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Effect of processing on the *in vitro* and *in vivo* protein quality of red and green lentils (*Lens culinaris*)

Matthew G. Nosworthy[a], Gerardo Medina[a], Adam J. Franczyk[a], Jason Neufeld[a], Paulyn Appah[b], Alphonsus Utioh[b], Peter Frohlich[c], James D. House[a,d,e,f,g,*]

[a] Department of Food and Human Nutritional Sciences, University of Manitoba, Winnipeg, MB R3 T 2N2, Canada
[b] Food Development Centre, Portage la Prairie, MB R1 N 3J9, Canada
[c] Canadian International Grains Institute, Winnipeg, MB R3 C 3G7, Canada
[d] Richardson Centre for Functional Foods and Nutraceuticals, University of Manitoba, Winnipeg, MB R3 T 2N2, Canada
[e] Department of Food Science, University of Manitoba, Winnipeg, MB R3 T 2N2, Canada
[f] Canadian Centre for Agri-Food Research in Health and Medicine, University of Manitoba, Winnipeg, MB R3 T 2N2, Canada
[g] Department of Animal Science, University of Manitoba, Winnipeg, MB R3 T 2N2, Canada

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ABSTRACT

In order to determine the effect of extrusion, baking and cooking on the protein quality of red and green lentils, a rodent bioassay was conducted and compared to an *in vitro* method of protein quality determination. On average, the Protein Digestibility-Corrected Amino Acid Score of red lentils (55.0) was higher than that of green lentils (50.8). Extruded lentil flour had higher scores (63.01 red, 57.09 green) than either cooked (57.40 red, 52.92 green) or baked (53.84 red, 47.14 green) flours. The average Digestible Indispensable Amino Acid Score of red lentils (0.54) was higher than green lentils (0.49). The Protein Efficiency Ratio of the extruded lentil flours (1.30 red, 1.34 green) was higher than that of the baked flour (0.98 red, 1.09 green). A correlation was found between *in vivo* and *in vitro* methods of determining protein digestibility (R^2 = 0.8934). This work could influence selection of processing method during product development.

1. Introduction

Lentils (*Lens culinaris)* are a pulse crop primarily produced in Canada and India (1.99 MT and 1.1 MT in 2014) (FAOSTAT, 2017). Consumed globally, this crop is considered to be rich in protein, fiber, carbohydrates, minerals and vitamins (Ermetice et al., 2006; Iqbal, Khalil, Ateeq, & Khan, 2006). Generally, consumers are demonstrating an increasing interest in plant-based sources of high quality protein. The factors which alter protein quality include protein content, amino acid composition and protein digestibility. The protein content of lentils has been documented to be 28.3%, significantly higher than that of cereals, however the range in lentil protein content has been shown to between 15.9% and 31.4% (Grusak, 2009). In contrast to cereal grains, lentils are rich in lysine but limiting in the sulfur amino acids methionine and cysteine (Sarwar & Peace, 1986). Similar to other pulse crops, lentils contain certain anti-nutritive factors, including trypsin inhibitors and tannins (Wang, Hatcher, Toews, & Gawalko, 2009). These anti-nutritive factors can alter protein bioavailability by inactivating key digestive enzymes (trypsin inhibitors) or complexing with dietary proteins to reduce their digestibility (tannins) (Adsule & Kadam, 1989; Chavan & Kadam, 1989). Processing of lentils provides an opportunity to increase protein digestibility and amino acid availability.

Boiling has been shown to increase the protein content of pulses (Candela, Astiasaran, & Belli, 1997; Wang, Hatcher, Tyler, Toews, & Gawalko, 2010), possibly due to the loss of carbohydrates during the boiling process (Verde, Frias, & Verde, 1992). With respect to lentils, some studies have found no difference in protein content between cooked and uncooked lentils (Candela et al., 1997; Hefnawy, 2011), while others have demonstrated an increase in the protein content after cooking (Wang et al., 2009). While there has been little work on *in vivo* protein digestibility, numerous studies have shown that cooking reduces the activity and concentration of anti-nutritive factors such as trypsin inhibitors, tannins, and phytic acid (Hefnawy, 2011; Sayeed & Njaa, 1985; Wang et al., 2009). As these compounds either inhibit the activity of digestive enzymes or sequester nutrients, thereby making them unavailable for digestion, any reduction in anti-nutritive

* Corresponding author at: Department of Food and Human Nutritional Sciences, University of Manitoba, Winnipeg, MB R3 T 2N2, Canada.
E-mail addresses: matthew.nosworthy@umanitoba.ca (M.G. Nosworthy), medinag@myumanitoba.ca (G. Medina), umfranc3@myumanitoba.ca (A.J. Franczyk), Jason.neufeld@umantioba.ca (J. Neufeld), Paulyn.Appah@gov.mb.ca (P. Appah), Alphonsus.Utioh@gov.mb.ca (A. Utioh), pfrohlich@cigi.ca (P. Frohlich), james.house@umanitoba.ca (J.D. House).

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factors would potentially increase dietary protein digestibility and thereby increase the bioavailability of the constituent amino acids.

Extrusion is a process by which ingredients are forced through a die of a particular shape and cut to a certain size by spinning blades after being exposed to expansion inducing temperatures. The effect of extrusion on the nutritional content and anti-nutritive factors has been investigated in beans (Al-Marzooqi & Wiseman, 2009; Arija et al., 1988; Batista, Prudencio, & Fernandes, 2010; Kelkar et al., 2012; Simons et al., 2015) and, to a lesser extent, peas (Alonso, Orúe, & Marzo, 1998; Frias et al., 2011; Roy, Boye, & Simpson, 2010). However, there has been little investigation on the impact of extrusion on the nutritional quality of lentils. Previous work has shown that extrusion reduced trypsin inhibitors by 99.54%, phytic acid by 99.30% and tannins by 98.83% increasing *in vitro* protein digestibility from 39.4% in raw lentil seed to 88.6% after extrusion, without altering protein content (Rathod & Annapure, 2016). Autoclaving is occasionally used to determine the impact of heat on protein quality rather than oven baking (del Cueto & Martinez, 1960; Marquardt, Campbell, Stothers, & Mckirdy, 1974; Srihara & Alexander, 1983; Umoren, Tewe, & Bokanga, 1997). Autoclaved lentils had lower concentrations and activities of trypsin inhibitors, tannins and phytic acid while protein content was not altered (Hefnawy, 2011).

The current study was undertaken to investigate whether processing (extrusion, cooking and baking) alters protein digestibility and/or the amino acid composition of red and green lentils. These two factors influence protein quality as measured by the Protein Digestibility Corrected Amino Acid Score (PDCAAS), currently used in the regulation of protein claims in the United States (FAO/WHO, 1991). The Digestible Indispensable Amino Acid Score (DIAAS), was calculated using true protein digestibility as currently recommended by the FAO/WHO (FAO/WHO, 2013). Additionally, an *in vitro* measurement of protein quality was determined in order to compare these values with those obtained via PDCAAS. As an additional measure of protein quality, the Protein Efficiency Ratio (PER), a bioassay used to assess the efficiency of weight gain in relation to protein consumption in rodents, was also determined due to the fact that it represents the approved method for assessing protein content claims in Canada (Health Canada., 1981).

2. Materials and methods

All procedures were approved by the University of Manitoba's Institutional Animal Care Committee, in accordance with guidelines established by the Canadian Council on Animal Care (CCAC, 2017).

2.1. Chemicals

All chemicals and reagents were purchased from Sigma (Oakville, ON, Canada).

2.2. Sample Procurement and preparation of extruded baked and cooked flours

Samples of red and green lentils were provided by SaskCan Pulse Trading (Regina, Saskatchewan), Thompsons Ltd. (Blenheim, Ontario) with an additional sample of green lentils provided by Diefenbaker Seed Processors (Elbow, Saskatchewan). Prior to processing samples of similar lentils from different suppliers were combined and thoroughly mixed. Milling of the combined samples was performed on a hammer mill (Jacobson 120-B hammer mill, Minneapolis, MN), with screen hole size of 0.050 inch (0.127 cm), round. The hammer mill and flour bin were vacuumed thoroughly after milling each sample. Extrudates were prepared using a Clextral Evolum® HT 25 twin screw extruder with a screw diameter of 25 mm L/D ratio of 40. The flours were extruded at 36 kg/hr with a moisture addition of 0.8 kg/hr. The screw speed was 650 rpm. The extrusion barrel temperatures were: 30–50 °C, 70–90 °C and 100–120 °C. After extrusion, samples were milled as described above.

The baking process was as follows; 4 kg each of red and green lentil flours were mixed for 4 min with 2 kg water, respectively. Specifically, the mixer (Hobart mixer, model D300DT) was set up with a dough hook attachment. After the water was incorporated, the dough was mixed at a set speed #1 for 1.5 min followed by speed #2 for 2.5 min. In the absence of suitable forming equipment, the dough was extruded into rod-like pieces (Biro, model 6642, attached with a 12.5 mm die, and two blades). Approximately 1.5–1.6 kg of the extruded pieces (≈12 mm diameter) were transferred to standard baking trays (18 × 26 × ½ inches) lined with parchment paper, and rested for approximately 30 min. A tray was placed in the preheated oven (Doyon® FC2-lll tunnel conveyor oven) at 380 °F, 380 °F and 330 °F to establish the bake time. The trays with the cut pieces were baked at set temperatures for 35 min. After the baked pieces cooled to room temperature, they were weighted to calculate loss during baking. The baked samples were milled on a hammer mill (Fitz mill – model #D comminutor VHP-506-55B), with screen hole size of 0.020 inch, round, with 24% opening. All samples were further screened through a 20 mesh screen on a sifter (Kason, Vibro Screen, K24 3 SS).

In the cooking process, lentils were soaked in tap water at a ratio of 1:4 (1.5 kg pulse:6 L water) for 16 h with the water being changed prior to cooking. The lentil/water mixture was brought to a boil and maintained until done, approximately 25–35 min. After cooking, green lentils were rinsed with 4:1 ratio of rinse water (2 × 3 L water aliquots) to halt cooking. The samples were drained, freeze dried and then milled on a hammer mill (Jacobson 120-B hammer mill, Minneapolis, MN), with screen hole size of 0.050 inch, round.

2.3. Analytical procedures

For all samples, percent crude protein (CP; N× 6.25) was determined through the use of a Dumas Nitrogen Analyzer (Dumatherm DT, Gerhardt Analytical Systems, Germany), percent dry matter (DM) and ash were determined according to standard procedures (AOAC, 1995). The percent crude fat was determined by extracting crude fat into hexane and by gravimetrics, while methionine and cysteine were determined using the AOAC Official Method 45.4.05 and other amino acids, excepting tryptophan were determined using AOAC Official Method 982.30 (AOAC, 1995). Tryptophan content was determined as previously described (Nosworthy, Franczyk et al., 2017)

2.4. Protein Digestibility-Corrected Amino Acid Score (PDCAAS)

A rat bioassay was used to determine the PDCAAS of the samples (FAO/WHO, 1991). Amino acid scores were determined according to FAO/WHO guidelines. True protein digestibility was determined using the AOAC Official Method 991.29 (AOAC, 1995), using casein as a reference standard, and correcting for endogenous protein losses using previously determined values. Male weanling laboratory rats (n = 70, 10 animals per treatment, 6 experimental diets and casein as a control; initial weight 70 g) were individually housed in suspended wire-bottomed cages, and treated as previously described with diets being formulated to contain 10% protein, supplied by the test sample (House, Neufeld, & Leson, 2010). True protein digestibility (TPD%) was calculated using the following equation:

$$\text{TPD\%} = ((\text{Nitrogen Intake} - (\text{Fecal Nitrogen Loss} - \text{Metabolic Nitrogen Loss}))/\text{Nitrogen Intake}) \times 100$$

The value for metabolic nitrogen loss was determined as the amount of fecal nitrogen produced by rats consuming a protein-free diet. The PDCAAS was calculated as the product of the amino acid score and TPD %.