Research Article

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Effect of heat treatment on protein quality of rapeseed protein isolate compared with non-heated rapeseed isolate, soy and whey protein isolates, and rice and pea protein concentrates

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Abstract

BACKGROUND: Rapeseed protein isolate is used in the food industry, and heating is often used during rapeseed processing. However, the digestible indispensable amino acid score (DIAAS) for heat-treated rapeseed protein isolate is unknown. The present study aimed to test the hypothesis that heating rapeseed protein isolate improves protein quality resulting in DIAAS that is greater than for pea and rice protein concentrates, and comparable to that of soy and whey protein isolates.

RESULTS: Standardized ileal digestibility (SID) of amino acids (AA), except leucine and methionine, was not different between heat-treated rapeseed protein isolate and soy protein isolate, but SID of most AA was greater ($P < 0.001$) for heat-treated rapeseed protein isolate than for brown rice protein concentrate, pea protein concentrate, rapeseed protein isolate and soy protein isolate, but not whey protein isolate. Non-heated rapeseed protein isolate had a reduced ($P < 0.001$) DIAAS for 6-month-old to 3-year-old children compared with soy protein isolate, but this was greater ($P < 0.001$) than for pea and brown rice protein concentrates. The DIAAS for heat-treated rapeseed protein isolate was greater ($P < 0.001$) than for non-heated rapeseed protein isolate for all age groups. Heat-treated rapeseed protein isolate and whey protein isolate had a DIAAS > 100 for individuals older than 3 years.

CONCLUSION: Rapeseed protein isolate had a DIAAS comparable to soy protein isolate, but heat-treated rapeseed protein isolate and whey protein isolate had DIAAS ≥ 100, qualifying these proteins as 'excellent'. Rice and pea protein concentrates had DIAAS < 75.

Keywords: digestible indispensable amino acid score; digestibility; plant-based proteins; processing

INTRODUCTION

The global protein ingredient market is projected to grow at a rate of 9.1% between the years 2020 and 2027 with protein concentrates [50–80% crude protein (CP)] and isolates (80–95% CP) from rice, pea, rapeseed, soy and whey providing a significant part of dietary protein supplements in food preparations including meat analogues, dairy alternatives and bakery products.[1-3] Protein concentrates are produced by processes such as dry fractionation from foods that yield protein-enriched ingredients with moderate carbohydrate and low fat concentrations.[4] Similarly, protein isolates are obtained through a wet extraction process in which the protein is solubilized in an aqueous environment, separated from the insoluble fraction, and precipitated or concentrated by ultrafiltration, ion-exchange or similar processes to obtain a highly pure protein with negligible amounts of carbohydrates and fat.[4]

The physical and chemical process (e.g. heating) used during the production of protein concentrates and isolates may negatively or positively affect amino acids (AA) concentration, digestibility and protein quality, depending on the method utilized.[5,6] Therefore, the effects of processing need to be evaluated to optimize protein utilization.[2,5-7] Plant proteins are also susceptible to Maillard reactions during processing and often contain anti-nutritional factors that negatively influence protein quality.[8,9]

To emphasize the importance of protein quality, the Food and Agriculture Organization of the United Nations (FAO) suggested the digestible indispensable amino acid score (DIAAS) methodology to assess protein quality of food proteins. The DIAAS values of protein are determined as a combination of protein concentration, AA concentration and AA digestibility.[7,9]

Rapeseed is the world's second most produced oilseed after soybean.[10,11] Cultivation of the crop is a result of human demand

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for the oil from certain varieties of rapeseed, also known as canola oil. Canola is a specific rapeseed variety with reduced levels of the anti-nutritional factors erucic acid and glucosinolates.[5] However, the co-product that is produced after oil extraction is a protein-rich meal that has a greater concentration of sulfur-containing AA compared with legumes and it contains much more lysine than cereal grains.[5,12-14] Rapeseed meal may also complement soy and pea protein or be used as an alternative to animal protein in the food industry to enhance protein quality of human diets because of its well-balanced AA profile,[5] which results in high metabolic utilization of AA.[15,16] However, to our knowledge, no DIAAS values have been reported for rapeseed protein isolate, and it is not known how this protein compares to protein concentrates or isolates from brown rice, pea, soy or whey, or how mild heat processing at 90 °C for 10 min influences protein quality in this protein. Therefore, the present study aimed to test the hypothesis that heat treatment of rapeseed protein isolate will increase digestibility of AA and result in a DIAAS (≥ 75) for rapeseed protein isolate and that DIAAS in heat treated rapeseed protein isolate is greater than that of pea and rice protein concentrate and comparable to soy protein or whey protein isolates.

MATERIALS AND METHODS

The protocol for the animal experiment was submitted to and approved by the Institutional Animal Care and Use Committee at the University of Illinois (Urbana-Champaign, IL, USA).

Materials

Six test ingredients were used in the experiment (Table 1) including whey protein isolate (Volactive®; Volac Int. Ltd, Royston, UK), soy protein isolate (Gushen Biological Technology Group Co., Ltd, Shandong, China), pea protein concentrate (Roquette Frères, Lestrem, France), brown rice protein concentrate (Oryzatein®; Axion Foods, Inc., Los Angeles, CA, USA) and two rapeseed protein isolates (i.e. non-heated and heated rapeseed protein isolate) (CanolaPRO®; DSM, Delft, The Netherlands).

Processing

Heat treatment of rapeseed protein isolate was performed by DSM (Delft, The Netherlands) and involved dissolving rapeseed protein isolate in osmosed water at 55 °C in a double-jacked tank with three axial impellers at 160 rpm. The product was left to hydrate overnight at 7 °C, mildly stirred at 80 rpm. The

Table 1. Analyzed nutrient composition of ingredients (as-fed basis)

Item (g kg^{-1})	Brown rice protein concentrate	Pea protein concentrate	Rapeseed protein isolate	Rapeseed protein isolate heat-treated	Soy protein isolate	Whey protein isolate
Dry matter	972.40	935.50	953.40	97.920	963.50	955.90
Crude protein	809.90	794.00	988.30	997.20	876.30	880.40
ΔH 40% solution (J g^{-1})†	–	–	5.70	–	–	2.50
Trypsin inhibitor activity	< 0.20	1.40	18.00	0.60	9.30	NM‡
Indispensable amino acids						
Arginine	61.10	67.30	63.00	63.30	65.00	17.20
Histidine	17.60	19.70	30.80	31.20	22.50	16.30
Isoleucine	36.00	40.40	37.90	38.00	43.40	68.30
Leucine	65.30	65.00	68.10	68.90	67.80	93.60
Lysine	21.90	60.30	59.10	59.90	54.60	88.10
Methionine	20.60	7.90	19.50	19.50	11.00	19.30
Phenylalanine	44.40	45.40	37.70	38.10	46.30	28.90
Threonine	26.30	27.50	32.60	33.10	31.20	69.70
Tryptophan	10.10	7.30	14.10	15.20	12.00	20.70
Valine	50.80	43.20	49.40	49.90	44.90	59.00
Total	354.10	384.00	412.20	417.10	398.70	481.10
Dispensable amino acids						
Alanine	43.90	33.80	40.60	41.10	37.00	46.40
Asparagine	67.10	89.40	55.10	55.10	95.40	105.60
Cysteine	17.60	8.00	34.30	33.30	10.10	23.90
Glutamic acid	136.10	128.80	220.90	222.50	159.70	167.80
Glycine	34.00	32.40	46.50	46.50	36.60	15.60
Proline	36.40	35.30	71.80	69.70	45.10	60.40
Serine	30.50	33.00	29.10	29.20	35.40	37.80
Tyrosine	41.10	30.20	20.10	20.10	32.90	26.80
Total	406.70	390.90	518.40	517.50	452.20	484.30
Total amino acids	760.80	774.90	930.60	934.60	850.90	965.40

† Differential scanning calorimetry by preparing 40% solutions/dispersions of protein isolate in water.
‡ NM, not measured.

temperature was increased to 90 °C and maintained for 10 min, then decreased to 60 °C and the mixture was treated in an in-line high shear mixer (ULTRA-TURRAX UTL 2000 Disperser; IKA, Staufen, Germany) to break up protein aggregates. The resulting suspension was spray dried (Extractis Spray Dryer, Dury, France) using an inlet temperature of 150 °C and an outlet temperature of 50 °C.

Experimental diets

Experimental diets were formulated to contain 13–14% CP (as-fed basis). Each test ingredient was included in one diet as the sole source of CP and AA, and a nitrogen-free diet was used to measure basal endogenous losses of CP and AA. In total, seven diets were, therefore, included in the experiment (Tables 2 and 3). Vitamins and minerals were included in all diets to meet or exceed current nutrient requirement estimates for growing pigs.[17] All diets also contained 0.40% titanium dioxide as an indigestible marker. A sample of each test ingredient and of all diets was collected at the time of diet mixing and used for later chemical analysis.

Digestibility trial

Seven growing pigs (initial body weight: 36.51 ± 1.61 kg) that were the offspring of PIC Camborough females and PIC Line 359 males (Pig Improvement Company, Hendersonville, TN, USA) were equipped with a T-cannula in the distal ileum and allowed a 7-day recovery period.[18] During the recovery period, pigs were fed a common grower diet based on corn and soybean meal. Feed was restricted the first 3 days after surgery, but from day 4 post-surgery, feed was provided on an *ad libitum* basis. Following the recovery period, pigs were randomly allotted to a 7 × 7 Latin square design with seven diets and seven 7-day feeding periods where each experimental diet was fed to one pig in each period. Therefore, pigs were fed experimental diets for 49 days in total and, at the end of the experiment, all pigs had received each diet in one period. Because no pig received the same diet more than once during the experiment, there were seven replicate pigs for each diet. Pigs were housed in individual pens (1.2 × 1.5 m) in an environmentally controlled room (22–25 °C and 60–70% humidity).

All pigs were fed their assigned diets in a daily amount of 3.3 times the estimated energy requirement for maintenance (i.e. 824 kJ metabolizable energy per $kg^{0.60}$).[17] Diets were provided two equal meals every day at 07.00 h and 16.00 h. Water was available at all times. Pig weights were recorded at the beginning of each period and at the conclusion of the experiment. The amount of feed supplied each day was recorded. The initial 5-days of each period was considered the adaptation period to the experimental diet, whereas ileal digesta were collected for 9 h on days 6 and 7 using standard procedures.[18,19]

Chemical analysis

Ileal digesta samples were lyophilized and finely ground prior to analysis. Samples of all ingredients, diets and ileal digesta were analyzed for dry matter (AOAC method 927.05)[20] and for N by combustion (AOAC method 990.03)[20] using a LECO FP628 analyzer (LECO Corp., Saint Joseph, MI, USA) with subsequent calculation of CP as N × 6.25. Diets and ileal digesta samples were also analyzed for AA [AOAC method 982.30 E (a–c)][20] and for titanium (method 990.08).[21] All ingredients except whey protein isolate were also analyzed for trypsin inhibitor activity (EN-ISO method 14 902:2001)[22] and differential scanning calorimetry was used to

Table 2. Ingredient composition of experimental diets (as-fed basis)

Item (%)	Brown rice protein concentrate	Pea protein concentrate	Rapeseed protein isolate	Rapeseed protein isolate heat-treated	Soy protein isolate	Whey protein isolate	Nitrogen-free
Test ingredient	16.00	16.00	13.50	13.50	15.00	14.75	–
Corn starch	53.30	53.30	55.75	55.75	54.45	54.85	69.25
Soybean oil	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Sucrose	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Limestone, ground	0.45	0.45	0.45	0.45	0.75	0.40	0.40
Dicalcium phosphate	1.70	1.70	1.75	1.75	1.25	1.45	1.80
Magnesium oxide	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Potassium carbonate	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Solka floc	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Sodium chloride	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Titanium dioxide	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin mineral premix†	0.15	0.15	0.15	0.15	0.15	0.15	0.15

† The vitamin-micromineral premix provided the following quantities of vitamins and microminerals per kilogram of complete diet: vitamin A as retinyl acetate, 11 136 IU; vitamin D_3 as cholecalciferol, 2208 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate and copper chloride; Fe, 126 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese sulfate; Se, 0.3 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc sulfate.

determine denaturation of the proteins. Values for enthalpies (ΔH) were determined by integration of the endothermal peak with STAR[e], version 12.10b (Mettler-Toledo, Columbus, OH, USA) normalized to J g^{-1} of a 40% protein solution.

Calculations

Values for apparent ileal digestibility, basal ileal endogenous losses and standardized ileal digestibility (SID) of CP and AA in each diet were calculated.[23] The DIAAS reference ratio was calculated for indispensable AA as previously explained,[13] and DIAAS values were calculated for three different age groups according to FAO 2013 AA reference values.[7]

Statistical analysis

Normality of residuals was verified and outliers were identified using the UNIVARIATE and BOXPLOT procedures, respectively (SAS Institute Inc., Cary, NC, USA). Outliers were removed if the value deviated from the first or third quartiles by more than three times the interquartile range.[24] Data were analyzed by analysis of variance using PROC MIXED of SAS (SAS Institute Inc.). The pig was the experimental unit for all analyses. Diet was the fixed effect and pig and period were random effects. Treatment means and pooled SEM were calculated using the LSMEANS statement in SAS and, if significant, means were separated using the PDIFF option in the PROC MIXED procedure. An alpha value of 0.05 was used to assess significance among means.

RESULTS

Pigs remained healthy throughout the experiment and accepted their assigned diets, with no leftovers were observed after the meals. However, during data analysis, three different pigs from different experimental periods fed diets containing rapeseed protein isolate, heat-treated rapeseed protein isolate, or whey protein isolate were identified as outliers (one pig for each diet) and, for these proteins, LSMEANS were calculated based on six replicates, whereas LSMEANS for the remaining proteins were based on seven replicates. At least five replicate pigs are required to obtain results that are representative for a food ingredient.[19]

Apparent ileal digestibility

Whey protein isolate had greater ($P < 0.001$) apparent ileal digestibility (> 90%) for all indispensable AA, except arginine, methionine and phenylalanine, compared with soy protein isolate and heat-treated rapeseed protein isolate (Table 4). The apparent ileal digestibility of all indispensable AA, except leucine and methionine, was not different between heat-treated rapeseed protein isolate and soy protein isolate. Heat-treated rapeseed protein isolate had greater ($P < 0.001$) apparent ileal digestibility (> 80%) of histidine, leucine, methionine, threonine, tryptophan, and valine than pea protein concentrate. Likewise, heat-treated rapeseed protein isolate had greater ($P < 0.001$) apparent ileal digestibility (> 85%) for all indispensable AA compared with conventional

Table 3. Analyzed nutrient composition of experimental diets (as-fed basis)

Item (g kg^{-1})	Brown rice protein concentrate	Pea protein concentrate	Rapeseed protein isolate	Rapeseed protein isolate heat-treated	Soy protein isolate	Whey protein isolate	Nitrogen-free
Dry matter	933.20	928.00	933.90	935.90	930.50	935.20	915.30
Crude protein	136.90	139.30	141.80	142.40	136.20	129.80	3.19
Indispensable amino acids							
Arginine	9.80	10.40	8.50	8.20	9.60	2.50	0.10
Histidine	3.20	3.20	4.30	4.30	3.40	2.40	0.00
Isoleucine	6.40	6.70	5.50	5.30	6.80	10.50	0.10
Leucine	11.60	11.00	9.90	9.60	10.80	14.50	0.30
Lysine	4.20	10.20	8.60	8.80	8.70	13.70	0.20
Methionine	3.50	1.30	2.70	2.60	1.70	2.70	0.00
Phenylalanine	7.70	7.60	5.60	5.30	7.30	4.50	0.20
Threonine	5.10	4.70	4.70	4.60	5.00	10.70	0.10
Tryptophan	1.70	1.20	2.10	2.10	2.00	3.30	0.20
Valine	8.80	7.00	7.00	6.80	6.90	8.90	0.10
Total	62.00	63.30	58.90	57.60	62.20	73.70	1.30
Dispensable amino acids							
Alanine	7.90	5.80	5.90	5.80	5.90	7.20	0.10
Asparagine	12.20	15.40	7.90	7.20	15.30	16.20	0.20
Cysteine	3.20	1.40	4.70	4.80	1.70	3.50	0.00
Glutamic acid	24.10	22.40	31.90	31.90	25.90	26.00	0.20
Glycine	6.50	5.60	6.70	6.50	5.90	2.50	0.10
Proline	7.00	6.20	10.80	10.50	7.40	9.70	0.40
Serine	7.10	5.90	4.60	4.50	6.00	6.20	0.10
Tyrosine	5.20	3.70	2.40	2.30	4.00	3.20	0.10
Total	73.20	66.40	74.90	73.50	72.10	74.50	1.20
Total amino acids	135.20	129.70	133.80	131.10	134.30	148.20	2.50

rapeseed protein isolate and brown rice protein concentrate. Soy protein isolate had greater ($P < 0.001$) apparent ileal digestibility (> 85%) of histidine, methionine, tryptophan, and valine than pea protein concentrate, but pea protein concentrate had a greater ($P < 0.001$) apparent ileal digestibility (> 80%) of all indispensable AA except tryptophan compared with brown rice protein concentrate and rapeseed protein isolate.

Standardized ileal digestibility

Apparent ileal digestibility was corrected for basal ileal endogenous losses (*i.e*. 20.84 g CP per kg dry matter intake), which enabled calculation of SID for each AA (Table 5). Whey protein isolate had greater ($P < 0.001$) SID (> 90%) of most indispensable AA compared with the remaining proteins, but the SID of threonine and tryptophan was not different among whey protein isolate, soy protein isolate, and heat-treated rapeseed protein isolate. However, heat-treated rapeseed protein isolate had greater ($P < 0.001$) SID (> 90%) of indispensable AA than rapeseed protein isolate and brown rice protein concentrate, and greater ($P < 0.001$) SID of all indispensable AA (> 90%) except arginine and lysine than pea protein concentrate. However, the SID of most indispensable AA was not different between heat-treated rapeseed protein isolate and soy protein isolate. Pea protein concentrate had greater ($P < 0.001$) SID (> 85%) of all indispensable AA except tryptophan than brown rice protein concentrate and rapeseed protein isolate.

DIAAS

For infants from birth to 6 months, whey protein isolate and soy protein isolate had the greatest ($P < 0.001$) DIAAS (67% and 68%, respectively) followed by heat-treated rapeseed protein isolate (DIAAS: 60%) (Table 6). Brown rice protein concentrate had the lowest ($P < 0.001$) DIAAS (29%) and rapeseed protein isolate had a greater ($P < 0.001$) DIAAS (43%) than brown rice protein concentrate, but the DIAAS for pea protein concentrate (49%) was greater ($P < 0.001$) than for rapeseed protein isolate and brown rice protein concentrate. The first limiting AA for the experimental proteins were: aromatic AA (rapeseed protein isolate, heat-treated rapeseed protein isolate and whey protein isolate), sulfur AA (soy protein isolate and pea protein concentrate) and lysine (brown rice protein concentrate).

For children from 6 months to 3 years, heat-treated rapeseed protein isolate had the greatest ($P < 0.001$) DIAAS (100%), whereas brown rice protein concentrate had the lowest ($P < 0.001$) DIAAS (35%). Whey protein isolate had a DIAAS (94%) that was less ($P < 0.001$) than the DIAAS for heat-treated rapeseed protein isolate, but greater ($P < 0.001$) than for soy

Table 4. Apparent ileal digestibility of crude protein (CP) and amino acids (AA) in experimental ingredients†

Item (%)	Brown rice protein concentrate	Pea protein concentrate	Rapeseed protein isolate	Rapeseed protein isolate heat-treate^{d}	Soy protein isolate	Whey protein isolate	Pooled SEM	*P*-value
Crude protein	60.9^{c}	80.9^{b}	65.2^{c}	82.8 a^{b}	82.9 a^{b}	86.9^{a}	1.90	< 0.001
Indispensable amino acid								
Arginine	78.4^{b}	89.7^{a}	66.0^{c}	88.0^{a}	92.9^{a}	81.3^{b}	1.93	< 0.001
Histidine	74.1^{c}	87.6^{b}	76.1^{c}	93.0^{a}	91.4^{a}	92.9^{a}	1.00	< 0.001
Isoleucine	73.5^{c}	86.9^{b}	65.1^{d}	89.3^{b}	89.2^{b}	95.6^{a}	1.14	< 0.001
Leucine	73.5^{d}	87.6^{c}	67.5^{e}	91.7^{b}	88.2^{c}	96.4^{a}	1.09	< 0.001
Lysine	63.7^{d}	91.2^{b}	76.7^{c}	90.1^{b}	91.8^{b}	96.1^{a}	1.50	< 0.001
Methionine	69.0^{e}	82.6^{c}	76.3^{d}	94.7^{a}	90.2^{b}	95.3^{a}	0.96	< 0.001
Phenylalanine	75.4^{c}	89.0^{b}	65.5^{d}	90.5^{ab}	90.2^{ab}	92.5^{a}	1.06	< 0.001
Threonine	66.7^{d}	76.4^{c}	59.9^{e}	81.8^{b}	79.2^{bc}	87.0^{a}	1.71	< 0.001
Tryptophan	84.3^{c}	82.4^{c}	71.3^{d}	93.4^{b}	92.5^{b}	97.5^{a}	1.11	< 0.001
Valine	72.3^{d}	81.4^{c}	63.8^{e}	87.2^{b}	84.9^{b}	90.9^{a}	1.23	< 0.001
Mean	73.2^{c}	86.9^{b}	68.4^{d}	89.6^{b}	89.1^{b}	93.3^{a}	1.06	< 0.001
Dispensable amino aci^{d}								
Alanine	67.6^{d}	79.7^{c}	64.1^{d}	84.3^{ab}	81.8 b^{c}	88.6^{a}	1.58	< 0.001
Asparagine	67.2^{d}	88.8^{b}	48.8^{e}	79.9^{c}	89.1^{b}	94.9^{a}	1.54	< 0.001
Cysteine	62.7^{c}	59.5^{c}	80.7^{b}	90.4^{a}	80.5^{b}	94.1^{a}	1.84	< 0.001
Glutamic aci^{d}	70.6^{c}	92.0^{a}	80.0^{b}	92.8^{a}	93.4^{a}	93.9^{a}	0.95	< 0.001
Glycine	48.9^{c}	59.4^{bc}	57.3^{c}	74.8^{a}	69.8^{ab}	59.4^{bc}	4.46	0.001
Proline	−64.0^{c}	−20.7^{bc}	20.6^{ab}	36.9^{a}	16.9^{ab}	54.6^{a}	22.06	< 0.001
Serine	75.3^{c}	82.4^{b}	65.0^{d}	85.0^{ab}	86.8^{a}	86.9^{a}	1.30	< 0.001
Tyrosine	68.7^{c}	84.3^{b}	55.2^{d}	84.9^{b}	89.4^{a}	92.4^{a}	1.32	< 0.001
Mean	54.9^{d}	74.9^{b}	63.0^{c}	80.1^{ab}	80.7^{ab}	86.8^{a}	3.07	< 0.001
Total amino aci^{d}	63.3^{c}	80.8^{b}	65.4^{c}	84.2^{b}	84.6^{b}	90.0^{a}	1.97	< 0.001

Note: $^{a–e}$Means within a row lacking a common lowercase letter are significantly different ($P < 0.05$).
† Data are least squares means of seven observations per treatment except for rapeseed protein isolate, rapeseed protein isolate heat-treated, and whey protein isolate that have six observations per treatment.

Table 5. Standardized ileal digestibility (SID) of crude protein (CP) and amino acids (AA) in experimental ingredients[†,‡]

Item (%)	Brown rice protein concentrate	Pea protein concentrate	Rapeseed protein isolate	Rapeseed protein isolate heat-treated	Soy protein isolate	Whey protein isolate	Pooled SEM	*P*-value
Crude protein	75.1 [c]	94.8 [b]	78.9 [c]	96.5 [b]	97.2 [ab]	101.9 [a]	1.90	< 0.001
Indispensable amino acid								
Arginine	86.2 [c]	97.1 [b]	75.0 [d]	97.3 [b]	100.9 [b]	111.9 [a]	1.93	< 0.001
Histidine	80.2 [d]	93.7 [c]	80.6 [d]	97.5 [b]	97.1 [b]	101.0 [a]	1.00	< 0.001
Isoleucine	78.5 [d]	91.7 [c]	70.8 [e]	95.3 [b]	93.9 [bc]	98.6 [a]	1.14	< 0.001
Leucine	78.1 [d]	92.5 [c]	73.0 [e]	97.3 [b]	93.2 [c]	100.1 [a]	1.09	< 0.001
Lysine	73.8 [d]	95.4 [ab]	81.6 [c]	94.9 [b]	96.8 [ab]	99.2 [a]	1.50	< 0.001
Methionine	71.1 [e]	88.3 [c]	79.1 [d]	97.6 [a]	94.6 [b]	98.0 [a]	0.96	< 0.001
Phenylalanine	79.7 [d]	93.4 [c]	71.5 [e]	96.8 [b]	94.8 [bc]	99.9 [a]	1.06	< 0.001
Threonine	77.8 [c]	88.5 [b]	71.9 [d]	94.2 [a]	90.5 [ab]	92.3 [ab]	1.71	< 0.001
Tryptophan	90.4 [b]	91.0 [b]	76.3 [c]	98.3 [a]	97.7 [a]	100.7 [a]	1.11	< 0.001
Valine	78.5 [d]	89.2 [c]	71.5 [e]	95.2 [ab]	92.7 [b]	97.0 [a]	1.23	< 0.001
Mean	79.4 [d]	93.0 [c]	74.9 [e]	96.3 [ab]	95.3 [bc]	98.5 [a]	1.06	< 0.001
Dispensable amino acid								
Alanine	75.9 [d]	91.0 [c]	75.2 [d]	95.6 [ab]	92.9 [bc]	97.6 [a]	1.58	< 0.001
Asparagine	73.6 [c]	93.9 [b]	58.7 [d]	90.7 [b]	94.2 [b]	99.7 [a]	1.54	< 0.001
Cysteine	68.4 [d]	72.5 [d]	84.6 [c]	94.2 [ab]	91.2 [b]	99.3 [a]	1.84	< 0.001
Glutamic acid	74.6 [c]	96.3 [a]	83.0 [b]	95.8 [a]	97.1 [a]	97.6 [a]	0.95	< 0.001
Glycine	78.5 [d]	93.7 [bc]	86.0 cd	104.4 [b]	102.4 [b]	136.3 [a]	4.46	< 0.001
Proline	54.7 [b]	113.3 [a]	97.6 [ab]	116.0 [a]	129.2 [a]	140.3 [a]	22.06	0.012
Serine	82.5 [c]	91.1 [b]	76.2 [d]	96.4 [a]	95.3 [a]	95.2 [a]	1.30	< 0.001
Tyrosine	73.7 [d]	91.4 [c]	66.0 [e]	96.2 [b]	95.9 [b]	100.5 [a]	1.32	< 0.001
Mean	73.4 [d]	95.4 [b]	81.1 [c]	98.5 [ab]	99.5 [ab]	105.1 [a]	3.07	< 0.001
Total amino acid	76.2 [c]	94.2 [b]	78.4 [c]	97.5 [ab]	97.6 [ab]	101.8 [a]	1.97	< 0.001

Note: [a–e]Means within a row lacking a common lowercase letter are significantly different ($P < 0.05$).
[†] Data are least squares means of seven observations per treatment except for rapeseed protein isolate, rapeseed protein isolate heat-treated, and whey protein isolate that have six observations per treatment.
[‡] Standardized ileal digestibility values were calculated by correcting values for apparent ileal digestibility for the basal ileal endogenous losses. Endogenous losses (g kg^{-1} of dry matter intake) of amino acids were as follows: crude protein, 20.84; arginine, 0.82; histidine, 0.21; isoleucine, 0.34; leucine, 0.58; lysine, 0.46; methionine, 0.08; phenylalanine, 0.36; threonine, 0.61; tryptophan, 0.11; valine, 0.58; alanine, 0.70; asparagine, 0.83; cysteine, 0.20; glutamic acid, 1.03; glycine, 2.06; proline, 8.90; serine, 0.55; tyrosine, 0.28.

protein isolate (83%). The DIAAS for rapeseed protein isolate (76%) was less ($P < 0.001$) than for soy protein isolate, but greater ($P < 0.001$) than for pea protein concentrate (60%), which had a DIAAS greater ($P < 0.001$) than brown rice protein concentrate. The first limiting AA for the experimental proteins were: leucine (rapeseed protein isolate), lysine (brown rice protein concentrate), histidine (whey protein isolate) and sulfur AA (soy protein isolate and pea protein concentrate). No limiting AA (DIAAS ≥ 100%) was observed for heat-treated rapeseed protein isolate.

For older children, adolescents and adults, whey protein isolate had the greatest ($P < 0.001$) DIAAS (117%) followed by heat-treated rapeseed protein isolate (110%). The DIAAS for rapeseed protein isolate (83%) was less ($P < 0.001$) than for soy protein isolate (97%), but greater ($P < 0.001$) than for pea protein concentrate (70%), which had a DIAAS greater ($P < 0.001$) DIAAS than brown rice protein concentrate (42%). The first limiting AA were: leucine (rapeseed protein isolate), lysine (brown rice protein concentrate) and sulfur AA (soy protein isolate and pea protein concentrate). No limiting AA (DIAAS ≥ 100%) was observed for heat-treated rapeseed protein isolate and whey protein isolate.

DISCUSSION

The calculation of DIAAS in food proteins is recommended to determine the protein quality of human foods[7] and details about conducting animal studies to determine DIAAS have been published.[19] The present study was conducted following the published procedures with the exception that the feeding level was calculated as 3.3 times the energy requirement for maintenance rather than 8% of the metabolic body weight and the level of CP in diets was slightly greater than the 10% recommended. These changes were made to supply sufficient energy and protein to the animals. There were also no antimicrobial control agents in collection bags because, if samples are frozen immediately after collection, there are no advantages of adding acids to the bags.[25] None of these deviations are expected to have influenced the results of the experiment.

Ingredients denoted as protein concentrates or isolates generally have a protein content (dry matter basis) of 50–80% or 80–95%, respectively.[2,26] The CP in all ingredients used in the present study was within the range of reported values.[4,14,16,27] Digestibility of CP and AA in brown rice and pea protein concentrates, as well as soy and whey protein isolates, was also in agreement with

Table 6. Digestible indispensable amino acid score (DIAAS) for experimental ingredients[†]

Item	Brown rice protein concentrate	Pea protein concentrate	Rapeseed protein isolate	Rapeseed protein isolate heat-treated	Soy protein isolate	Whey protein isolate	Pooled SEM	*P*-value
Infant (birth to 6 months)[‡]								
DIAA reference ratio								
Histidine	0.83	1.11	1.20	1.45	1.19	0.89		
Isoleucine	0.63	0.85	0.49	0.66	0.85	1.39		
Leucine	0.66	0.79	0.52	0.70	0.75	1.11		
Lysine	0.29	1.05	0.71	0.83	0.87	1.44		
Sulfur AA	1.00	0.49	1.37	1.53	0.68	1.47		
Aromatic AA	0.86	0.94	0.43	0.60	0.92	0.67		
Threonine	0.57	0.70	0.54	0.71	0.73	1.66		
Tryptophan	0.66	0.49	0.64	0.88	0.79	1.39		
Valine	0.89	0.88	0.65	0.87	0.86	1.18		
DIAAS (%)	29[e] (lysine)	49[c] (sulfur AA)	43[d] (aromatic AA)	60[b] (aromatic AA)	68[a] (sulfur AA)	67[a] (aromatic AA)	0.88	< 0.001
Child (6 months to 3 years)[§]								
DIAA reference ratio								
Histidine	0.87	1.16	1.26	1.52	1.25	0.94		
Isoleucine	1.09	1.46	0.85	1.13	1.45	2.39		
Leucine	0.95	1.15	0.76	1.02	1.09	1.61		
Lysine	0.35	1.27	0.86	1.00	1.06	1.74		
Sulfur AA	1.22	0.60	1.67	1.87	0.83	1.79		
Aromatic AA	1.56	1.70	0.78	1.08	1.66	1.22		
Threonine	0.82	0.99	0.77	1.01	1.04	2.35		
Tryptophan	1.33	0.98	1.28	1.76	1.57	2.78		
Valine	1.14	1.13	0.83	1.11	1.10	1.51		
DIAAS, %	35[f] (lysine)	60[e] (sulfur AA)	76[d] (leucine)	100[a]	83[c] (sulfur AA)	94[b] (histidine)	1.19	< 0.001
Older child, adolescent, adult[¶]								
DIAA reference ratio								
Histidine	1.09	1.45	1.57	1.90	1.56	1.17		
Isoleucine	1.16	1.55	0.91	1.21	1.55	2.55		
Leucine	1.03	1.24	0.83	1.10	1.18	1.74		
Lysine	0.42	1.51	1.02	1.19	1.26	2.07		
Sulfur AA	1.43	0.70	1.96	2.20	0.97	2.11		
Aromatic AA	1.98	2.15	0.99	1.38	2.10	1.55		
Threonine	1.01	1.23	0.95	1.25	1.29	2.92		
Tryptophan	1.71	1.27	1.65	2.27	2.03	3.58		
Valine	1.23	1.21	0.90	1.19	1.19	1.62		
DIAAS, %	42[f] (lysine)	70[e] (sulfur AA)	83[d] (leucine)	110[b]	97[c] (sulfur AA)	117[a]	1.38	< 0.001

Note: [a–f] Means within a row lacking a common lowercase letter are significantly different ($P < 0.05$).

[†] First-limiting AA is in parentheses. Aromatic AA (phenylalanine and tyrosine); Sulfur AA (methionine and cysteine).

[‡] DIAAS were calculated using the recommended AA scoring pattern for an infant (birth to 6 months). The indispensable AA reference patterns are expressed as mg AA g^{-1} protein: histidine, 21; isoleucine, 55; leucine, 96; lysine, 69; sulfur AA, 33; aromatic AA, 94; threonine, 44; tryptophan, 17; valine, 55.[14]

[§] DIAAS were calculated using the recommended AA scoring pattern for a child (6 months to 3 years). The indispensable AA reference patterns are expressed as mg AA g^{-1} protein: histidine, 20; isoleucine, 32; leucine, 66; lysine, 57; sulfur AA, 27; aromatic AA, 52; threonine, 31; tryptophan, 8.5; valine, 43.[14]

[¶] DIAAS were calculated using the recommended AA scoring pattern for an older child, adolescent, and adult. The indispensable AA reference patterns are expressed as mg AA g^{-1} protein: histidine, 16; isoleucine, 30; leucine, 61; lysine, 48; sulfur AA, 23; aromatic AA, 41; threonine, 25; tryptophan, 6.6; valine, 40.[14]

published data.[14,27] Amino acid digestibility in protein concentrates and isolates from plant proteins is expected to be greater compared with their meal or flour form as a result of the removal of fiber and anti-nutritional factors,[8,28] resulting in an improved protein quality.

The increase in digestibility of CP and AA that was observed for rapeseed protein isolate after heat treatment may be a result of the decrease in trypsin inhibitor activity observed for the heat-treated rapeseed protein isolate (0.06%) compared with the non-heat-treated rapeseed protein isolate (1.8%). Anti-nutritional factors are generally removed during protein extraction, whereas heat treatments are commonly used to modify protein structure and may be more effective than extraction in removing anti-nutritional factors.[29] Increased digestibility in heat-treated rapeseed protein isolate compared with non-heat-treated rapeseed may also be a result of irreversible denaturation of proteins, which is more likely to occur at temperatures above 70 °C and may result in increased digestibility of CP and AA.[8,30] Temperatures ranging from 50 to 90 °C can be used to control moderate protein denaturation and promote protein unfolding and, as a result, increased accessibility to intramolecular bonds for digestive enzymes, depending on protein concentration, pressure, denaturation, transition, temperature range and bond strength.[30,31] Because rapeseed proteins contain disulfide bonds, hydrolysis of these bonds by heating may allow easier access for digestive enzymes.[32,33] The observation that lysine digestibility in the heat-treated rapeseed protein isolate was greater than in the non-heat-treated rapeseed protein isolate is a strong indication that the heat treatment did not initiate Maillard reactions, because the Maillard reaction usually results in reduced digestibility of lysine.

As expected, whey protein isolate had greater DIAAS for children older than 3 years, adolescents and adults than the plant proteins, and whey protein is considered a high-quality protein for humans.[14] Animal proteins usually have a greater DIAAS compared with plant proteins,[8,9] but pulses and oilseeds have a greater DIAAS compared with cereal grains.[28,34] The ingredients used in this experiment follow this generalization, with whey protein isolate having the greatest DIAAS, soy protein isolate, rapeseed protein isolate and pea protein concentrate with a lesser DIAAS, and brown rice protein concentrate having the least DIAAS. Values for DIAAS for these proteins are in agreement with DIAAS reported in the literature.[14,27,28] However, to our knowledge, the DIAAS for rapeseed protein isolate or rapeseed in any form has not been reported, but the DIAAS values of 76% and 100% for rapeseed protein isolate and heated rapeseed protein isolate, respectively, for children 6 months to 3 years are in agreement with reported values for DIAAS in soy (83–92%) and milk (90–109%) proteins.[14,27,28] The lower DIAAS in brown rice and pea protein concentrates compared with the isolates (i.e. whey, soy, and rapeseed) may be a result of the greater concentration of non-protein substances, such as fiber and anti-nutritional factors, in concentrates than in isolates.[35] The DIAAS for rice protein concentrate was lower than that reported for oat protein concentrate,[36] which is in agreement with the observation that intact dehulled oats has a greater DIAAS than polished white rice.[13]

The observation that heat processing of rapeseed protein isolate improved DIAAS to a value greater than that for soy protein isolate and close to that for whey protein isolate demonstrates the value of mild heat processing. An increase in DIAAS or nutritive value was also observed when peas and rapeseed products were processed from their raw form into a concentrate product;[27,29] however, a decrease in DIAAS was observed when nuts (e.g. pistachios) or cereal grains (e.g. rice, oats, wheat) were processed at temperatures over 100 °C, which may cause heat damage and protein aggregation, resulting in decreased CP and AA digestibility.[26,37,38] These different responses to heating illustrates the importance of correct heat treatment because overheating results in degradation of lysine and often other AA, which results in a reduction in both digestibility and post-absorptive utilization of absorbed AA.[13] Nevertheless, based on DIAAS cut-off values,[7] heat-treated rapeseed protein isolate and whey protein isolate are both 'excellent' protein sources for humans and can be used as the sole protein source or to supplement lower quality proteins to meet the physiological requirements for CP and AA for individuals older than 6 months.

CONCLUSIONS

According to FAO protein quality guidelines, no claims regarding protein quality can be made for brown rice and pea protein concentrates because their DIAAS were less than 75. Rapeseed, soy, and whey protein isolates, on the other hand, can be considered high-quality protein sources for individuals over the age of 6 months. The results of this experiment demonstrated that the protein quality of rapeseed protein isolate was comparable to that of soy protein isolate. In addition, mild heating improved AA digestibility and increased DIAAS for heat-treated rapeseed protein isolate, qualifying it as an 'excellent' protein and allowing it to be used to supplement low-quality protein ingredients and provide a complete meal with indispensable AA for individuals older than 6 months.

AUTHOR CONTRIBUTIONS

HHS conceptualized the research. HMB conducted the animal part of the experiment, analyzed the data and wrote the first draft of the manuscript. HHS and NSF revised the manuscript and prepared the final version submitted for publication.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

Research data are not shared.

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