS.

3. Results

3.1. Nitrogen and free NH_2 group contents

Unsoaked raw CB had a higher nitrogen content than the other unsoaked raw legumes (47 vs. 33–38 g/kg DM respectively) (Table 1). In general, unsoaked and soaked B5 and unsoaked A5 legumes had a similar nitrogen content to that of their unsoaked raw counterparts. In contrast, the nitrogen content of the soaked

Table 1
Nitrogen content (g/kg DM) in selected types of legumes after being heat treated (either autoclaving or boiling) and/or soaked.

Legume[2]	Soaking	Heating treatment[1]		
		Raw	B5	A5
CB	Unsoaked	46.6	46.4	46.7
	Soaked	43.4	45.8	31.5
LP	Unsoaked	37.6	37.6	38.2
	Soaked	34.8	37.0	29.1
PVU	Unsoaked	33.9	34.2	34.6
	Soaked	32.5	31.2	29.8
RVU	Unsoaked	34.6	35.0	35.8
	Soaked	32.0	31.4	28.5
WVU	Unsoaked	33.3	35.0	34.7
	Soaked	30.4	29.8	28.8

Values are means of duplicate determinations.
[1] B5, legumes were boiled at 96 °C for 5 min; A5, legumes were autoclaved at 121 °C for 5 min.
[2] CB, *Canavalia brasiliensis*; LP, *Lablab purpureus*; PVU, RVU and WVU, *Vigna unguiculata* with pink, red and white coat, respectively.